THESIS

COLOR, CAROTENOID CONTENT AND SENSORY PERCEPTIONS IN POTATO GERMPLASM FROM THE COLORADO POTATO BREEDING AND SELECTION PROGRAM

Submitted by

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ABSTRACT

COLOR, CAROTENOID CONTENT AND SENSORY PERCEPTIONS IN POTATO GERMPLASM FROM THE COLORADO POTATO BREEDING AND SELECTION PROGRAM

Field-grown potato tubers were evaluated for tuber flesh color, focusing on hue and chroma, total carotenoid content and identification and quantification of individual carotenoids. A total of 138 clones/cultivars from the Colorado Potato Breeding and Selection Program were evaluated to determine the chroma and hue of the tuber flesh. A subset of 100 entries, 65 tetraploids and 35 diploids, were analyzed for total carotenoid content and eight select entries for individual carotenoid content. Volatile flavor compounds were analyzed in 12 select entries, including two diploid entries with high carotenoid levels, using both microwaved and steamed cooking methods. Five entries from the volatile compound analysis were selected for a sensory evaluation. The relationship between tuber flesh chroma and carotenoid content was analyzed. Total carotenoid content was positively correlated ($r = 0.72$) with chroma for the subset of 100 entries. The range in total carotenoid content was 16 to 2741 µg/100 gfw (grams fresh weight). Diploid entries had a total carotenoid content three times higher than tetraploid entries. There was a significant entry by year interaction for total carotenoid content. Lutein was the major carotenoid detected among the eight entries analyzed. For the volatile flavor compounds, limonene was quantified and alpha-copaene, decanal, isovaleraldehyde, and 2-pentanone were detected in 12 select entries. The relationship between volatile compounds and sensory scores was analyzed. Limonene was not detected in the two diploid entries with high carotenoid levels.

The sensory evaluation revealed higher sensory scores for the three tetraploid entries than the two diploid entries with high carotenoid levels. The recently named cultivar Masquerade received the highest score for overall acceptability for both steamed and microwaved cooking methods. The use of diploid potato entries will be a target for future breeding efforts in order to increase carotenoid levels. Further research is needed to identify entries with promising flavor characteristics in order to develop cultivars with greater carotenoid levels and enhanced flavor.

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1.1 Introduction

Studies have suggested that the consumption of carotenoids in the human diet have beneficial health effects. Carotenoids may provide protection against chronic diseases (Gaziano et al. 1995) including cardiovascular disease, coronary heart disease, diabetes, and certain cancers (Colditz et al. 1985). Carotenoids contain antioxidant properties that may help protect cellular systems from oxidative damage which will lower the risk of chronic diseases (Liu 2003). Intake of specific carotenoids, lutein and zeaxanthin, appear to provide protection against age-related macular degeneration (Krinsky et al. 2003; Beatty et al. 2004). Age-related macular degeneration is the leading cause of severe visual impairment and blindness in the United States (Department of Health and Human Services et al. 1997).

The potato (*Solanum tuberosum*) is rich in calories and is often denigrated for its calories in today's society where physically active lifestyles have greatly decreased. This provides a strong impetus to study and characterize other compounds of the tuber (Brown 2008). Breeding efforts largely focus on agronomic practices and efforts to change the nutrient composition of potato are lagging behind (Nesterenko and Sink 2003). Carotenoids are a group of compounds that accumulate in the flesh of the tuber along with another group of antioxidants called anthocyanins. The level of carotenoids determines whether the tuber flesh is white, yellow, or orange, whereas the anthocyanin levels lead to red, purple or blue flesh color (Morris et al. 2008). Carotenoids are one of the many phytochemicals that potato contains. Along with them, the potato also encompasses flavonoids, flavonols, phenolic compounds, vitamins and minerals (Woolfe 1987).

Even though potato may be seen from a negative aspect for being calorie rich, it is the most important non-cereal food crop worldwide (Nesterenko and Sink 2003) and in the U.S., with a per capita consumption of 117 lbs (53.1 kg) reported in 2010 (National Potato Council 2011). However, this consumption has decreased within the last ten years, from 138 lbs (62.6 kg) reported in 2000. This is most likely due to several factors, one of them is that consumers believe potatoes are high in calories and will cause weight gain. This is unfortunate since potatoes not only contain antioxidants, but significant levels of vitamin C, fiber and protein (Woolfe 1987). An average potato (5.3 ounces/150.3 g) contains only 110 calories, 0 g of fat, about 620 mg of potassium, and 10% of the daily value of vitamin B6 (United States Potato Board 2010). Through continued research and marketing efforts, consumer knowledge of the many positive attributes of potato is slowly increasing.

Xanthophylls are the most abundant carotenoids in potato. Fat soluble xanthophylls are antioxidants associated with membranes in the cell and have half-lives of several days in humans (Brown 2008). They are not provitamin A compounds, but are components of the human retina. Potatoes contain varying concentrations of lutein, zeaxanthin, and violaxanthin among others (Brown et al. 2007). There are other sources of these xanthophylls but potato is one source that is frequently consumed in the U.S. The major dietary sources of zeaxanthin and lutein are dark green leafy vegetables. These are generally not consumed by Americans in large amounts (Lu et al. 2001).

While the nutritional content of potatoes is important, taste and flavor are also important factors to consider. The marketplace has a strong influence in determining whether a new cultivar is acceptable to consumers (Wang and Kays 2003). A cultivar may have high levels of carotenoids but the taste may not be acceptable to consumers. This thesis will focus on

carotenoid content and will look at the relationship between carotenoid content, volatile flavor compounds and consumer preference of potatoes.

This research study had four objectives:

1. To evaluate and compare the tuber-flesh color of 138 entries in the Colorado Potato Breeding and Selection Program,
2. To measure select entries for total and individual carotenoid content,
3. To evaluate 12 select entries for volatile flavor compounds present using both steamed and microwaved cooking methods,
4. To perform a sensory analysis for 5 select entries using both steamed and microwaved cooking methods.

1.2 Potato History and Characteristics

The potato (*Solanum tuberosum* L.) is native to South America and was domesticated in the Andes Mountains (Brown et al. 2007). Potatoes were cultivated from Chile to New Granada before the discovery of the New World (Decoteau 2000). There is general agreement that the greatest diversity in potato exists in the Southern Peru-Bolivian Altiplano (Bohl and Johnson 2010). Prevailing evidence suggests that the Spaniards introduced the potato from an Andean location in South America to Spain by 1580, or possibly as early as 1565. It was taken into Italy about 1585, Belgium and Germany by 1587, Austria by 1588, and France soon after 1600. It returned to North America with the colonists who settled along the Atlantic coast. However, potatoes were not a significant food source there until Irish immigrants came in the early 1700's (McMahon et al. 2007).

An assumption exists that potato taken from the central Andes would have lacked long-day adaptation (Bohl and Johnson 2010). Selection was needed to obtain an appropriate day

length response and a respectable tuber yield before they were adopted as “second bread” (Brown 1999). Potatoes became America’s favorite carbohydrate food because of their low cost and acceptance by many people. They are currently grown on every continent, in every state of the United States and are harvested somewhere in the United States every month of the year (McMahon et al. 2007).

The potato is classified in the family *Solanaceae*, or the nightshade family which includes many other important commercial plants such as tomato, green peppers, and eggplant. The family includes about 90 genera, the largest being the genus *Solanum* and about 100 species of *Solanum* are tuber-bearing. These species are polyploids, ranging from diploids to hexaploids with 75% of them being diploid (Sleper and Poehlman 2006). Tetraploid potatoes are the most diverse, widespread and highest yielding (Brown 1999).

The potato is classified as an annual due to it being planted and harvested in one year. It can however, persist in the field vegetatively as potato tubers resulting in volunteer plants growing in subsequent years after planting. This creates problems for the next season when dealing with pest management. Potato is also a dicot and contains the characteristics of dicotyledons such as stems with vascular bundles placed in circular arrangement and layers of xylem and phloem (Bohl and Johnson 2010).

The potato plant produces purple, white or blue flowers. When the flowers are pollinated they may develop small green berries containing seeds. The seeds produce new types of potato plants different from the parent plant and each other in many respects (McMahon et al. 2007). The tuber of the potato is not a root but an enlarged portion of an underground stem referred to as a stolon. Tubers originate from the tips of stolons and occasionally tubers form along the stolon itself (Bohl and Johnson 2010). The potato tuber contains characteristics of

normal stems. This includes dormant true buds (eyes) with leaf scars and lenticels or stem pores where air penetrates to the stem interior. The buds form a spiral pattern on the tuber and are generally concentrated at the apical end of the tuber.

1.3 Potato Production and Growth Cycle

Potatoes are one of the most productive and widely grown food crops that feed the world's population. They produce approximately twice as many calories per hectare as rice or wheat (Sleper and Poehlman 2006). The crop is grown commercially throughout North America and harvested potato land area in North America expanded and peaked in the first quarter of the 20th century. United States potato area harvested was in the range of 1.2-1.3 million acres in the 1980's and rose to 1.350 million acres in 2000, but dropped again in 2005 to 1.085 million acres. The decline in land planted to potatoes in the United States is partly due to the steady increase in yields that have occurred. United States average yields improved more than seven fold during the twentieth century, from 52 to 381 hundredweight (cwt) per acre. In just the last quarter of the twentieth century U.S. potato yields increased 50 percent.

Potato is a cool-season crop that is slightly tolerant of frost, but can easily be damaged by freezing weather near maturity (McMahon et al. 2007). Generally, a growing season of 90 to 120 frost-free days is required. They can grow in areas with shorter seasons because it can be compensated for by long days and by higher light intensities at higher elevations. Potatoes can grow on a variety of soil types but well-drained, fertile sandy loam soils are preferred. Good water penetration and aeration are necessary for proper potato plant growth and tuber formation.

The potato plant generally grows to a height of 24 to 30 inches (61.0 to 76.2 cm), a spread around 24 inches (61.0 cm) and a root depth of 2 feet (61.0 cm) (Decoteau 2000). The initiation of young tubers at the tips of the stolons usually occurs when the plants are 6 to 8

inches (15.2 to 20.3 cm) high, or 5 to 7 weeks after planting. Several environmental factors affect tuberization and a large amount depends on the translocation and storage of carbohydrate reserves (Bohl and Johnson 2010). This is in excess of that needed by other parts of the plant in its growth and metabolism. Potato plants will form tubers without any flowers and is not dependent upon flowering. Tuber formation generally occurs during long days of high light intensity and maximum yields of high-quality tubers are produced during a growing season with a mean temperature between 15°C and 18°C (59°F and 64°F) (McMahon et al. 2007).

There are different stages in the development of a potato plant. These include: sprout development, plant establishment, tuber initiation, tuber bulking and tuber maturation (Stark and Love 2003). Tubers will begin sprouting once dormancy is broken and environmental conditions are favorable. Vegetative growth then starts to occur with the development of both roots and shoots. When conditions are right new tubers will begin to form which usually occurs during the same time as flowering for most cultivars. There is no causal relationship between the two though. In cultivated potato, flowers mostly open early in the morning, with a few continuing to open throughout the day. For flowering to occur, potatoes require abundant rainfall, cool temperatures and long days (McMahon et al. 2007). The berry that develops from the flower will only form when a precise climate and long day length period is met. Cross-pollination is most often accomplished by bumblebees, which are the main carriers of pollen.

Tuber bulking is a critical time during growth so good tuber yield and quality can be achieved. The cultivar Russet Burbank typically adds about 6 to 10 cwt per acre per day during active growth when grown in southern Idaho under ideal conditions (Stark and Love 2003). Two major factors shown in research to influence tuber yield are the length of the linear tuber growth phase and the photosynthetic activity and duration of the leaf canopy. It is also important to note

that the tubers are often competing with the vines for limited nutrient resources. One of the many factors that can affect the balance between vine and tuber growth is temperature. Tuber growth can be delayed by high soil temperatures. As tubers mature, the vines die back and the tuber skin thickens to provide greater protection. In addition, specific gravity or dry matter increases and free sugars are converted to starch. With proper maturity, tubers put into storage have lower respiration rates. This results in less dry matter loss and tubers that remain dormant longer and sprout later. Tuber quality is improved when specific gravity increases and the conversion from free sugars to starch allows for lighter colored chips and fries. Storing tubers when they are at proper maturity produces better quality for both processing and fresh market consumption.

1.4 Nutrition and Health Attributes

As important as potatoes have been in the North American diet, perceptions of consumers have shifted from lowly to fattening to healthy to high carbohydrate (Bohl and Johnson 2010). In the past the potato was mostly consumed by people with a low income because it was cheaper than other foods. The potato has also been given the label of fattening, healthy or high carbohydrate throughout the years. The United States Potato Board (USPB) was created to help educate consumers about the health benefits of potatoes. This was done in the 1970s to improve the image of potato and increase per capita consumption (Bohl and Johnson 2010). Consumption increased to the year 2000 but with it came a change in product form. Fresh consumption declined while consumption of processed products grew. From 1960 to 1990, consumption of processed products, on a farm-weight basis, tripled. The shifts in potato consumption occurred from a number of different factors. Two major factors are the decrease in

household size along with an increase in the number of households with two or more members employed outside the household reducing the amount of time for food preparation.

In the early 2000's the United States went through a low-carbohydrate diet obsession causing a slight noticeable decrease in potato consumption (U.S. Potato Board 2012). Efforts in consumer marketing were increased by the potato industry in order to educate the public. Throughout the 2000's, consumers also believed that potatoes were high in calories and fat compared to other carbohydrate sources such as rice or pasta. This is an incorrect assumption because potato has negligible fat and a low energy density similar to legumes (Camire et al. 2009). In 2007 the USPB adopted an industry-wide signature, "Potatoes…Goodness Unearthed," to promote the nutritional benefits of the potato. This was the first unified endeavor by the entire U.S. potato industry to clearly identify the U.S. potato as a "nutrition powerhouse" (U.S. Potato Board 2012).

Many consumers are unaware of the important contribution that relatively small amounts of potato make to North American nutrition today. Modern nutritionists call it the high "nutrient density" of potato, meaning that for each calorie of potato eaten there is an ample return of essential nutrients (Bohl and Johnson 2010). A medium potato (5.3 oz/150.3 g) with the skin contains 45% of the daily value of Vitamin C. Vitamin C has many benefits to the human body. It acts as an antioxidant, helping to prevent cellular damage. Vitamin C also assists with the absorption of iron, aids in collagen production and may help support the body's immune system. Data has shown that potatoes rank in the top five for dietary sources of vitamin C for Americans (U.S. Potato Board 2012).

Potassium is a nutrient that provides heart disease protection and reduces the risk of hypertension and stroke when accompanied in a diet with low sodium. Potatoes provide one of

the most concentrated and affordable sources of potassium, as much or more than either bananas, spinach or broccoli (U.S. Potato Board 2012). With the skin, potatoes supply 18% of the daily value of potassium (Bohl and Johnson 2010). There are at least 12 essential vitamins and minerals present in the potato plus protein. Some of these are vitamin B6, which potatoes provide 10% of the daily value, along with trace amounts of thiamin, riboflavin, folate, magnesium, phosphorous, iron and zinc.

A cooked potato with the skin left on is a good dietary source of carbohydrates. Dietary fiber is a complex carbohydrate that has several health benefits, such as regulating blood glucose, improving blood lipid levels and increasing satiety. One medium potato provides 8% of the daily value recommended for fiber (United States Potato Board 2012). Resistant starch is also present in potatoes, providing health benefits within the colon. It is important to note that potatoes are gluten-free since an estimated 3 million Americans suffer from side effects associated with gluten-containing foods. The majority of the nutrients contained in potatoes are within the tuber flesh and not in the skin. Cooking does have an impact on these nutrients and nutrient loss is greatest when the cooking method involves extended periods of time and/or water. Steaming and microwaving are the best cooking methods to use in order to maintain the most nutrition (Perla et al. 2012).

In addition to the many vitamins and minerals present in potatoes, they also contain a variety of phytochemicals with antioxidant potential. Varying amounts of anthocyanins and carotenoids are in the tuber skin and flesh (Brown et al. 2007). Purple and red potatoes contain the greatest amount of anthocyanins while carotenoids are largely found in yellow and red potatoes, with small amounts present in white potatoes (Brown et al. 2004). Anthocyanins are important in plant and human health, contributing beneficial antioxidant properties (Camire et al.

2009). Carotenoids may protect against a variety of chronic diseases, including cardiovascular disease and certain cancers (Lu et al. 2001).

1.5 Tuber Flesh Color

Yellow-flesh potatoes contain higher levels of total carotenoids than white-flesh and dominate the majority of the world's production of potatoes (Brown et al. 2007). Although Americans are more familiar with white-flesh U.S. cultivars, several yellow-flesh potato cultivars have been developed and released recently. The determination of white or yellow flesh is thought to be controlled by a single gene which maps to chromosome 3 for the yellow flesh factor (*Y/y*) (Bonierbale et al. 1988; Gebhardt et al. 1989). The yellow flesh state with allele *Y* is dominant over the white flesh state with allele *y* (Fruwirth 1912). Brown and colleagues (1993) discovered the orange flesh trait in diploid populations and was found to be controlled by the *Or* allele at the *Y* locus, with *Or* being dominant over *Y* and *y*. The concentration of carotenoids can vary greatly in different entries but certain carotenoids have been found to be associated with certain tuber flesh colors. Violaxanthin and lutein are identified as the major carotenoids found in yellow-flesh potatoes (Iwanzik et al. 1983), whereas zeaxanthin and lutein are identified as major carotenoids in orange-flesh potatoes (Brown et al. 1993).

1.5.1 L, a, b Values, Hue and Chroma

When measuring flesh or skin color on fruits and vegetables, a reflectance colorimeter is used to determine various values associated with a color. Many scientists use instruments such as the Hunter colorimeter or various Minolta chroma meters, which generate a set of Cartesian coordinates that pinpoint the measured color in a three-dimensional space (McGuire 1992). For the Hunter scale, L measures lightness and varies from 100 for the perfect white to 0 for black and the coordinates, a and b, locate the color on a rectangular-coordinate grid perpendicular to

the L axis and they are the chromaticity dimensions (HunterLab 2003; McGuire 1992). Color at the grid origin represents grays (colors of little or no chromaticity) and on the horizontal axis a positive a coordinate indicates a hue of red-purple and a negative a bluish-green. The b coordinate is on the vertical axis, a positive b indicates yellow while a negative b blue (Voss 1992; McGuire 1992). Chroma indicates the intensity or saturation of a color and hue measures true color and varies from 0 to 360 on a color wheel (Cantwell et al. 2004). Coordinates a and b indirectly reflect hue and chroma and are difficult to infer separately. Chroma denotes the hypotenuse of a right triangle by joining the points (0,0), (a,b) and (a,0) and hue is the angle between the hypotenuse and 0° on the a axis (McGuire 1992). To calculate chroma, $[a^2+b^2]^{1/2}$ is used and arctan (b/a) is used for hue angle, where arctangent uses positive values in the first and third and negative values in second and fourth quadrants (Steed and Truong 2008; McGuire 1992).

Both white-skin, white-flesh potatoes and yellow-skin, yellow-flesh potatoes have relatively high L and hue values. This contrasts with red-skin, red-flesh potatoes, which show low L and hue values and purple-skin, purple-flesh potatoes have very high hue values (Cantwell et al. 2004). Lu et al. (2001) found evidence suggesting that selecting for greater yellow flesh intensity will result in higher levels of carotenoids. Thus, measurement of yellow intensity can provide a quick and efficient way to screen a large number of entries for carotenoids in order to narrow it down to those that contain high carotenoid levels for further analysis.

1.6 Carotenoids and Potato

Plants contain both primary and secondary metabolites. Primary plant metabolites are necessary building blocks for growth and development, whereas secondary metabolites are used for signaling and defense against abiotic and biotic stresses (Watson et al. 2009). Secondary

metabolites are divided into three major groups based on biosynthetic origins: terpenoids, alkaloids, and the phenylpropanoids and allied phenolic compounds (Croteau et al. 2000). These groups are further divided into several chemical classes of compounds. Of the 14 classes that categorize secondary metabolites, carotenoids are a part of the tetraterpene class (Wink 2003).

Terpenoids are classified by the number of five-carbon units they contain also known as an isoprene (Croteau et al. 2000). Tetraterpenes contain eight isoprene units or 40 carbons. The most prevalent of the tetraterpenes are carotenoids. The class of carotenoids contains more than 600 naturally occurring pigments synthesized by plants, algae and photosynthetic bacteria (Hidgon 2005). Carotenoids are also lipophilic molecules and are components of photosynthetic machinery, intermediates in the biosynthesis of abscisic acid and other apocarotenoids and in floral and fruit tissue, they act as colored pigments (Fraser and Bramley 2004; Taylor and Ramsay 2005). The stage of plant development can influence the carotenoid content. One study found higher carotenoid concentrations in swelling stolons and developing tubers than in mature tubers (Payyavula et al. 2013).

1.6.1 Xanthophylls

Carotenoids can be broadly classified into two classes, carotenes and xanthophylls (Higdon 2005). In potato, xanthophylls are the most abundant carotenoids which do not contain vitamin A activity. Xanthophylls have an important function as accessory pigments, capturing certain wavelengths of sunlight not absorbed by chlorophylls, increasing the overall absorbance of sunlight (Niyogi et al. 1997). These pigments provide photoprotection for plants when they are exposed to high solar radiation through a process known as the xanthophyll cycle. Violaxanthin is present in the plastids of the plant at dawn and then as the intensity of the sunlight increases the compound is changed through an intermediary compound called

antherxanthin into zeaxanthin. Zeaxanthin is able to absorb excessive energy that chlorophyll cannot use so the photosynthetic apparatus is not damaged.

Different carotenoids are detected in potatoes and they vary depending on the flesh color and ploidy level. Lu et al. (2001) detected six carotenoids, neoxanthin, violaxanthin, lutein-5,6-epoxide, lutein, zeaxanthin and an unknown, in eleven diploids and two tetraploid entries. Total carotenoid content in diploids was from 136µg (micrograms)/100gfw to 1435µg/100gfw (fresh weight) while the two tetraploids were 64µg/100gfw and 111µg/100gfw. Entry by environment interactions also need to be considered when measuring carotenoid content. It was found that total carotenoid content ranged from 101 to 511µg/100gfw in nine tetraploid entries grown in both Florida and Maine (Haynes et al.2010). However, the estimate of broad-sense heritability for total carotenoid (H=0.96) is similar to the estimate for yellow-flesh intensity (H=0.93) (Haynes et al. 1996). Due to these estimates the role of environment and entry x environment interactions on other agronomic traits will probably be more important than the environment and entry x environment interactions on yellow-flesh intensity and total carotenoid content (Haynes et al. 1996).

1.6.2 Health Benefits

Reduction of cardiovascular diseases, some cancers, and macular degeneration has been seen as there is continued expansion of the understanding of carotenoids in human health which supports the promotion of increased consumption of fresh fruits and vegetables (Mayne 1996). Carotenoids are among the many phytochemicals found in various fruits and vegetables. These phytochemicals are characterized as antioxidants, which prevent oxidation and protect against damage by reactive species (Halliwell et al. 1995). Among others, this is one of the mechanisms that have been assumed to halt the progression and proliferation of chronic illnesses, including

cancer, cardiovascular disease, diabetes, macular degeneration, and cataracts (Willcox et al. 2004). Forty to fifty carotenoids have been reported to be metabolized, absorbed, or used by humans (Khachik et al. 1991). All are antioxidants, some contain provitamin A activity, some enhance immune function, have anti-inflammatory properties, promote cell-to-cell communication, protect skin from ultraviolet light, and improve mental acuity (Haynes et al. 2010).

The major carotenoids in humans are β-carotene, α-carotene, lycopene, phytoene, phytofluene, and xanthophylls, lutein, zeaxanthin, α-cryptoxanthin, and β-cryptoxanthin (Sies and Stahl 2004). Other dietary carotenoids such as violaxanthin are rarely found in the blood of humans because of direct metabolism or poor absorption. They are present in the skin and eye, target sites of light-induced damage and carotenoid levels vary between different skin areas on the human body. Normal human skin color is significantly contributed to by carotenoids, especially the presence of yellowness.

The specific carotenoid, lutein, has been correlated with improvement in visual function in patients suffering from macular degeneration and cataracts when supplemented in the diet (Olmedilla et al. 2001). Lutein and zeaxanthin circulate in human blood plasma and are concentrated in the macula region of the eye. Age-related macular degeneration (AMD) is the leading cause of severe visual impairment and blindness in the United States (Lu et al. 2001). A risk factor for AMD is low pigment density and the number of Americans with AMD is expected to increase as babyboomers get into their sixties, creating social and economic impacts. Studies have shown that consumption of foods rich in lutein and zeaxanthin can substantially increase pigment density in the eyes of humans (Hammond et al. 1997). Americans do not frequently consume major dietary sources of lutein and zeaxanthin which are dark green leafy vegetables.

One solution to increasing the consumption of these carotenoids is to enhance the lutein and zeaxanthin content of the most commonly consumed vegetables, like the potato. The potato is the most commonly consumed vegetable in the U.S. and could aid in protecting human health and quality of life.

1.7 Sensory and Potato

Potato is one of the most popular vegetables worldwide and has a diversity of uses due to the many ways it can be prepared. The flavor of potato results from the taste, aroma and texture. Flavor precursors consist mainly of sugars, amino acids, RNA, and lipids and levels of these constituents and the enzymes that react with them to produce flavor compounds are influenced by plant entry, production environment and storage environment (Jansky 2010). Tubers do not emit volatile flavor compounds at maturity, but instead develop flavor from compounds in tissues when they are sliced or heated. The three components of flavor (taste, aroma, and texture) in potato interact to produce a flavor response.

Taste incorporates bitter, sour, sweet, salty and umami (Japanese meaning delicious) and potatoes contain all of these components except salty. Potato tubers have mechanisms to deter consumption by herbivores which are why some wild potato tubers have bitter-tasting glycoalkaloids (Jansky 2010). Cultivars still contain some glycoalkaloids but domestication has selected against bitterness. Glycoalkaloids and phenolic compounds may contribute to bitterness and larger amounts are found in the skin, making cooked potatoes with the skin on potentially more bitter. The starch in potato influences texture and interacts with flavor compounds during cooking. The low amounts of sugar in tubers contribute to a sweet taste which has become a desirable flavor due to consumers generally preferring sweet foods. Ribonucleotides are released by the enzymatic hydrolysis of RNA when tubers are heated during cooking. They are

precursors for umami compounds and levels and types of ribonucleotides vary among cultivars. The natural mixture of glutamic acid and other amino acids, plus the guanosine-5'-monophosphate (5'-GMP) and other 5'ribonucleotides contribute to the taste of cooked (boiled) potatoes (Halpern 2000). Cooking oil is an important component of taste for processed potato products (Jansky 2010). Some oils can give a unique flavor during processing while a few sensory evaluations did not show a major effect on potato chip flavor. Another influence of taste is the color of potato. The color of both cooked and fried products can affect the perception of taste. The taste threshold for many compounds varies significantly among panel members. Some panelists may detect a certain taste such as sour while other panelists do not.

Texture is an important quality attribute of potatoes and affects consumer preference. Generally, it is described in terms of mealiness, hardness, sloughing, moisture, and graininess (Ochsenbein et al. 2010). Texture influences the release of volatile flavor compounds during chewing and is controlled by many factors, including dry matter content, specific gravity, sugars, protein, amylose, and nitrogen levels in tubers (Jansky 2010). The size and structure of starch grains in raw tuber tissue has an effect on texture. During cooking the starch gelatinizes and causes pressure in cells as it expands. The amount of gelatinized starch in each cell influences the moistness of the tuber. The effects of ploidy on cell size has also shown an influence on texture where diploid entries had a more floury texture than related tetraploid entries (Jansky 2010).

Hundreds of aroma compounds are produced from cooked potatoes. Important compounds are produced by the Maillard reaction, lipid degradation and/or sugar degradation during heating (Jansky 2010). These aroma compounds exhibit a wide range of concentrations and odor thresholds. There are several extraction methods that can be used to isolate compounds

crucial to food aroma in order to characterize the aroma profile of a food product. A few of them are: SDE (simultaneous distillation and extraction), SAFE (solvent-assisted flavor evaporation), or headspace methods such as static and dynamic headspace, and SPME (solid-phase microextraction) (Majcher and Jelen 2009). Microwave-baked potatoes have lower levels of volatiles than boiled or oven-baked potatoes, tend to be more bland and receive lower ratings in sensory analyses (Jansky 2010).

1.7.1 Hedonic Scale

The traditional method used to assess the flavor of foods is the hedonic scale. For more than half a century, the 9-point hedonic scale, in its various formats, has been widely used to assess the average degree of liking or disliking of foods or consumer products across a large number of subjects (Lim et al. 2009). It was originally introduced as an aid to menu planning for U.S. soldiers in their canteens (Nicolas et al. 2010). The 9-point hedonic scale comprises a series of nine verbal categories ranging from 'like extremely' to 'dislike extremely.' For statistical analysis, the verbal categories are converted to numerical values with 'like extremely' being equivalent to 9 and 'dislike extremely' equivalent to 1. Advantages of this scale are that panelists can respond meaningfully without prior experience, it's suitable for a wide range of populations, the data can be handled by the statistics of variables, and the results are meaningful for indicating general levels of preference (Peryam and Pilgrim 1957).

1.8 Flavor and Potato

After cost, consumer preference is greatly influenced by the flavor of the potato. However, potato flavor is difficult to assess in breeding programs, which is common with many food crops (Morris et al. 2008). The volatiles produced by raw and cooked potatoes have been studied extensively (reviewed by Maga 1994; Taylor et al. 2007) and over 250 compounds have

been identified. Attempts have been made to discriminate which compounds are important for potato flavor and which are specific to the method of cooking, cultivar differences, effects of agronomic conditions and the effects of storage (Ducreax et al. 2008). Soluble cellular constituents are also important for flavor with cooked potatoes (Halpern 2000) and they define the basic taste parameters, sweet, sour, salty, bitter and umami (Morris et al. 2008). Generally, umami compounds give the impression of creaminess and viscosity to savory dishes by enhancing flavor and mouthfeel. Compounds that have umami-like sensory characteristics include adenosine 5'-monophosphate (5'-AMP), inosine 5'-monophosphate (5'-IMP), guanosine 5'-monophosphate (5'-GMP), several process-derived glutamate glycoconjugates, and monosodium glutamate (MSG) (Morris et al. 2007). Another compound likely to be a determinant of flavor in tubers and contributes to the umami taste is glutamate. Glutamate is the most potent umami amino acid and when it interacts with 5'-ribonucleotides, the umami taste intensity increases exponentially. Intensity can be enhanced with salts including sodium, potassium and magnesium and with certain organic acids like succinate (Morris et al. 2010).

The *S. tuberosum* group, Phureja, has been differentiated from S. tuberosum group, Tuberosum, when comparing a number of important tuber quality traits including flavor, color, texture, and reduced tuber dormancy (De Maine et al. 1993, 1998; Morris et al. 2004; Dobson et al. 2004; Ghislain et al. 2006). According to Ducreux et al. (2008), boiled Phureja tubers have much higher levels of the sesquiterpene, α-copaene, than Tuberosum tubers. Alpha-copaene is one of many sesquiterpene volatiles produced by plants and is an important aroma compound in several food plants such as sweet potatoes, carrots and lettuce. One research study by Morris et al. (2011) was successful in engineering potato tubers to accumulate high levels of α-copaene.

However, when a sensory analysis was completed the results suggested that α-copaene was not a major component of potato flavor (Morris et al. 2011).

Sensory profiles of Phureja entries and Tuberosum cultivars showed that Phureja scored higher on the acceptability scale, which correlated highly with traits such as creaminess and flavor intensity. Volatile profiles of the two groups that were analyzed using principal component analysis (PCA) revealed compounds α-copaene, pentanal, hexanal, and pentyl-furan contributed to most of the variation between the two when they were boiled. A study done by Morris and his colleagues (2010) characterized changes in cooked tuber flavor following storage. Significant changes in flavor related to storage were suggested by sensory scores and main metabolites driving these changes were identified using PCA. Propanal, 2-hexanal, and 5-methylhexanal were aldehydes found at enhanced levels following storage. The overall benefit from the study completed by Morris and his colleagues was that it is now possible to associate groups of metabolites with different flavor attributes.

Solid-phase microextraction (SPME) is an extraction technique that can be used to extract potato volatile flavor compounds from cooked potato. The compounds can be quantified and then analyzed by gas chromatography/mass spectrometry (GC/MS). SPME has many advantages over conventional analytical methods by combining sampling, preconcentration, and direct transfer of the analytes into a standard gas chromatograph (Tsai and Chang 2003). It is a simple, sensitive and solvent-free sample extraction and concentration technique. One example that applied this method was aldehydes in breath and blood. They are difficult to measure due to their volatility and activity. The results from researchers Deng and Zhang (2004) demonstrated that GC/MS and SPME with on-fiber derivatization is a simple, rapid, sensitive and solvent-free method for the determination of aldehydes in lung cancer blood.

Since potatoes that contain higher levels of carotenoids will have more health benefits it is important to evaluate entries for flavor. By identifying the major flavor compounds in potato tubers, breeders can then select for enhanced flavor (Jansky 2010) along with high carotenoid levels.

CHAPTER 2: COLOR AND CAROTENOID CONTENT IN POTATO GERMPLASM

2.1 Introduction

Potato (*Solanum tuberosum L.*) originated in the Andes Mountains of South America where the earliest farming of the modern potato began in about 1400 BC (Spooner et al. 2006; United States Potato Board 2012). The mountainous regions of Peru made potato an ideal crop due to its hardiness. The potato was first introduced to Europe in the early 1500's by Spanish conquistadors who brought potatoes back to their homeland. However, the potato remained a poorly understood plant for nearly a century and worldwide distribution occurred over several centuries (Hawkes 1992). Today, the potato is a major food crop in the world and is grown in all 50 states of the U.S. and about 125 countries throughout the world (United States Potato Board 2012). In South America, cultivated potato is represented by diploid, triploid, tetraploid, and pentaploid cultivars (Brown et al. 2007). Tetraploid cultivars have the widest geographic distribution and the greatest number of accessions. Domestication and selection have provided higher yield and characteristics suitable for fresh market and processing. Potato is the highest consumed vegetable crop in the U.S. with a per capita consumption of about 50.8 kg (112 lbs) (National Potato Council 2012). Colorado ranked fourth in 2012 for potato production in the United States, producing 1.04 million tons per year.

Potatoes contain numerous nutrients and antioxidants. They are rich in vitamin C and contain more potassium than either bananas, spinach or broccoli (United States Potato Board 2012). Potatoes have no fat, cholesterol or sodium and only contain 110 calories per serving. They are also a good source of vitamin B_6 and fiber. In addition, they contain a variety of phytonutrients with many having antioxidant activities. These phytonutrients include a highly

diverse list of phenolic compounds, flavonoids, flavonols, and carotenoids (Woolfe 1987). Carotenoids and anthocyanins are pigments that are antioxidants and impart color to the tuber flesh and skin (Brown 2008).

Carotenoids are secondary metabolites in plants found in fruits and vegetables (Watson et al. 2009; Mayne 1996). The carotenoids in tubers are primarily xanthophylls and vary in concentration among different genotypes, with lutein being the most common xanthophyll (Brown et al. 2006). Cultivated potatoes contain varying amounts of carotenoids in tuber flesh and skin (Brown et al. 2007). Reduction of cardiovascular disease, some cancers, diabetes, cataracts, and macular degeneration has been seen in human health from carotenoids. These health benefits support the promotion of increasing the consumption of fresh fruits and vegetables (Mayne 1996; Willcox et al. 2004). Lutein and zeaxanthin circulate in human blood plasma and are concentrated in the macula region of the eye (Konschuh et al. 2005). Studies have shown that consumption of foods rich in lutein and zeaxanthin can substantially increase pigment density in the eyes of humans and is inversely related to age-related macular degeneration. Potato contributes a significant portion to human carotenoid consumption due to the large quantities of potatoes that are consumed (Lu et al 2001). Yellow flesh potatoes were first reported to have carotenoid levels reaching 560 µg/100 gfw with more recent studies finding some entries that exceed 2000 µg (Brown et al. 2006).

Carotenoids, predominantly xanthophylls, produce yellow flesh color in potato tubers (Brown et al. 1993; Iwanzik et al. 1983). When measuring tuber flesh color, a reflectance colorimeter is used to determine values of color, L, a and b (HunterLab 2003). For the Hunter scale, L measures lightness and varies from 100 for white to 0 for black and the coordinates, a and b, locate the color on a rectangular-coordinate grid perpendicular to the L axis and are

chromaticity dimensions (HunterLab 2003; McGuire 1992). At the horizontal axis, a positive a coordinate indicates a hue of red-purple and a negative bluish-green while the b coordinate on the vertical axis indicates yellow with a positive coordinate and blue with a negative coordinate (Voss 1992; McGuire 1992). Chroma indicates the intensity or saturation of a color and hue measures true color and varies from 0 to 360 on a color wheel (Cantwell et al. 2004). Coordinates a and b indirectly reflect hue and chroma, respectively.

Carotenoid content has not been studied in entries developed from the Colorado Potato Breeding and Selection Program located in the San Luis Valley in Colorado. The purposes of this study were to evaluate and compare tuber-flesh color of 138 potato entries in the Colorado Potato Breeding and Selection Program and identify and quantify carotenoids in 100 entries selected based on color. This was done to identify breeding material that would be used to develop new entries with elevated carotenoid levels.

2.2 Materials and Methods

This research project was conducted at the Colorado State University (CSU) San Luis Valley Research Center (SLVRC) and at CSU – Fort Collins. Field-grown potato tubers were evaluated for tuber flesh color, focusing on hue and chroma, total carotenoid content and identification and quantification of individual carotenoids. These variables were compared across entries.

Plant Material

The potato tubers utilized in this study were grown for two field seasons (2011, 2012) at the SLVRC, CSU, Center, Colorado. The same agronomic practices were used to minimize differences between the years. Studying multiple entries during multiple years made analysis for significant interactions possible. The majority of the potato entries utilized in this study were

developed by the CSU Potato Breeding and Selection Program. Others were from the United States Department of Agriculture-Agricultural Research Service (USDA-ARS: Prosser, WA and Beltsville, MD) and breeding programs from Canada, Idaho, Oregon, and Texas. There were a total of 138 entries, including nine commercial cultivars and fifty-nine entries developed in Colorado. Commercial cultivars included in the study were Agria, Chipeta, Inka Gold, Purple Majesty, Rio Grande Russet, Rose Valley, Russet Nugget, Sierra Gold, and Yukon Gold.

Entries were planted in individual plots and randomly located in the field. The plots were selection plots that were non-replicated. Cultural management practices were uniform among all of the plots. Plots were of varying lengths depending on the stage of selection the entries were at with 34 inches (86.4 cm) of spacing between rows and 12 inches (30.5 cm) of spacing between hills. Potatoes were grown in a sandy loam soil from mid-May to early September. During the 2011 field season, planting was done on May 20, the vines were killed 112 days after planting using Reglone and harvesting was done on September 28 and 29. During the 2012 field season, planting was done on May 16, the vines were killed 107 days after planting using sulfuric acid and harvesting was done on September 24 and 25. Total applied fertilizer each year included 54 kg (120 lbs) N, 27 kg (60 lbs) P_2O_5, 18 kg (40 lbs) K_2O, 11 kg (25 lbs) S, and 1.1 kg (2.5 lbs) Zn/A and was based on soil tests. Irrigation was performed using a center pivot. In 2011 there was a gross application of 20.8 inches (52.8 cm) and in 2012 the gross application was 17.7 inches (45.0 cm). The application frequency and amount was based on evapotranspiration.

Tuber Flesh Color Analysis

To determine the tuber flesh color a HunterLab MiniScan XE colorimeter (HunterLab, Reston, VA) was used. The analysis of the tuber flesh color was performed on 138 entries. These included 128 yellow, 3 white, 2 purple, and 5 red-yellow flesh entries. Thirty-five of the

entries were diploids with the rest being tetraploids. Raw potato tubers that had been in cold storage (4.4°C/40°F) for approximately one month after harvest were utilized for flesh color measurements. Potato tubers were removed from cold storage and put at room temperature for analysis. Potato tubers were sliced in half with a knife and flesh color measurements were taken at the stem end, center and bud end of each tuber half. The outer cortex and the darker pith area down the center were avoided if possible. The colorimeter produced values of L, a, and b. Hue and chroma values were determined from the a and b values. To calculate chroma the equation $[a^2+b^2]^{1/2}$ was used and to calculate hue the equation arctan (b/a) was used (Steed and Truong 2008; McGuire 1992). If $a<0$ and $b>0$, 180 is added to the hue equation and if $a>0$ and $b<0$, 360 is added to the hue equation. Yellow and white flesh entries grown in 2011 were also rated using a standard color chart used in the Western Regional Potato Variety Trials. A scale of 0-3 (0 = white, 3 = dark yellow) was used for those grown in 2012. Tubers were then placed into a bag labeled with the sample identification and put back into cold storage at 4.4°C (40°F).

Analysis of Carotenoids

To determine total carotenoid content and identification and quantification of individual carotenoids a Molecular Devices Spectramax Plus spectrophotometer, a Shimadzu HPLC (high-performance liquid chromatography) instrument and a Waters Acquity UPLC (ultra-high performance liquid chromatography) instrument were used. The study of total carotenoid content was performed on 100 entries. A subset of eight entries was analyzed for identification and quantification of individual carotenoids. The subset included four entries from the top twenty and four from the bottom twenty entries for total carotenoid content when averaged over both years. Tubers were taken from cold storage (4.4°C/40°F), rinsed and then placed into small plastic containers labeled with the sample identification. They were then peeled, sliced using a

French fry slicer (or knife if too small) and at least 300 g was weighed. The weight was recorded and samples were then double bagged. Samples were double bagged so holes could be placed in the inner bag for processing and then the inner bag could be placed in the outer bag when samples were transported. The bags were labeled with the sample identification, month and year. These were then placed in insulated boxes with dry ice and shipped to be freeze-dried at the Apex Lyo, Inc. facility in Washington. Freeze-drying is also known as lyophilization and it is a dehydration process used to preserve the samples. Samples were frozen and then dried by direct sublimation of the ice under reduced pressure, going from a solid phase to a gas phase (Rey 1975). Once samples were returned, they were stored at -20°C (-4°F) until grinding. Samples were first weighed and a dry weight was recorded. They were then ground into a fine powder using a blender. The sample was then put through a No. 20 sieve, larger pieces were put through the grinder and the sieve once more and the sample was then poured into a new bag. Samples were double bagged using the same outer bag from freeze-drying which were labeled with the sample identification, month and year. The blender and sieve were cleaned between each sample using 70% acetone and paper towels. Samples were stored at -20°C (-4°F) until extraction.

For carotenoid extraction, ground tissue was weighed (~200 mg per extraction) into a 2 mL Eppendorf tube labeled with sample identification and replication number. Samples were placed in plastic Eppendorf tube racks. The carotenoid extraction protocol is based on Lopez et al. (2008). An 80% acetone solution was prepared for carotenoid extraction and chilled in the refrigerator along with ethyl acetate and distilled water. A plastic container was filled with ice to keep the solvents and samples cool. The solvents and samples were placed in the container. The extraction was performed in a low light setting and 16 extractions were conducted at one time. A

solution of 0.8 mL of 80% acetone was added and samples were vortexed for one minute using a Thermolyne Maxi-Mix III Type 65800 vortex at speed 2000. A solution of 0.5 mL ethyl acetate was added and samples were vortexed for 30 seconds followed by the addition of 0.5 mL distilled water. Samples were centrifuged for ten minutes at 13,000 X g at 4°C (39°F) using a Galaxy 16 centrifuge (VWR International, Radnor, PA). The upper non aqueous layer was transferred to a new 1.5 mL Eppendorf tube and labeled with the sample identification and replication number. A 0.5 mL ethyl acetate solution was added to the remaining aqueous fraction and centrifuged as above. The upper layer was combined with the previous non aqueous layer and samples were placed at -20°C (-4°F) until settled. The bottom acetone layer was removed and discarded. The extraction was then dried to completion under vacuum in an Eppendorf Vacufuge (Brinkmann Instruments, Westbury, NY) at 30°C (86°F). Carotenoids were resuspended in 200 microliters (µL) ethyl acetate and stored at -80°C (-112°F) until analysis.

To determine total carotenoid content a Molecular Devices Spectramax Plus spectrophotometer (Molecular Devices Corporation, Sunnyvale, CA) was used. Samples were removed from -80°C (-112°F) and diluted by a factor of 10. To dilute samples, 20 µL was put into one well of a 96-well glass plate and 180 µL of ethyl acetate was added. Total carotenoid content was determined by the absorbance of the extracts at 450 nm, respectively (Reddivari et al. 2007). Total carotenoid content was calculated using a lutein standard curve. The lutein curve was prepared by determining the absorbance of lutein standard solution ranging in concentration from 0-50 µg/mL at 450 nm. Total carotenoid content was expressed as micrograms of lutein equivalents per 100 g of fresh weight tuber (µg of LE/100 gfw). The 2011

samples were extracted in 2012, the 2012 samples were extracted in 2013 and all of the samples were analyzed on the spectrophotometer in 2013.

To determine carotenoid composition for 2011 samples, a Shimadzu HPLC instrument (Shimadzu, Columbia, MD) was used, equipped with a system controller (Shimadzu SCL-10A), a binary pump system (Shimadzu LC-10AD), a degasser (Shimadzu DGU-14A), an autoinjector (Shimadzu SIL-10A), and a diode array detector (DAD) (Shimadzu SPD-M10A). Concentrated samples were filtered through a 0.22 µm (13mm) syringe filter using a 3 mL syringe. Samples were filtered into a vial insert that was placed inside a vial and vials were capped and stored at -20°C (-4°F) until injection. Vials were labeled with sample identification, replication number and date. The protocol was based on the method used by Breithaupt et al. (2002). A 10 µL sample was injected into the HPLC. Solvent A consisted of methanol:water:triethylamine (90:10:0.1 v/v/v) and solvent B consisted of methanol:methyl tert-butyl ether (MTBE):water:triethylamine (6:90:4:0.1 v/v/v/v). A YMC carotenoid column (4.6 x 250 mm, 3 µm, C-30 reverse-phase) was used with a column heater set to 35°C (95°F) with a 0.8 mL/min flow rate (YMC, Japan). For analysis of carotenoids, the following gradient system was used: gradient (min/%B) 0/1, 6/1, 35/0, 40/1, 49.59/1. The column was brought back to initial conditions and allowed to equilibrate before the following injection. Four entries from the top twenty entries and four from the bottom twenty entries for total carotenoid content were analyzed for carotenoid composition. Antheraxanthin, lutein, neoxanthin, violaxanthin, and zeaxanthin were five carotenoids used as standards to identify and quantify the samples. The peaks were identified by matching the spectra and retention times and quantified using standard curves.

To determine the carotenoid composition for 2012 samples, a Waters Acquity UPLC System equipped with a bio-sample manager, bio-quaternary solvent manager with a DAD was

used (Waters, Milford, MA). Samples were filtered through a 0.22 µm (13mm) syringe filter using a 3 mL syringe. Samples were filtered into a vial insert placed inside a vial and vials were capped and stored at -20°C (-4°F) until injection. The vials were labeled with sample identification, replication number and date. The protocol was based on the method used by Lefsrud and colleagues (2007). A 10 µL sample was injected into the UPLC. Solvent A, the mobile phase, was fresh Millipore water and solvent B was acetonitrile. A BEH (ethylene bridged hybrid) UPLC column (2.1x150mm, 1.7 µm, C18) was used with a 0.6 mL/min flow rate (Waters, Ireland). The sample temperature was set to 4.4°C (40°F) and the column temperature was set to 60°C (140°F). For analysis of carotenoids, the following gradient system was used: gradient (min/%B) 0/50, 8/100, 12.25/50, 15/50. The same eight entries analyzed for carotenoid composition in 2011 were analyzed in 2012. Cryptoxanthin, lutein, lutein isomer, neoxanthin, violaxanthin, and zeaxanthin were the carotenoids used as standards to identify and quantify the samples. Carotenoids were detected at 450 nm with continuous monitoring of the peak spectra between 190-500 nm.

Experimental Design and Statistical Analysis

Potato tubers for this study were grown for two years (2011, 2012) at the SLVRC and 138 entries were selected for analysis. A random set of tubers was selected within the plot. Three biological replicates within the plot were used for the tuber flesh color analysis and each replicate was represented by a tuber chosen at random. Randomly selected tubers were freeze-dried to be used for carotenoid extraction. Three technical replicates were performed for total carotenoid analysis. Three technical replicates were performed for HPLC and three technical replicates were run as duplicates for UPLC to measure individual carotenoid content. Treatment means were compared using Tukey's test. Pearson correlation coefficients were conducted to

determine if chroma and total and individual carotenoid content were associated using the SAS Proc Corr procedure. All statistical analyses were performed using SAS Statistical Analysis System, v.9.3 software (SAS Institute Inc., Cary, NC).

2.3 Results

Tuber Flesh Color Analysis

The entry by year interaction was significant for tuber flesh chroma ($p<0.0001$). Tuber flesh chroma ranged from 7 in CO08390-1P/P to 41 in BDC741-4-3W/Y among the 138 entries tested when averaged across years. Diploid entries had higher mean chroma ($p<0.0001$) than tetraploid entries. The range in chroma for diploids was 22 to 46 with a mean of 36. The range in chroma for tetraploids was 6 to 41 with a mean of 22. Figure 2.1 illustrates the twenty entries with the highest and lowest chroma values when averaged over both years and the standard check, Yukon Gold. The ten with high chroma values include all diploid entries except for one tetraploid, CO07131-1W/Y. Two purple flesh entries were ranked in the bottom ten for chroma. The remaining entries had white or yellow flesh for the top and bottom ten. BDC741-4-3W/Y had the highest chroma in 2011 with a value of 46 and CO08390-1P/P had the lowest with a value of 9. For 2012, entry CO07131-1RW/Y had the highest chroma with a value of 41 and CO08390-1P/P had the lowest with a value of 6. Variation is seen between 2011 and 2012, with some entries having higher values in 2011 and others having higher values in 2012. The three entries, Yukon Gold, Chipeta, and CO08390-1P/P, had significantly different chroma values compared to the rest of the top and bottom ten entries ($p<0.05$).

The hue values for the entries in this study fall within the visible colors of yellow, reddish yellow, yellowish red, and bluish red. The hue values for yellow range from greater than 68 to 113, reddish yellow range from greater than 45 to 68, yellowish red range from greater than 23 to

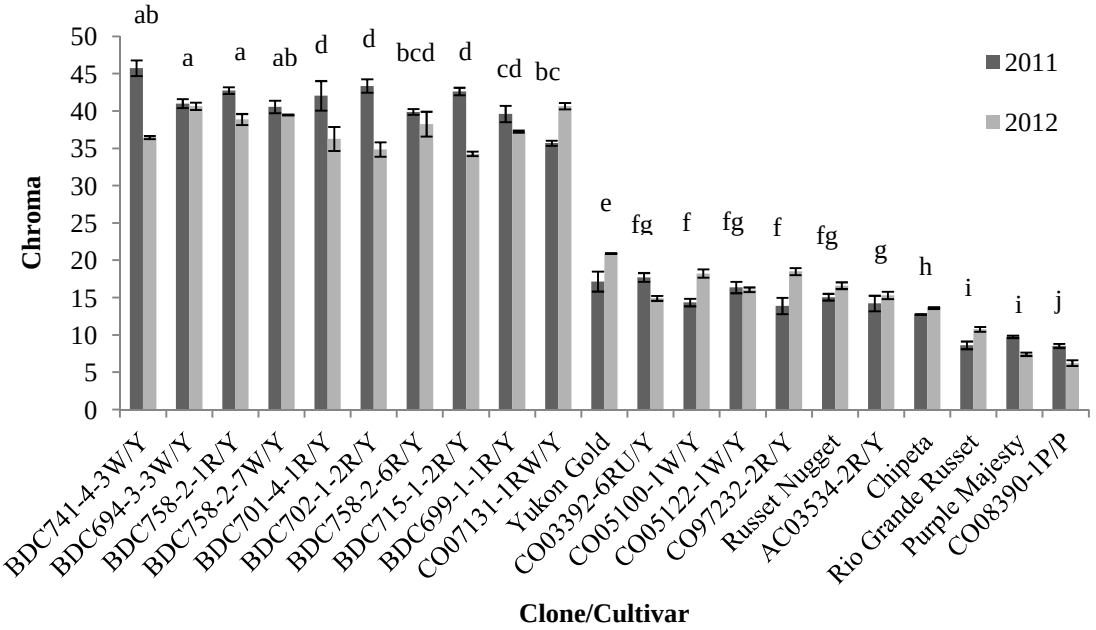

Figure 2.1: Chroma† values among the top and bottom ten entries (n= 138) in 2011 and 2012. Chroma values were averaged over both years to obtain the top and bottom ten. Means with the same letter are not significantly different among entries ($p>0.05$). Error bars represent S.E. (standard error of the mean, 3 reps).
† Chroma indicates the intensity or saturation of a color and is calculated with the equation, $(a^2+b^2)^{1/2}$, where a is the coordinate indicating red(+)/green(-) and b is the coordinate indicating yellow(+)/blue(-).

45, and bluish red range from greater than 315 to 338. Tuber flesh hue ranged from 24 in CO05085-5R/RY to 328 in CO08390-1P/P among the 138 entries tested. Entry was significant ($p<0.0001$) and the year was not significant ($p=0.1876$). Since the two years were not significantly different the average values are shown in Figure 2.2. The range in hue for diploids was 35 to 92 with mean 81. The range in hue for tetraploids was 24 to 328 with mean 95. Two diploids, one with reddish yellow flesh and one with yellowish red flesh, two tetraploids with bluish red flesh, and three tetraploids, two with reddish yellow flesh and one with yellowish red flesh, were included among the rest of the yellow/white flesh entries. Figure 2.2 illustrates the hue values for the same twenty entries and the check, Yukon Gold, seen in Figure 2.1. Purple Majesty and CO08390-1P/P have significantly higher hue values due to purple flesh. Hue values

of 322 and 328 indicate a visible color of bluish red. The hue values for the rest of the entries indicate a visible color of yellow.

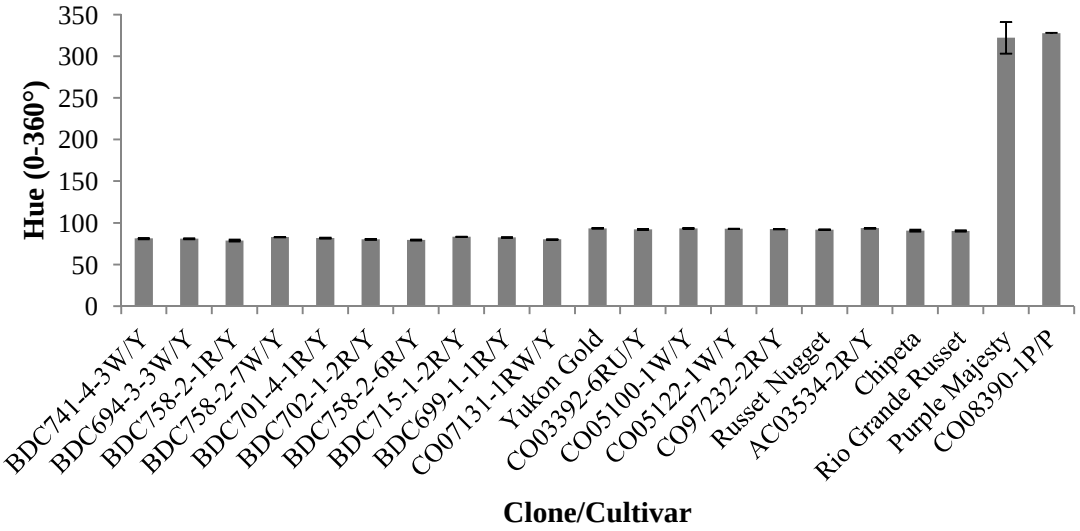

Figure 2.2: Hue† values among the top and bottom ten entries (n= 138) ranked by chroma values. Error bars represent S.E. (standard error of the mean, 3 reps).
† Hue indicates the true color and varies from 0 to 360° on a color wheel.

Analysis of Carotenoids

Spectrophotometric readings for samples at 450 nm were converted into lutein equivalents based on the following equation: $y = 17.34x - 0.0836$, where x = absorbance at 450 nm and y = µg lutein equivalents/mL. The R^2 value for this curve was 0.9993. The value for y was then used to calculate µg lutein equivalents/100gfw.

There was a significant entry by year interaction for total carotenoid content ($p<0.0001$). The total carotenoid content for all of the entries showed variation between the two years. Figure 2.3 shows a scatterplot comparing 2011 and 2012 total carotenoid levels. Greater variation is associated with those entries that have a higher carotenoid content. The majority of entries with lower levels of carotenoids are similar between the two years. When levels reach 1000 µg/100 gfw or greater, the entries become quite variable between years. BDC715-1-1R/Y increased significantly, from 1761 µg/100 gfw in 2011 to 2741 µg/100 gfw in 2012. BDC758-2-

2RW/Y also had an increase in carotenoid content, with 974 µg/100 gfw in 2011 to 1869 µg/100 gfw in 2012. BDC699-1-2W/Y showed a significant decrease in carotenoid content, with 2083 µg/100 gfw in 2011 to 1510 µg/100 gfw in 2012. Another entry that decreased in carotenoid content was 4X91E22, with 1590 µg/100 gfw in 2011 to 988 µg/100 gfw in 2012.

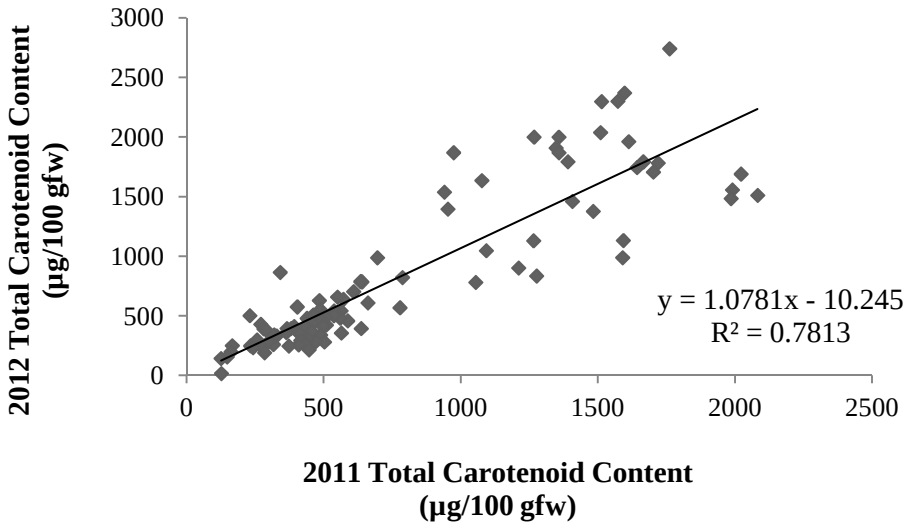

Figure 2.3: Scatterplot comparing total carotenoid content for 2011 and 2012.

Total carotenoid content ranged from 71 µg/100 gfw in entry CO08390-1P/P to 2251µg/100 gfw in entry BDC715-1-1R/Y among the 100 entries tested when averaged over years. Figure 2.4 illustrates the ten entries with the highest carotenoid content and the ten entries with the lowest carotenoid content based on the average of both years. The standard check, Yukon Gold, was one of the entries in the bottom ten. The ten with high carotenoid content include all diploid entries except for one tetraploid, PA4X137-12. The ten with low carotenoid content include all tetraploid entries. Entry BDC699-1-2W/Y had the highest total carotenoid content in 2011 with 2083 µg/100 gfw and Rio Grande Russet had the lowest with 126 µg/100 gfw. In 2012, entry BDC715-1-1R/Y had the highest total carotenoid content with 2741 µg/100 gfw and CO08390-1P/P had the lowest with 16 µg/100 gfw. The top ten entries had a higher

carotenoid content than Yukon Gold for both years. Nine of the top ten entries with high carotenoid content and seven of the bottom ten entries with low carotenoid content were different between years. Entry BDC715-1-1R/Y has significantly more carotenoids than any other clones evaluated ($p<0.05$).

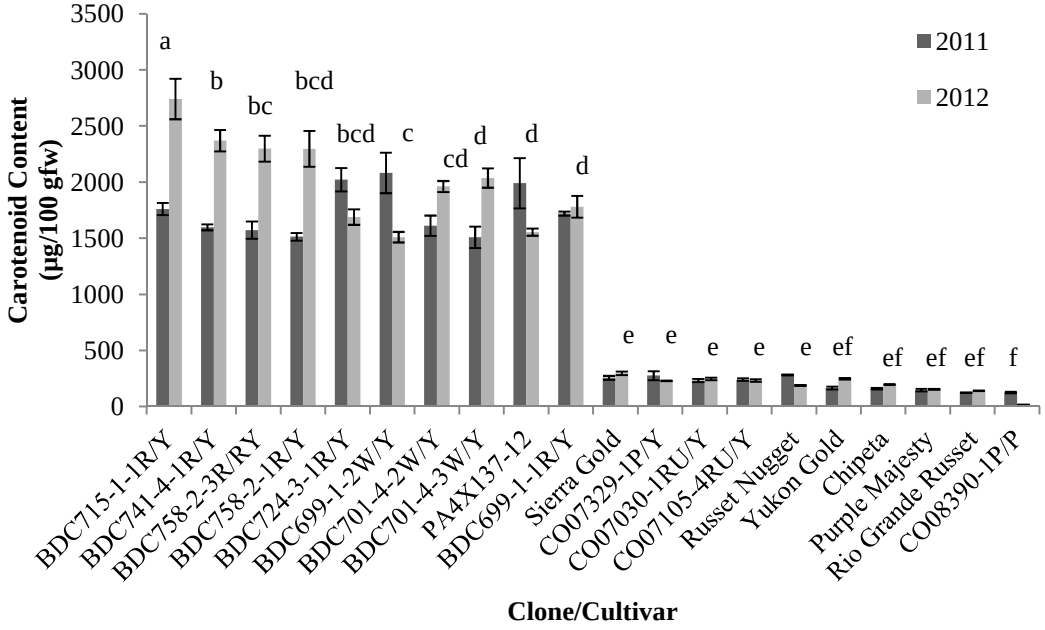

Figure 2.4: Total carotenoid content among the top ten and bottom ten potato entries in 2011 and 2012. Total carotenoid content was averaged across both years to obtain the top and bottom ten. Means with the same letter are not significantly different among entries ($p>0.05$). Error bars represent S.E. (standard error of the mean, 3 reps).

Four entries from the top twenty with high carotenoid content and four entries from the bottom twenty with low carotenoid content in 2011 were analyzed for carotenoid composition (Table 2.1). Total carotenoid content was averaged over both years to obtain the top and bottom twenty entries. Content for the carotenoid standards used were measured based on the following equations: antheraxanthin ($y = 41769x – 8881.6$ with $R^2 = 0.9995$); lutein ($y = 23373x – 14605$ with $R^2 = 0.9959$); neoxanthin ($y = 14285x – 1450.8$ with $R^2 = 0.9999$); violaxanthin ($y = 47443x + 1107.1$ with $R^2 = 0.9996$); zeaxanthin ($y = 14433x – 10135$ with $R^2 = 0.9938$).

Antheraxanthin was detected in five entries, three of the top four and one of the bottom four of those selected from the top and bottom twenty for total carotenoid content. Antheraxanthin content ranged from 39 µg/100 gfw in Yukon Gold to 256 µg/100 gfw in BDC715-1-1R/Y. Lutein was detected in three of the bottom four entries. Lutein content ranged from 41 µg/100 gfw in Chipeta to 212 µg/100 gfw in Russet Nugget. Neoxanthin was detected in five entries, the top four and one of the bottom four entries. The content of neoxanthin ranged from 24 µg/100 gfw in Yukon Gold to 1399 µg/100 gfw in BDC701-4-3W/Y. Violaxanthin was detected in three of the top four entries. Violaxanthin content ranged from 104 µg/100 gfw in BDC758-2-3R/RY to 169 µg/100 gfw in BDC715-1-1R/Y. Zeaxanthin was detected in two entries, one of the top four and one of the bottom four entries. Zeaxanthin content ranged from 145 µg/100 gfw in Russet Nugget to 160 µg/100 gfw in BDC701-4-3W/Y. The total content of the five carotenoids analyzed ranged from 48 µg/100 gfw in Rio Grande Russet to 1560 µg/100 gfw in BDC701-4-3W/Y.

Table 2.1: Individual[a] and total carotenoid content[b] among the eight selected potato entries grown at the San Luis Valley Research Center, Center, Colorado in 2011.

Clone/Cultivar	Anth	Lut	Neo	Vio	Zea	Total
			µg/100 gfw[c]			
BDC701-4-3W/Y			1399		160	1559
BDC715-1-1R/Y	256		160	169		585
BDC741-4-1R/Y	191		211	111		513
BDC758-2-3R/RY	205		130	104		439
Chipeta	50	41				91
Rio Grande Russet		48				48
Russet Nugget		212			145	357
Yukon Gold	39		24			63

[a] Anth = antheraxanthin, Lut = lutein, Neo = neoxanthin, Vio = violaxanthin, Zea = zeaxanthin
[b] The carotenoid content for each entry was the average of three technical replicates.
[c] fw, fresh weight.

The same eight entries analyzed for carotenoid composition in 2011 were also analyzed in 2012 (Table 2.2). However, cryptoxanthin was one of the carotenoids analyzed instead of

antheraxanthin and a lutein isomer was added. Content for the carotenoid standards used were measured based on the following equations: cryptoxanthin ($y = 139638x - 58955$ with $R^2 = 0.9932$); lutein and lutein isomer ($y = 86265x - 35804$ with $R^2 = 0.9971$); neoxanthin ($y = 113105x - 51685$ with $R^2 = 0.9957$); violaxanthin ($y = 89154x - 53121$ with $R^2 = 0.9991$); zeaxanthin ($y = 142157x - 53121$ with $R^2 = 0.9946$).

The six standards were detected in all eight entries. Cryptoxanthin content ranged from 43 µg/100 gfw in Russet Nugget to 84 µg/100 gfw in BDC701-4-3W/Y. Lutein content ranged from 114 µg/100 gfw in Russet Nugget to 635 µg/100 gfw in BDC701-4-3W/Y. The content of the lutein isomer ranged from 43 µg/100 gfw in Russet Nugget to 316 µg/100 gfw in BDC758-2-3R/RY. Neoxanthin content ranged from 46 µg/100 gfw in Chipeta to 53 µg/100 gfw in BDC741-4-1R/Y. Violaxanthin content ranged from 27 µg/100 gfw in Russet Nugget to 268 µg/100 gfw in BDC701-4-3W/Y. Zeaxanthin content ranged from 37 µg/100 gfw in Russet Nugget to 54 µg/100 gfw in BDC758-2-3R/RY. Total content for the five carotenoids and the isomer analyzed ranged from 312 µg/100 gfw in Russet Nugget to 1346 µg/100 gfw in BDC701-4-3W/Y.

Table 2.2: Individual[a] and total carotenoid content[b] among the eight selected potato entries grown at the San Luis Valley Research Center, Center, Colorado in 2012.

Clone/Cultivar	Cryp	Lut	Lut Iso	Neo	Vio	Zea	Total
				µg/100 gfw[c]			
BDC701-4-3W/Y	84	635	261	52	268	46	1346
BDC715-1-1R/Y	75	468	307	52	167	50	1119
BDC741-4-1R/Y	56	433	305	53	166	42	1055
BDC758-2-3R/RY	72	405	316	48	119	54	1014
Chipeta	44	122	45	46	29	38	324
Rio Grande Russet	44	117	45	46	28	37	317
Russet Nugget	43	114	43	47	27	37	311
Yukon Gold	45	174	49	46	30	38	381

[a] Cryp = cryptoxanthin, Lut = lutein, Lut Iso = lutein isomer, Neo = neoxanthin, Vio = violaxanthin, Zea = zeaxanthin
[b] The carotenoid content for each entry was the average of three technical replicates that were run as duplicates.
[c] fw, fresh weight.

Diploid entries had higher total carotenoid content than tetraploid entries ($p<0.0001$). In 2011, diploid entries ranged from 538 µg/100 gfw to 2083 µg/100 gfw with mean 1302 µg/100 gfw. Tetraploid entries ranged from 126 µg/100 gfw to 1991 µg/100 gfw with mean 493 µg/100 gfw. In 2012, diploid entries ranged from 540 µg/100 gfw to 2741 µg/100 gfw with mean 1522 µg/100 gfw. Tetraploid entries ranged from 16 µg/100 gfw to 1556 µg/100 gfw with mean 431 µg/100 gfw. Figure 2.5 compares the total carotenoid content by ploidy level on both a fresh weight and dry weight basis. Diploids had higher carotenoid levels than tetraploids on both a fresh and dry weight basis. The mean carotenoid content on a fresh weight basis was approximately three times greater for diploid entries than for tetraploid entries in 2011 and three and a half times greater for diploids in 2012. For dry weight, the carotenoid content was approximately two times greater for diploids than tetraploids in 2011 and three times greater for diploids in 2012. Diploids and tetraploids produced similar values for total carotenoid content for 2011 and 2012.

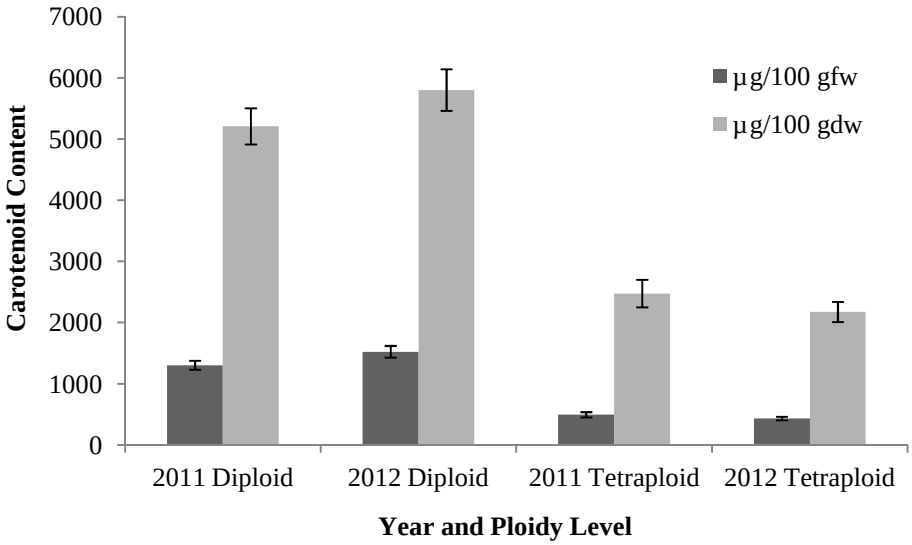

Figure 2.5: A fresh weight (fw) and dry weight (dw) comparison of total carotenoid content by ploidy level in potato tubers (n=100). There were 35 diploids and 65 tetraploids. Error bars represent S.E. (standard error of the mean).

There was a positive correlation between chroma and total carotenoid content among the 100 entries analyzed ($r = 0.72$, $p < 0.01$). For 2011, the correlation among individual carotenoids, total carotenoid content and chroma for the eight entries analyzed is shown in Table 2.3. There was a positive correlation between antheraxanthin and violaxanthin ($p<0.01$). The correlation between the five carotenoids and total carotenoid content were all positive except for lutein, where there was a negative correlation and zeaxanthin, where there was no correlation. The same correlations were seen between the five carotenoids and chroma. The correlation between total carotenoid content and chroma was positive ($p<0.01$).

Table 2.3: Correlation coefficients between individual[a] and total carotenoid content and chroma in potato tubers in 2011 (n=8).

	Anth	Lut	Neo	Vio	Zea	Total[b]	Chroma
Anth		- 0.47	- 0.20	0.98**	- 0.54	0.75*	0.71
Lut			- 0.30	- 0.41	0.53	- 0.50	- 0.50
Neo				- 0.12	0.64	0.48	0.51
Vio					- 0.43	0.79*	0.77*
Zea						0.02	0.07
Total							0.96**
Chroma							

[a] Anth = antheraxanthin, Lut = lutein, Neo = neoxanthin, Vio = violaxanthin, Zea = zeaxanthin
[b] Total Carotenoid Content (µg/100 gfw)
*Significant at $p<0.05$, ** significant at $p<0.01$

For 2012, the correlation among individual carotenoids, total carotenoid content and chroma for the eight entries analyzed is shown in Table 2.4. A positive correlation was seen between all carotenoids, total content and chroma. Cryptoxanthin has a strong positive correlation with lutein ($p<0.01$). Lutein also has a strong positive correlation with violaxanthin ($p<0.01$). Strong positive correlations were seen between the individual carotenoids and total carotenoid content, especially for the lutein isomer ($p<0.01$). Lutein, lutein isomer, neoxanthin, and violaxanthin showed a strong positive correlation ($p<0.01$) with chroma and total carotenoid content. There continues to be a positive correlation between total carotenoid content and chroma ($p<0.01$).

Table 2.4: Correlation coefficients between individual[a] and total carotenoid content and chroma in potato tubers in 2012 (n=8).

	Cryp	Lut	Lut Iso	Neo	Vio	Zea	Total[b]	Chroma
Cryp		0.95**	0.85**	0.73*	0.91**	0.85**	0.86**	0.75*
Lut			0.89**	0.88**	0.99**	0.74*	0.90**	0.91**
Lut Iso				0.84**	0.83*	0.88**	0.99**	0.88**
Neo					0.90**	0.52	0.87**	0.94**
Vio						0.63	0.84**	0.89**
Zea							0.86**	0.59
Total								0.88**
Chroma								

[a] Cryp = cryptoxanthin, Lut = lutein, Lut Iso = lutein isomer, Neo = neoxanthin, Vio = violaxanthin, Zea = zeaxanthin
[b] Total Carotenoid Content (µg/100 gfw)
*Significant at $p<0.05$, ** significant at $p<0.01$

2.4 Discussion

Chroma is a measurement of color most closely associated with tuber color intensity. The chroma of tuber flesh showed a wide variation in the entries tested. A study by Cantwell et al. (2004) measured internal and external color in specialty potato lines. They reported similar chroma values for both yellow and colored flesh in tetraploid entries. Two of the same entries were evaluated by Cantwell et al., POR00PG4-1 and VC1009-1W/Y, and chroma values were higher for these two entries in this study. The entry by year interaction was significant for chroma. However, a high heritability of 0.93 for yellow-flesh intensity has been reported in potatoes (Haynes et al. 1996). Diploid entries had higher chroma values than tetraploids. There is a large variation in tuber flesh color for cultivated diploid potatoes (Lu et al. 2001). The tetraploid entry, CO07131-1W/Y, was in the top ten chroma values while the rest were diploids. This is most likely due to the parentage of CO07131-1W/Y (PA4X137-12 x 4X91E22) where both parents are doubled diploids.

Hue is a standard color term that defines the true color of an object and the color wheel is a visual representation of hue. A significant difference for entry was seen because colored flesh clones with red or purple were included among the entries studied. There was a larger range in

hue values among tetraploids than diploids. This is because of two purple-fleshed entries and one red-yellow fleshed entry whose hue value fell within the corresponding visible color of red for one of the years. The hue range for diploids is larger than the range corresponding to the visible color of yellow because of the two entries with red-yellow flesh. Their hue values fell within the visible color of yellowish red.

There was a wide variation in total carotenoid content for the 100 entries tested over 2 years. Diploid entries showed greater total carotenoid content than tetraploids. It has been reported in previous studies that diploids contain more carotenoids than tetraploids. Brown et al. (1993) reported combined levels of lutein and zeaxanthin from a diploid hybrid population that were four to five times higher than the highest total xanthophyll content in tetraploid German cultivars. Another study found that eleven selected yellow-fleshed diploid entries contained three to thirteen times more carotenoids than Yukon Gold (Lu et al. 2001). The diploids that were in the top ten entries for carotenoid content in this study contained six to thirteen times more carotenoids than Yukon Gold. This includes data from both 2011 and 2012. The tetraploid entry, PA4X137-12, ranked in the top ten for carotenoid content. This is most likely due to its background which shows it is a doubled diploid. There was a significant entry by year interaction for total carotenoid content. Greater variation was seen in entries that contained more carotenoids. Genetic variation for individual and total carotenoid content is present in potatoes and significant entry by environment interactions have been seen in previous work (Haynes et al. 2010).

Four entries from the top twenty entries and four from the bottom twenty entries for total carotenoid content were analyzed for carotenoid composition for both 2011 and 2012. Carotenoids were analyzed using a HPLC instrument for 2011 material. Due to complications

with the instrument, 2012 material was analyzed for carotenoids using a UPLC instrument. Instrument problems with the HPLC also occurred during analysis of 2011 samples. This may be why consistency was not always seen between reps. Antheraxanthin was only analyzed in 2011 samples. For the entries it was detected in, higher levels are seen in the diploids that had a higher total carotenoid content. Cryptoxanthin and the lutein isomer were only analyzed in 2012 samples. There were lower cryptoxanthin levels compared to other carotenoids measured and levels in the four entries from the top twenty were slightly higher than the four from the bottom twenty. The lutein isomer levels were about five to seven times higher in the top four entries compared to the bottom four.

The detected lutein levels in 2011 for the three entries, Chipeta, Rio Grande Russet, and Russet Nugget were lower than the levels detected in 2012 for Chipeta and Rio Grande Russet but were higher in Russet Nugget. Higher levels of lutein were observed in 2012 for eight entries than any other carotenoids analyzed. This indicates that lutein is one of the major carotenoids present. Neoxanthin levels are higher in 2011 compared to 2012 for the five entries it was detected in. The neoxanthin content in BDC701-4-3W/Y was significantly high compared to the other entries. The neoxanthin levels in 2012 samples were consistent among the eight entries. For violaxanthin content, we see similar levels between years for the three entries, BDC715-1-1R/Y, BDC741-4-1R/Y, and BDC758-2-3R/RY. For 2012 there are higher violaxanthin levels in the top four entries compared to cryptoxanthin, neoxanthin, and zeaxanthin. Violaxanthin could also be a major carotenoid present in the top four entries which were also diploids. These results are similar to those of Lu et al. (2001) where the main carotenoids in eleven diploid clones were violaxanthin, lutein, and lutein-5,6-epoxide. The two

entries, BDC701-4-3W/Y and Russet Nugget, had higher levels of zeaxanthin in 2011. Similar zeaxanthin levels were seen among the entries in 2012.

Diploid entries were found to have about two to three times more carotenoids than tetraploids. A study done by Brown et al. (2007) also found that twenty-three diploid native potato cultivars had almost four times more carotenoids compared to eight tetraploids. Total carotenoid content was compared on both a fresh weight and dry weight basis to account for tuber size differences. Tetraploids are generally larger in tuber size than diploids, indicating they may contain more water. Diploids showed higher levels of carotenoids than tetraploids for both fresh weight and dry weight comparisons. Total carotenoid content had the highest correlation with violaxanthin in 2011 and with lutein and a lutein isomer in 2012. Correlation differences between the two years for chroma, individual, and total carotenoid content could be due to using different instruments.

Total carotenoid content was positively correlated with tuber chroma values. The literature also indicates that flesh color is positively correlated with individual and total carotenoid content. A positive but weaker correlation (r^2=0.57) was found between total carotenoid content and chroma for a study done in Alberta (Konschuh et al. 2005). Carotenoids are the yellow or orange pigment found in the flesh of potatoes indicating there would be an association between total carotenoid content and tuber flesh chroma. A study by Konschuh et al. (2007) found that flesh color was more closely correlated with total carotenoid content (r^2=0.46) than with the carotenoid, lutein (r^2=0.30) but neither correlation was very strong. Their results also found that correlations depended on time of harvest, location and may relate to tuber development. In 2011 the highest correlation for chroma occurred for total carotenoid content, followed by violaxanthin. In 2012, chroma and neoxanthin had the highest correlation, followed

by lutein and chroma. The correlation of individual and total carotenoid content with yellow-flesh intensity was reported by Lu et al. (2001). The correlation of lutein (r = 0.66) with yellow-flesh intensity was weaker than the correlation of lutein with chroma in 2012 for this study. However, the correlation of total carotenoid content (r = 0.83) with yellow-flesh intensity was slightly stronger than the correlation of total carotenoid content and chroma. Compounds other than carotenoids can influence the chroma of potatoes and variation in location, year or harvest date may indicate variability in flesh color.

The work done by Lu et al. (2001) looked at the correlation of tuber size with yellow-flesh intensity and total carotenoid content. Their results show a negative correlation of yellow-flesh intensity with tuber size and a negative correlation of total carotenoid content with tuber size. Diploid potato entries produce smaller tubers than tetraploid entries. The negative correlation between tuber size and total carotenoid content suggests an explanation for why diploids have a higher total carotenoid content. The smaller tuber size of diploids indicates an increase in yellow-flesh intensity and total carotenoid content. However, their smaller size may make them less marketable.

2.5 Conclusions

This research work provides insight into the carotenoid content produced by entries developed from the Colorado Potato Breeding and Selection Program. Due to the time and cost of extraction and analysis of carotenoid content, the association between tuber flesh chroma and the total carotenoid content suggests the opportunity for indirect selection for high carotenoid content based on chroma. The varying levels seen in carotenoid content indicates genetic variation is present in the breeding program. The high carotenoid content produced in diploid potato entries makes them useful as potential breeding material. A recent study by Haynes et al.

(2011) used three diploid entries with high, moderate and low carotenoid levels and crossed them with a light yellow-fleshed tetraploid to determine the inheritance of carotenoid content. Their results showed a continuous distribution of carotenoid concentration, with high and low carotenoid segregants in all three families. The results from the study done by Haynes et al. indicate that smaller tuber size is not the reason for an increase in total carotenoid content. More research needs to be done to determine if there is a significant entry by environment interaction for carotenoid content. It would also be beneficial to obtain more data to determine the major carotenoids present in the entries with high total carotenoid content. This would provide more information about the health benefits of the potato entries. Increasing carotenoid levels through the use of diploid potato entries will be a target for future breeding efforts.

CHAPTER 3: SENSORY PERCEPTIONS IN POTATO GERMPLASM

3.1 Introduction

The potato (*Solanum tuberosum* L.) is the fourth most widely grown crop in the world after wheat, rice and corn (Gilsenan et al. 2010). It is the world's third most important food crop and is produced on all continents except Antarctica (Birch et al. 2012). Colorado is ranked fourth in potato production in the United States, producing 1.04 million tons per year (National Potato Council 2012). Potato is gaining importance as a staple food crop to meet the demands of the increasing human population. In the past two decades, potato production has increased significantly in developing countries (Birch et al. 2012). Understanding of key genes and mechanisms underlying potato development, water and nutrient use efficiency, physiology and resistance to stresses has increased in the genomics era.

Potatoes are a nutrient dense vegetable with a variety of antioxidants. For each calorie of potato eaten there is an ample return of essential nutrients (Bohl and Johnson 2010). Potatoes are carbohydrate-rich, have high-quality protein and have a significant level of vitamin C (Brown 2005). They also contain a large amount of potassium as well as vitamin B6. Dietary fiber and resistant starch are present in potato tubers and provide health benefits such as regulating blood glucose and maintaining a healthy colon (United States Potato Board 2012). Carotenoids, flavonoids, and anthocyanins are a few antioxidants present in the potato tuber. A lower incidence of heart disease, certain cancers, macular degeneration, and severity of cataracts has been associated with diets rich in carotenoids and flavonoids (Brown 2005). The majority of the nutrients contained in potatoes are within the tuber flesh and not in the skin. Cooking does have an impact on these nutrients and nutrient loss is greatest when the cooking method involves

extended periods of time and/or water. Steaming and microwaving are the best cooking methods to use in order to maintain the most nutrition (United States Potato Board 2012).

The flavor is defined as the overall sensation resulting from the impact of the food on the chemical sense receptors in the nose and mouth. It is created by aromatic volatile compounds that are biosynthesized during metabolic processes in the plant and are further modified by cooking and processing (Dresow and Bohm 2009). When a person eats, odorous volatile substances pass from the mouth to the nose through inner passages to create the complex sensation of taste and odor known as flavor. However, odor is the most important characteristic of flavor which is apparent when a person is sick with a cold. Taste and odor are an assessment of the person sensing a specific compound rather than an inherent property of it. This means a specific compound can be sensed differently between people or at different times by the same person.

The aroma and perceived flavor of a food is determined by the volatile compounds present and their concentration. Volatile compounds primarily in potatoes include aldehydes, alcohols, ketones, acids, esters, hydrocarbons, amines, furans, and sulphur compounds (Dresow and Bohm 2009). Hundreds of aroma compounds are produced from cooked potatoes. Important compounds are produced by the Maillard reaction, lipid degradation and/or sugar degradation during heating (Jansky 2010). Cooked potatoes contain more volatiles than raw potatoes. About 159 volatiles have been identified in raw potatoes. 2-methoxy-3-isopropylpyrazine was the most identified methoxypyrazine in raw potato profiles.

Potato flavor, color and texture are important traits for consumer acceptability. Internal color, intensity of aroma, mustiness, hardness, moistness, earthiness, adhesiveness, sweetness, and aftertaste are sensory attributes that define cooked potato quality (Gilsenan et al. 2010).

Taste incorporates bitter, sour, sweet, salty and umami (Japanese meaning delicious) and potatoes contain all of these components except salty. Another influence of taste is the color of potato. The color of both cooked and fried products can affect the perception of taste. Colored potatoes have attracted investigators and consumers because of their antioxidant activity, taste and appearance (Murniece et al. 2013). Texture influences the release of volatile flavor compounds during chewing and is controlled by many factors, including dry matter content, specific gravity, sugars, protein, amylose, and nitrogen levels in tubers.

The traditional method used to assess the flavor of foods is the hedonic scale. The 9-point hedonic scale comprises a series of nine verbal categories ranging from 'like extremely' to 'dislike extremely.' The verbal categories can be converted to numerical values with 'like extremely' being equivalent to 9 and 'dislike extremely' equivalent to 1. Advantages of this scale are that panelists can respond meaningfully without prior experience, it's suitable for a wide range of populations, the data can be handled by the statistics of variables, and the results are meaningful for indicating general levels of preference (Peryam and Pilgrim 1957).

Solid-phase microextraction (SPME) is an extraction technique that can be used to extract potato volatile flavor compounds from cooked potato. The compounds can be quantified and then analyzed by gas chromatography/mass spectrometry (GC/MS). SPME has many advantages over conventional analytical methods by combining sampling, preconcentration, and direct transfer of the analytes into a standard gas chromatograph (Tsai and Chang 2003). It is a simple, sensitive and solvent-free sample extraction and concentration technique.

A volatile compound profile and sensory assessment has not been studied in entries developed from the Colorado Potato Breeding and Selection Program located in the San Luis Valley in Colorado. However, volatile compound data has been collected and presented on some

entries. The entries for this research study were chosen based on a previous study done that evaluated tuber-flesh color and carotenoid content. The purposes of this study were to evaluate 12 select entries for volatile flavor compounds present and perform a sensory analysis for five select entries. Both of these objectives will be completed using steam and microwave cooking methods. Entries will be selected based on their background, if they have good flavor properties, and some will be selected based on having a high carotenoid content. The study was done to examine the relationship between consumer preference, volatile compounds present and carotenoid content.

3.2 Materials and Methods

Plant Material

Field grown tuber tissue was utilized for identification and quantification of volatile flavor compounds present and for a sensory evaluation. The study involved material from the 2012 field season at the CSU SLVRC located in Center, Colorado. Entries were planted in individual plots and randomly located in the field. The plots were selection plots that were non-replicated. Cultural management practices were uniform among all of the plots. Plots were of varying lengths depending on the stage of selection the entries were at with 34 inches (86.4 cm) of spacing between rows and 12 inches (30.5 cm) of spacing between hills. Potatoes were grown in a sandy loam soil from mid-May to early September. During the 2012 field season, planting was done on May 16, the vines were killed 107 days after planting using sulfuric acid and harvesting was done on September 24 and 25. Total applied fertilizer each year included 54 kg (120 lbs) N, 27 kg (60 lbs) P_2O_5, 18 kg (40 lbs) K_2O, 11 kg (25 lbs) S, and 1.1 kg (2.5 lbs) Zn/A and was based on soil tests. Irrigation was performed using a center pivot. In 2012 there was a

gross application was 17.7 inches (45.0 cm). The application frequency and amount was based on evapotranspiration.

The volatile flavor compound analysis was performed on a subset of 12 entries from a previous study that focused on carotenoid content. This subset included a variety of entries where they either had good flavor properties, different genetic background, high carotenoid content, or a diploid background. Yukon Gold was included as the standard cultivar for comparison purposes. Two diploids were selected because they ranked in the top ten for total carotenoid content from the previous study. The sensory evaluation was performed on a subset of five entries that were used in the volatile flavor compound analysis. These included Yukon Gold, a check cultivar, CO05030-5W/Y, a tetraploid known to have good flavor and sensory properties, Masquerade, a bi-color tetraploid recently named cultivar, and BDC701-4-3W/Y and BDC758-2-1R/Y, the two diploids that ranked in the top ten for total carotenoid content.

Volatile Flavor Compound Analysis

Tubers were removed from 4.4°C (40°F) storage and rinsed. A fresh weight measurement was recorded before tubers were cooked. Tubers that were microwaved were pierced with a fork twice on each side and cooked on the outer edge of the rotating table of the microwave oven. Smaller tubers were cooked for 2 ½ - 3 minutes, three at a time and large tubers were cooked for 8-10 minutes, three at a time. Tubers were turned over about every minute. For tubers that were steamed a sieved double-boiler was used. The bottom pan of the double-boiler was filled halfway with water. Once the water came to a boil, tubers were placed in steamer insert and covered with the lid. Enough tubers were used to fill the bottom of the steamer insert. The stove burner was set to medium and cooked for 1 hour.

The weight of the tubers after cooking was recorded. Enough tubers were cooked to make sure at least 500 grams/entry/cooking method was present. Tubers were cut into small cubes and 100 grams was weighed out for one replication. A total of five replications were weighed out for one cooking method. Each replication was placed into a metal bowl and cooled to room temperature. Liquid nitrogen was poured into the bowl and pieces were stirred to ensure they were completely frozen. More liquid nitrogen was added if needed. Each replication was wrapped in foil labeled with sample identification, entry name, cooking method and the date. The five replications were placed into a gallon size plastic bag labeled with the sample identification, entry name, and date. Bags were placed in a cooler with dry ice and then transported and stored at -80°C (-112°F) until analysis.

A solid phase micro extraction (SPME) technique was used to extract volatile compounds which were then measured using a Varian 2000 Gas Chromatography-Mass Spectrometry (GC-MS) instrument (Varian, Inc.) with a DB wax column (30 m long, 30.25 mm I.D., 0.25 ml film thickness). Helium was used as a carrier gas at a flow rate of 1.5 mL/min. Standards were measured first to determine the appropriate program settings for the GC-MS. A total of ten standards generally associated with potato that were prominent and measurable compounds such as, alpha-copaene, carene, decanal, furfural, isobutyl-3-methoxypyrazine, isopropyl-3-methoxypyrazine, isovaleraldehyde (3 methyl butanal), limonene, 2-pentanone, and pinene were used. Whatman filter paper was cut up into pieces of 4 and one piece was placed into a clean, 16 ounce glass canning jar. The jar had a specially made lid that a carboxen/polydimethylsiloxane (PDMS) SPME fiber could be inserted into. A 4 µl sample of each standard was placed onto the piece of filter paper and the jar was immediately sealed. The jars were labeled with the standard name. For successive analysis of samples, the SPME fiber was always first heated in the GC

injector and a blank run was performed beforehand to make sure the fiber was clean as well as to avoid carryover effects. The fiber was left in the machine for the first 5 minutes of the program and was then taken out. For each standard the SPME fiber was inserted into the jar for 5 minutes and inserted into the GC-MS injector for the first 5 minutes of the program. A blank was run in between each standard. Retention time, major ion fragments and peak area were recorded. Since three of the standards did not have a peak in the chromatogram the start time of the program was adjusted to 3 minutes instead of 6 minutes. A standard mix was then performed. The program was altered to adjust for retention times falling too close together. The settings of the final program developed were an initial hold at 50°C (122°F) for 1 minute, the GC oven increased at 5°C (41°F) /minute up to 210°C (410°F) with a 5 minute hold and decreased back down at 100°C (212°F) /minute to 50°C (122°F) with a hold for 3 minutes. Total program time was 42.60 minutes.

Potato samples that were frozen in liquid nitrogen and stored at -80°C (-112°F) were transported from the Colorado State University campus to the SLVRC in insulated boxes with dry ice. They were stored at -80°C (-112°F) and were then moved to -20°C (-4°F) for a day and then to 4.4°C (40°F) for a couple days and finally put at room temperature when samples were analyzed. Clean jars were labeled with sample identification and replication number. One replication was placed into the jar and the jar was placed into a hot water bath (70°C/158°F) for 2 minutes. After 2 minutes the SPME fiber was placed into the jar for 5 minutes. The fiber was then inserted into the GC-MS machine for the first 5 minutes of the program. A blank was run before each sample. The file from each sample was saved and named with the sample identification, cooking method, and replication date. Retention time, major ion fragments and peak area were recorded for each sample. When analyzing each sample, the major ion fragment

of each standard was entered to see if the standard was present in the sample by comparing the retention time and ion fragments. A calibration curve was developed for the standard limonene in order to quantify the amount present in the samples.

Sensory Evaluation

Tubers were removed from 4.4°C (40°F) storage and rinsed. They were microwaved and steamed for evaluation. The process used for microwaving and steaming was the same process used for the volatile compound analysis. Once cooked, the skin was peeled off, tubers were cut into small pieces and placed into a labeled bowl. Two pieces were then placed in the corresponding cups on each tray when samples were ready to be served.

Before cooking, scorecards were printed and plastic 2 ounce cups were labeled with the random number given to each entry. For microwaved samples, cups were labeled with blue marker and for steamed samples, cups were labeled with red marker. Blue scorecards corresponded to microwaved samples and yellow scorecards corresponded to steamed samples. Trays were laid out with a napkin, fork, pencil, a cup of water, ten sample cups, two unsalted tops crackers, and a granola bar placed on each one. Cups were ordered so everyone did not taste samples in the same order. Two pieces of each sample were placed into the corresponding cup and trays were handed out to students and faculty. The evaluation was explained and scorecards were handed in once completed. Panelists were randomly assigned to start with microwaved samples first or steamed samples first. They were told to begin from left to right and to cleanse their palette between each sample with a bite of cracker and drink of water. Panelists kept their granola bar as a gift, pencils and trays were handed in and the rest was discarded. The evaluation was performed over two days in four classes of about 20 students and

other various faculty and students. There were a total of 105 panelists but seven scorecards were not fully completed so they were dropped for a total of 98 panelists.

The sensory attributes evaluated were flesh color, texture, taste, flavor, and overall acceptability. These were rated using a 9 point Hedonic scale with 1 being dislike extremely and 9 being like extremely. An overall ranking was also given as well as any comments on why they chose the sample they ranked first.

Experimental Design and Statistical Analysis

Five replications for each cooking method were performed for volatile compound analysis. Results were expressed as mean ± standard error (SE). Pearson correlation coefficients were calculated for the two cooking treatments used in the sensory evaluation, as well as for sensory attributes, total carotenoid content, and chroma using the SAS Proc Corr procedure. All statistical analyses were performed using SAS Statistical Analysis System, v.9.3 software (SAS Institute Inc., Cary, NC).

3.3 Results

3.3.1 Volatile Compound Analysis

The volatile compound analysis included ten standards. A typical chromatogram with nine of the standards identified is shown in Figure 3.1. The standard not shown is isovaleraldehyde and it has an earlier retention time than the other standards. The quantitative analysis of the standard, limonene, revealed varying concentrations present in 10 of the 12 entries. The other two did not show consistency between the replications, with limonene only being identified in one or two reps. These two entries were the diploids with a high carotenoid content. Figure 3.2A shows the calibration curve used to quantify limonene in the samples. Limonene content among the ten entries ranged from 0.43 μg/g to 2.14 μg/g for microwaved

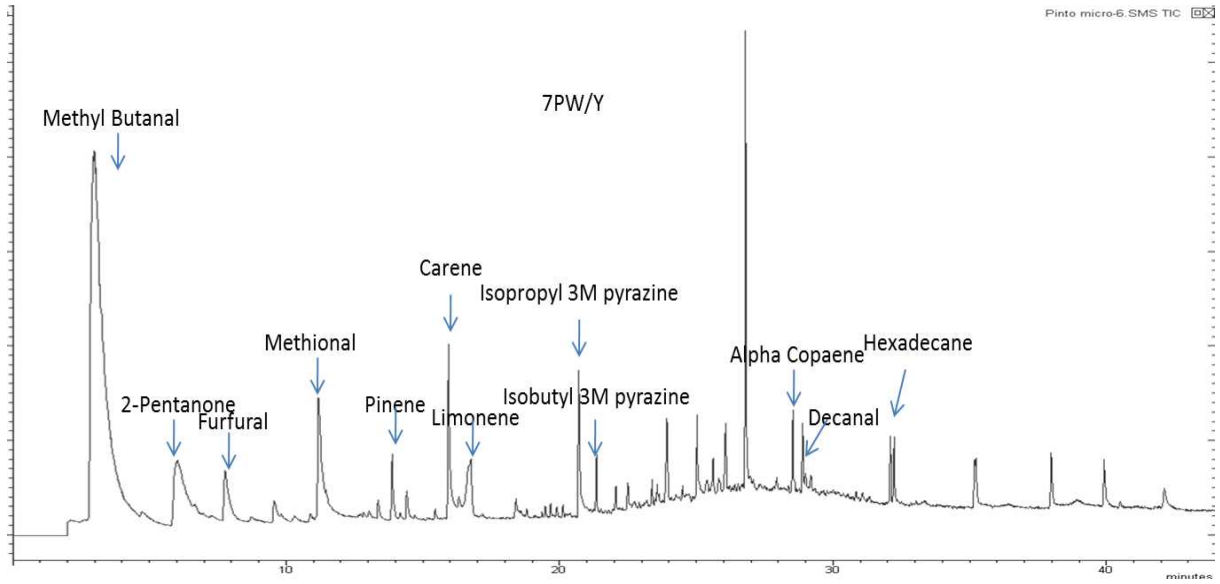

Figure 3.1: A representative chromatogram of the separation of volatile compound standards via GC/MS.

samples and 0.88 µg/g to 2.76 µg/g for steamed samples. Limonene was consistently higher in the steamed samples compared to the microwaved samples (Figure 3.2B). Entry, CO07044-3RU/Y, does not have data for steamed because limonene was only identified in one of the five reps. CO07109-1W/Y and Masquerade had the highest limonene content for both microwaved and steamed samples. Masquerade had the highest content for microwaved with 2.14 µg/g and CO07109-1W/Y had the highest content for steamed with 2.76 µg/g.

A qualitative analysis was performed for the other nine standards used in this study. One of the standards, isopropyl-3-methoxypyrazine, did not appear in the chromatogram of the standard mix and there was no isobutyl-3-methoxypyrazine to be used for the analysis. The results from the other seven standards showed that four of the standards were detected in the samples. The fours standards identified were alpha-copaene, decanal, isovaleraldeyhyde, and 2-pentanone.

Alpha-copaene was present in all 12 entries for both cooking methods (Figure 3.3). Peak area for microwaved samples ranged from 9,860 in Russet Nugget to 18,648 in BDC758-2-1R/Y. Peak area for steamed samples ranged from 9,799 in Russet Nugget to 27,057 in BDC701-4-

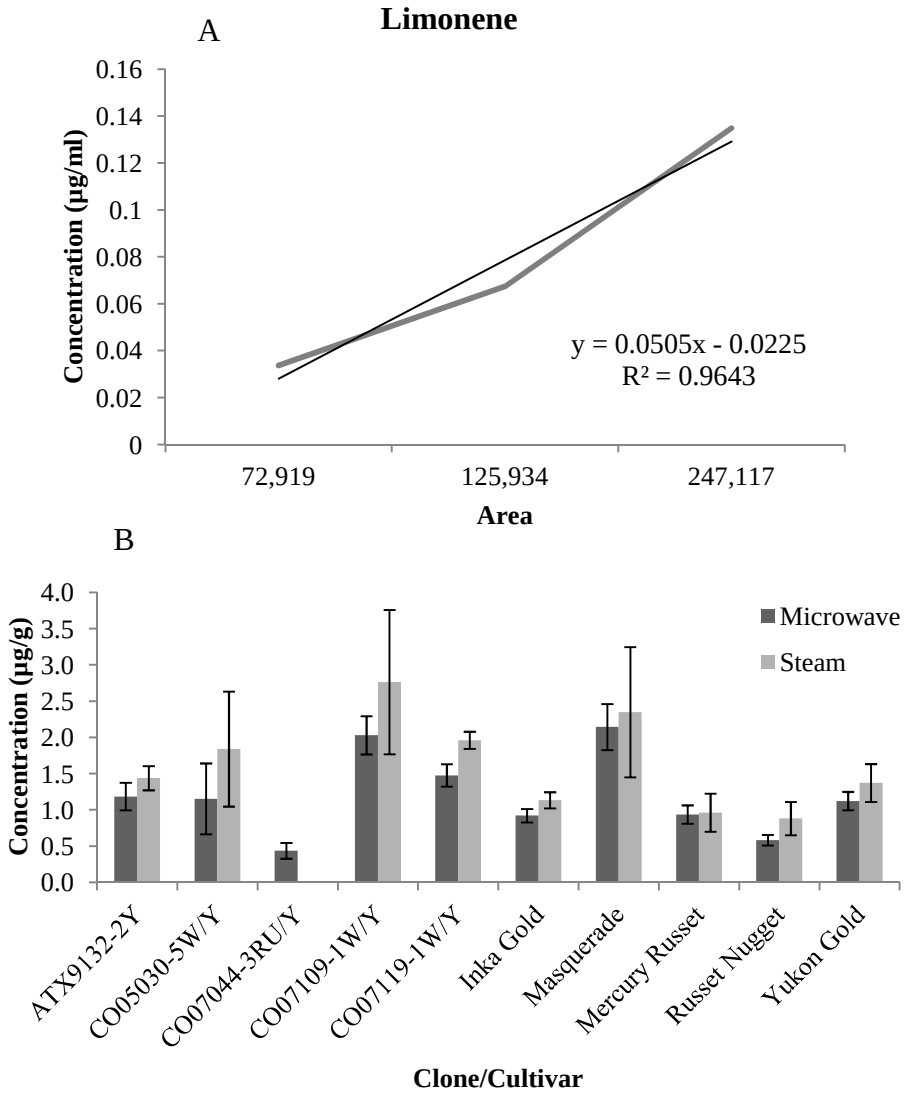

Figure 3.2: Calibration curve for the standard limonene (A) and limonene content of tubers cooked by microwaving and steaming (B). Error bars represent S.E. (standard error of the mean, generally 5 reps with one instance of 3 reps).

3W/Y. There were no significant differences between the two cooking methods for all entries. Decanal was present in four entries for both cooking methods (Figure 3.4). Peak area for microwaved samples ranged from 1,072 in Inka Gold to 1,830 in Yukon Gold while steamed samples ranged from 1,227 in Inka Gold to 3,977 in Yukon Gold. There was a significant difference between the two cooking methods for Yukon Gold, where steamed samples had a higher peak area.

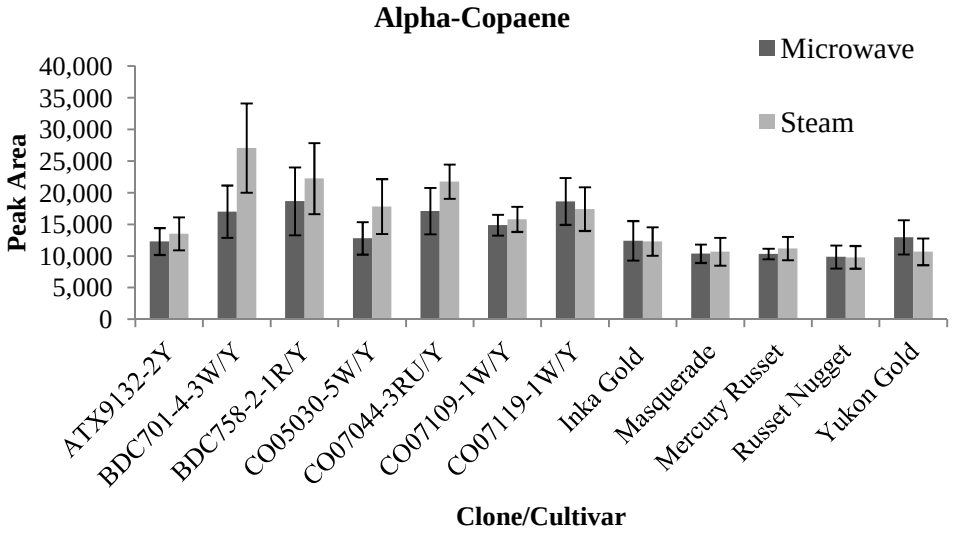

Figure 3.3: Alpha-copaene content of tubers cooked by microwaving and steaming. Error bars represent S.E. (standard error of the mean, minimum of 3 reps).

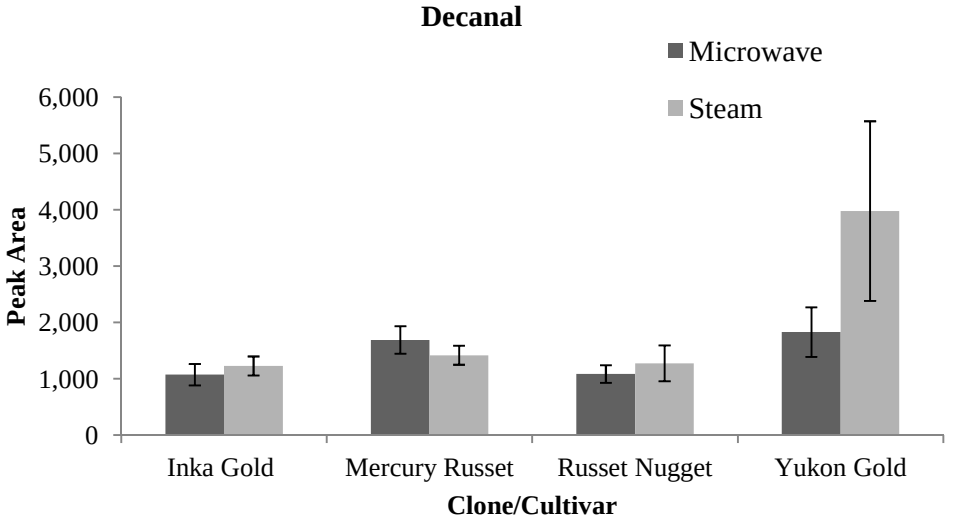

Figure 3.4: Decanal content of tubers cooked by microwaving and steaming. Error bars represent S.E. (standard error of the mean, minimum of 3 reps).

Isovaleraldehyde was present in ten entries for both cooking methods (Figure 3.5). Peak area for microwaved samples ranged from 8,172 in Russet Nugget to 55,345 in CO07044-3RU/Y. For steamed samples, peak area ranged from 5,471 in Mercury Russet to 57,471 in CO07109-1W/Y. There was variation between the two cooking methods, with some entries

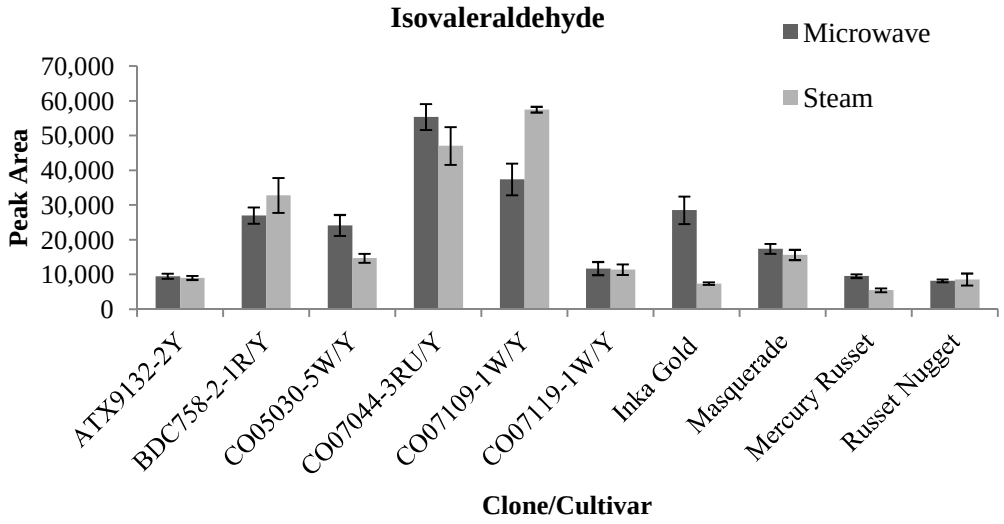

Figure 3.5: Isovaleraldehyde content of tubers cooked by microwaving and steaming. Error bars represent S.E. (standard error of the mean, minimum of 3 reps).

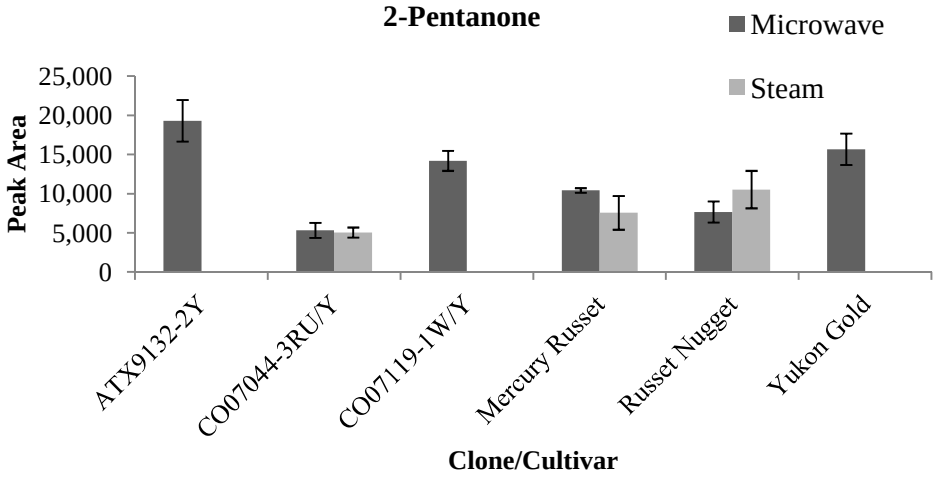

Figure 3.6: 2-Pentanone content of tubers cooked by microwaving and steaming. Error bars represent S.E. (standard error of the mean, minimum of 3 reps).

having a higher peak area for microwaved samples and others having a higher peak area for steamed samples. 2-pentanone was present in six entries for microwaved samples and 3 entries for steamed samples (Figure 3.6). Peak area for microwaved samples ranged from 5,318 in entry

CO07044-3RU/Y to 19,317 in entry ATX9132-2Y. For steamed samples, peak area ranged from 5,043 in entry CO07044-3RU/Y to 10,529 in Russet Nugget.

Limonene, alpha-copaene and isovaleraldehyde were detected in the majority of the entries for both microwaved and steamed samples (Figure 3.7). Limonene and isovaleraldehyde had the highest peak areas in most of the entries for both cooking methods. Mercury Russet and Russet Nugget were the only entries that had all five compounds detected in both microwaved and steamed samples. Limonene was not detected in steamed samples of CO7044-3RU/Y but was in microwaved samples. 2-Pentanone was not detected in steamed samples of ATX9132-2Y, CO07119-1W/Y, or Yukon Gold but was in microwaved samples.

The five entries that were used for the sensory evaluation show differences for limonene content. Limonene was not detected in the two diploids with high carotenoid content, BDC701-4-3W/Y and BDC758-2-1R/Y. It was detected in the three tetraploids, CO05030-5W/Y, Masquerade, and Yukon Gold, with steamed samples having a higher amount than microwaved samples. 2-Pentanone and decanal were only detected in Yukon Gold for the entries used in the sensory evaluation. Isovaleraldehyde was detected in BDC758-2-1R/Y, CO05030-5W/Y, and Masquerade but not in BDC701-4-3W/Y and Yukon Gold. Alpha-copaene was detected in all five entries.

3.3.2 Sensory Evaluation

Sensory evaluations of steamed and microwaved tubers from a subset of five entries that were used in the volatile flavor compound analysis were carried out by an untrained panel. These included Yukon Gold, a check cultivar, CO05030-5W/Y, a tetraploid known to have good flavor and sensory properties, Masquerade, a bi-color tetraploid recently named as a cultivar, and BDC701-4-3W/Y and BDC758-2-1R/Y, two diploids that ranked in the top ten for total

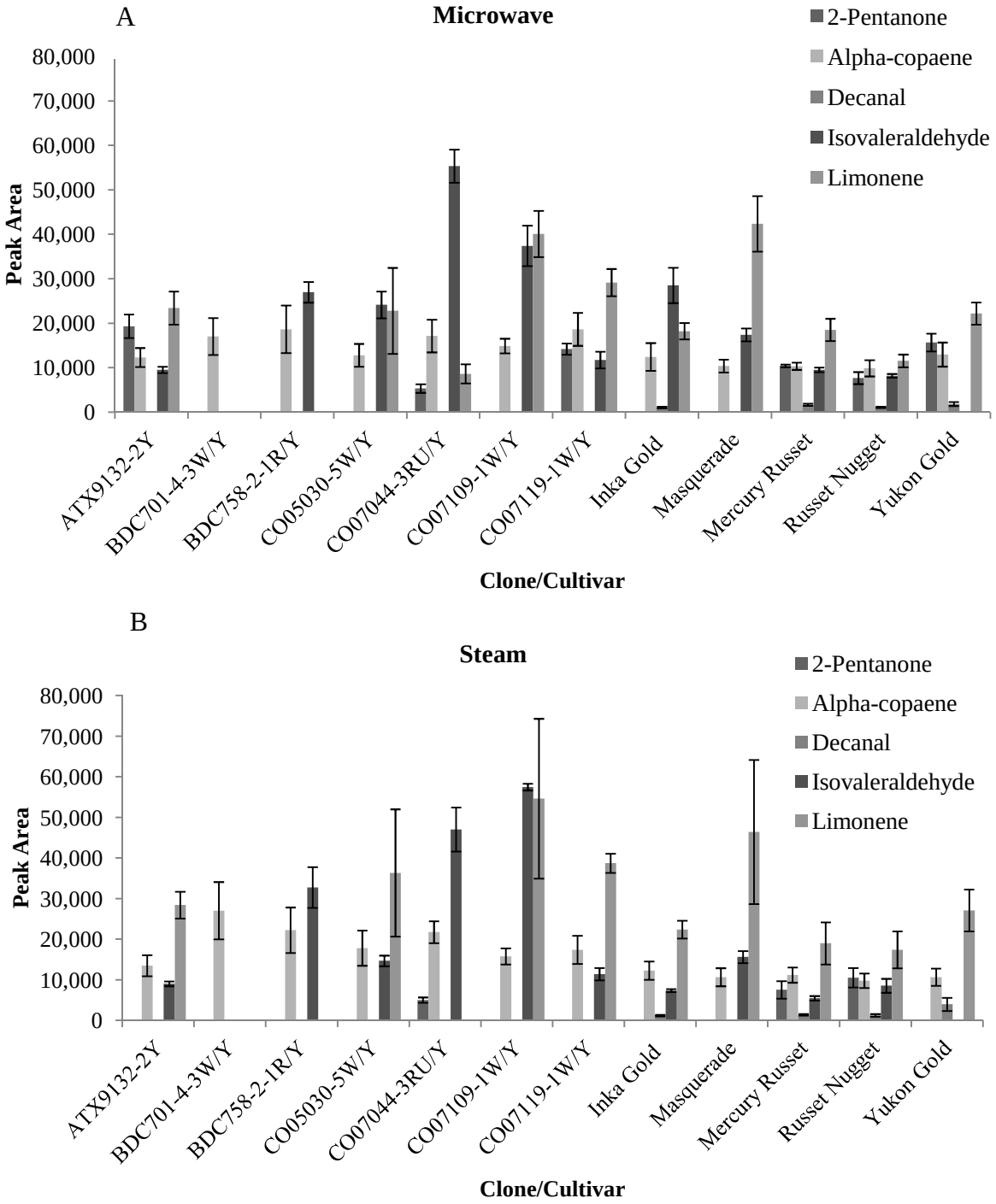

Figure 3.7: Volatile content of tubers cooked by microwaving (A) and steaming (B). Error bars represent S.E. (standard error of the mean, minimum of 3 reps).

carotenoid content. All entries had yellow flesh color. Overall acceptability and sensory attributes, flesh color, texture, taste, and flavor were scored on a scale of 1-9 for microwaved

samples (Figure 3.8A) and steamed samples (Figure 3.8B). Yukon Gold, Masquerade, and CO05030-5W/Y had similar scores for overall acceptability and all sensory attributes for both cooking treatments. BDC701-4-3W/Y and BDC758-2-1R/Y also had similar scores to each

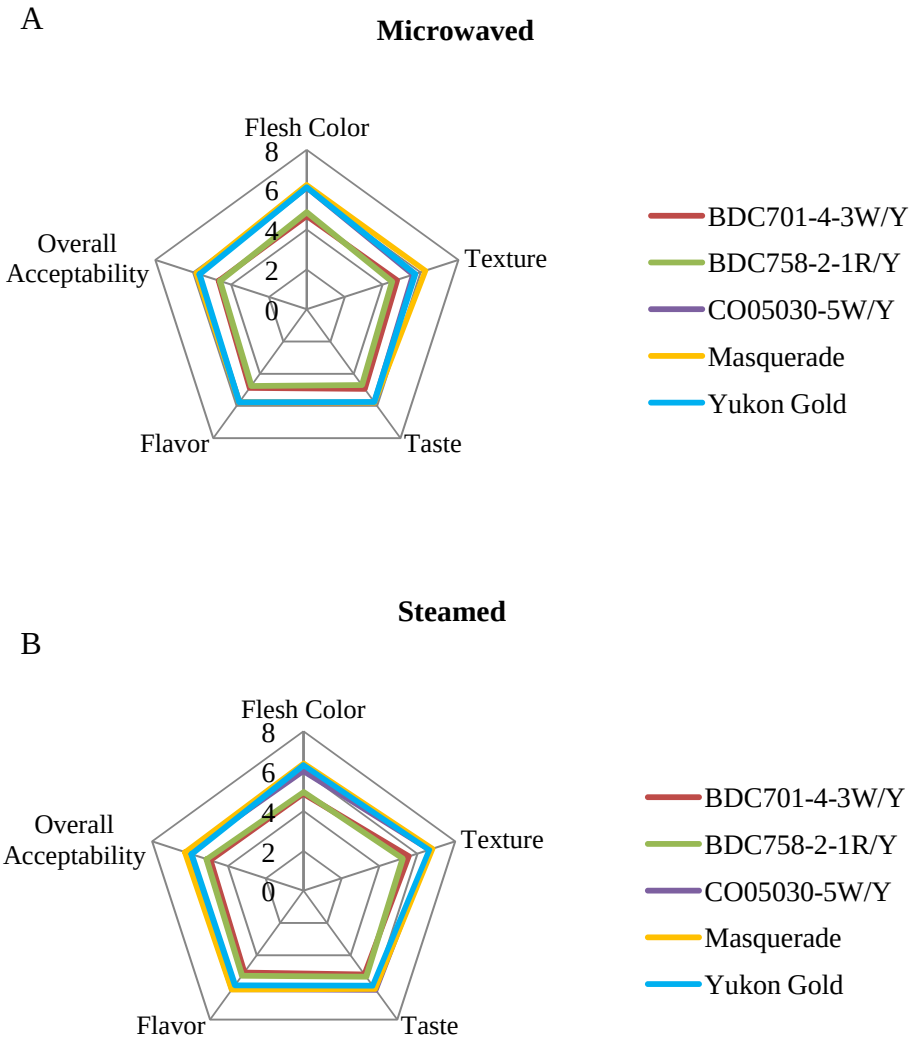

Figure 3.8: Comparison of sensory attributes among cooked potato entries (n=5). Samples were cooked by microwaving (A) and steaming (B). Sensory attributes were rated on a 9 point Hedonic scale with 9 being like extremely and 1 being dislike extremely. Ratings include 98 untrained panelists.

other for both cooking treatments. Yukon Gold, Masquerade, and CO05030-5W/Y, all tetraploids, received higher scores than the two diploids, BDC701-4-3W/Y and BDC758-2-1R/Y for both steamed and microwaved samples. For microwaved samples, Masquerade had the highest scores for flesh color, texture and overall acceptability and both Masquerade and CO05030-5W/Y had the highest scores for taste and flavor. BDC758-2-1R/Y had the lowest scores for texture, taste, flavor and overall acceptability while BDC701-4-3W/Y had the lowest score for flesh color. For steamed samples, Masquerade had the highest scores for overall acceptability and all sensory characteristics except taste where it followed CO05030-5W/Y, which had the highest score. BDC701-4-3W/Y had the lowest scores for all expect texture where BDC758-2-1R/Y scored the lowest for steamed samples.

Sensory evaluations also included an overall ranking of the five potato entries for both cooking treatments (Table 3.1). Panelists ranked entries from 1 to 5 with 1 being the top rank or best sample and 5 being the bottom rank or worst sample. For microwaved samples,

Table 3.1: Overall ranking of microwaved and steamed potato entries. Entries were ranked 1 to 5 with 1 being the top rank.

Clone/Cultivar	Microwaved	Steamed
BDC701-4-3W/Y	3.32	3.48
BDC758-2-1R/Y	3.12	3.56
CO05030-5W/Y	3.00	2.71
Masquerade	2.77	2.43
Yukon Gold	2.80	2.82

Masquerade was again ranked as the best sample and BDC758-2-1R/Y was ranked as the worst. For steamed samples, Masquerade was ranked as the best sample overall and BDC701-4-3W/Y was ranked as the worst. The correlation of all sensory attributes, overall acceptability and rank between the two different cooking treatments was examined in Table 3.2. The correlations between the sensory attributes, overall acceptability and overall rank for the two cooking treatments all show a high positive correlation. The highest correlation was flesh color ($r = 0.99$)

while the lowest correlation was taste (r = 0.94). When comparing overall acceptability and flesh color for both cooking methods to total carotenoid content and chroma, a high negative correlation is observed (Table 3.3).

Table 3.2: Correlation coefficients for the sensory attributes, overall acceptability and rank between microwaved and steamed potato samples (n=5).

Sensory Attributes	Correlation	Prob > |r|
Flesh Color	0.99	0.002
Texture	0.96	0.009
Taste	0.94	0.017
Flavor	0.96	0.011
Overall Acceptability	0.98	0.003
Overall Rank	0.96	0.008

Table 3.3: Correlation coefficients between flesh color and overall acceptability for microwaved and steamed samples, total carotenoid content and chroma (n=5).

	Color (Steamed)	Color (Microwaved)	Overall Acceptabilit-y (Steamed)	Overall Acceptability (Microwaved)	Carotenoid Content	Chroma
Color (Steamed)		0.99**	0.96**	0.97**	-0.97**	-0.92*
Color (Microwaved)			0.99**	0.99**	-0.98**	-0.93*
Overall Acceptability-(Steamed)				0.98**	-0.95*	-0.89*
Overall Acceptability-(Microwaved)					-0.99**	-0.95*
Carotenoid Content						0.98**
Chroma						

***** Significant ($p<0.05$), ** ($p<0.01$).

3.4 Discussion

Flavor is an important driver for consumers when considering what food to purchase. The preference of consumers is a factor that must be considered when developing new potato cultivars. Since the aroma and perceived flavor of a food is determined by the volatile compounds present, it is beneficial to determine what volatile compounds are found in potatoes preferred by consumers. Previous studies have looked at the relationship between the volatile compound profile and sensory scores in various potato germplasm. The results from a study by Morris et al. (2010) showed it was possible to associate groups of metabolites with different

flavor attributes. Carotenoid content was another aspect added to this study. The relationship between carotenoid content, sensory scores, and volatile compounds has not been previously observed.

The volatile compounds used in this study have different flavors associated with them. Alpha-copaene has a woody flavor, decanal gives off a citrus waxy odor, isovaleraldehyde has a malty and nutty flavor, limonene has a citrus flavor, and 2-pentanone has a sweet banana like flavor. These compounds were quantified based on ions and retention time. Limonene was detected in the majority of the entries. The cultivar Masquerade had the highest content of microwaved samples. This is similar to a study done by Jayanty and Holm (2012) where samples were microwaved and Masquerade was found to have the highest peak area for limonene out of four entries studied. Steaming appeared to be a better cooking method for the retention of the compound limonene. Limonene was not present in the two entries that had high carotenoid levels. It would be interesting to study more potato germplasm with high carotenoid levels to see if there is a relationship between carotenoid and limonene content in cooked tubers.

Alpha-copaene was detected in all 12 entries for both microwaved and steamed samples. The two diploids had the highest peak areas for alpha-copaene, one was highest for microwaved and the other highest for steamed samples. However, not much variation was seen between the entries or the two cooking methods for alpha-copaene. Collecting data on more entries with high carotenoid content would give more information on the relationship between alpha-copaene and carotenoid content. Decanal was not a main volatile compound detected in the entries. The compound isovaleraldehyde was detected in the majority of the entries except for one of the diploids and Yukon Gold. The two entries that were consistently higher for both microwaved and steamed cooking methods, CO07044-3RU/Y and CO07109-1W/Y, have a diploid

background. However, both of these entries had lower carotenoid levels. 2-Pentanone was not consistent between cooking methods and was more quantifiable in microwaved samples. The three entries that 2-pentanone was detected in for both cooking methods were russets. 2-pentanone could be one of the main volatile compounds in russet cultivars. The two diploids with high carotenoid content did not have 2-pentanone detected in their volatile profiles.

A comparison of cooking methods shows that limonene, alpha-copaene, and isovaleraldehyde were detected for both methods in the majority of the entries. Some compounds weren't detected in both microwaved and steamed samples among the entries. These compounds were present in microwaved samples but not steamed samples. The results indicate that compounds are more quantifiable in microwaved than steamed potatoes. An article by Jansky (2010) noted that microwave-baked potatoes had lower levels of volatiles than oven-baked or boiled potatoes. Lower levels were not seen in the results of this study but instead similar levels are seen between microwave-baked and steamed potatoes.

Sensory evaluations provide information to the potato breeder about consumer preference. It is important to consider consumer preference when developing new cultivars because if consumers don't like the potato then they won't buy it. The flavor of a potato is the second most important concern to consumers after cost. The sensory evaluation performed for this study included steamed and microwaved cooking methods. This is because microwaving is one of the most popular ways to cook potatoes and steaming and microwaving are the best cooking methods to use in order to maintain the most nutrition (Perla et al. 2012; United States Potato Board 2012). The main purpose of this sensory evaluation was to look at the results of potato entries that contain high levels of the phytonutrients, carotenoids, compared to entries that contain lower levels and are known to have good sensory properties. Yukon Gold was also

included as a standard check for comparison. The entries with a high carotenoid content were also diploids while the entries with a lower content were tetraploids. A recent similar study looked at the antioxidant profiles and sensory preferences for white- (Russet Burbank), yellow- (PORO3PG6-3) and purple-flesh (PORO4PG82-1) potato cultivars (Kaspar et al. 2013). This was the first study on sensory preferences for different pigmented potato cultivars and relating the antioxidant profiles to the sensory preferences. The study done by Kaspar et al. (2013) used baked samples which were heated up in a microwave oven right before serving to 60 untrained panelists for the sensory evaluation. Consumers ranked the aroma and appearance of white and yellow potatoes higher than purple ($P< 0.05$) but no significant differences were observed for flavor or overall acceptance between the cultivars.

The results of this study show that the two tetraploids with a lower carotenoid content along with our standard, Yukon Gold, are preferred over the two diploids with a higher carotenoid content. The bi-color tetraploid, Masquerade, received the highest score for overall acceptability for both steamed and microwaved cooking methods. These results are promising since it was recently named. The sensory evaluation results also showed a high positive correlation between the two cooking methods for all sensory attributes, overall acceptability and rank. This indicates there were no significant differences between steamed and microwaved samples for these five entries.

Another study done by James and Brown (2006) observed no significant differences among their array of specialty potato selections in taste, texture or smell for any of the preparation methods. The potato selections included mottled purple, dark purple, yellow, mottled red and white flesh colors and samples were baked, fried wedges, or included in a salad. These previous studies provide beneficial information about consumer preference and indicate

that pigmented potatoes are acceptable to consumers. However, the study by James and Brown (2006) did not measure carotenoid content and neither study measured the volatiles present in the potatoes.

The results from the volatile compound analysis and sensory evaluation were compared for the five entries chosen for the sensory evaluation (refer to Figures 3.2 to 3.6 and Figure 3.8). Differences were found for limonene content. Limonene was not detected in the two diploids with high carotenoid content. These two entries received the lowest sensory scores for all attributes. The results indicate a positive relationship between limonene and sensory scores. No relationship was seen for alpha-copaene, decanal, isovaleraldehyde, and 2-pentanone when compared to sensory score results. This is similar to a previous study by Morris et al. (2010), which compared potato germplasm that was different in several attributes determined by sensory evaluation with results from a phytochemical analysis. Correlations between sensory scores and decanal and alpha-copaene were not significant. There was, however, a positive correlation between furfural and aroma. Furfural was not detected in the entries used in this study. Another study by Thybo et al. (2006) also correlated metabolites and sensory scores but on pre-peeled tubers and their results also found no significant correlation between decanal and flavor. The results from a study by Morris et al. (2011) showed successful engineering of potato tubers to accumulate high levels of alpha-copaene, but their sensory analysis suggests that it is not a major component of potato flavor. These results are in agreement with the results from this study where no relationship was observed between any of the sensory attributes and alpha-copaene.

3.5 Conclusions

This research work provides insight into the volatile profiles and sensory assessment of various entries from the Colorado Potato Breeding and Selection Program. It also provides

information on carotenoid content for those entries selected for the sensory evaluation. Limonene was detected in all entries except those with a high carotenoid content which were also diploids. These results combined with sensory scores indicate that limonene may be contributing the most flavor. More research would be beneficial to determine if a relationship is present between limonene and carotenoid content in cooked tubers. Alpha-copaene was detected in all entries but no relationship was seen in any of the sensory attributes. 2-Pentanone was only detected in the three russet cultivars, indicating that it may be the main volatile in russets. There may also be a relationship between 2-pentanone and carotenoid content but further research is needed. Volatile compounds were more quantifiable in microwaved tubers than steamed tubers. Compounds that were detected in both microwaved and steamed samples showed similar levels between the two cooking methods.

The sensory evaluation showed that CO05030-5W/Y, Masquerade, and Yukon Gold received higher scores than BDC758-2-1R/Y and BDC701-4-3W/Y, which both had high carotenoid levels. Masquerade is a cultivar that has recently been named and received the highest sensory scores for the majority of the attributes, overall acceptability, and rank for both cooking methods. A positive correlation was seen between the two cooking methods for the sensory evaluation scores. It is unfortunate that the entries with high carotenoid content were not preferred by consumers since they contain more nutrients for the human body. However, it is important to keep in mind that the previous study done which looked at carotenoid content included a limited selection of diploids. The two diploids selected for this study may not have shown favorable flavor results but there may be other diploids with high carotenoid levels that have promising flavor characteristics. The results from this study indicate that entries with high carotenoid content can be used as breeding material to develop cultivars with both greater

nutrient levels and consumer acceptance. However, more research needs to be done to find entries with high carotenoid content and enhanced flavor.

CHAPTER 4: GENERAL DISCUSSION

4.1 Conclusions

Potato is the fourth most widely grown crop in the world and is grown in all 50 U.S. states and about 125 countries throughout the world (Gilsenan et al. 2010; United States Potato Board 2012). The potato contains numerous nutrients and antioxidants. Carotenoids are phytonutrients contained within the tuber flesh of the potato. They are known to help with reduction of cardiovascular disease, some cancers, diabetes, cataracts, and macular degeneration (Mayne 1996; Willcox et al. 2004). Xanthophylls are the primary carotenoids in potato tubers (Brown et al. 2006) and produce a yellow or orange flesh color. Previous studies have found a positive correlation between yellow-flesh intensity and carotenoid content. The Colorado Potato Breeding and Selection Program has not analyzed their material for carotenoid content or looked at the relationship between carotenoid content, volatile flavor compounds and consumer preference.

This study focused on the analysis of tuber flesh color, carotenoids, volatile flavor compounds, and sensory attributes of potato entries. The majority of these entries were developed from the Colorado Potato Breeding and Selection Program, with others from the USDA and breeding programs in Canada, Idaho, Maryland, Oregon, Texas, and Washington.

Material from two field seasons (2011 and 2012) at the SLVRC, Colorado State University in Center, Colorado was utilized in this study. Tuber flesh color was analyzed on 138 entries with three replications per entry. Total carotenoid content was analyzed on a subset of 100 entries and included three technical replicates. For 2011, individual carotenoid content was analyzed on a subset of eight entries using HPLC with three technical replicates. For 2012,

individual carotenoid content was analyzed on the same subset of eight entries using UPLC with three technical replicates ran as duplicates. A subset of 12 entries was analyzed in 2012 for volatile flavor compounds, using five replicates per cooking method. A sensory evaluation was completed on a subset of five entries from these 12 entries.

Chroma is the intensity or saturation of a color (Cantwell et al. 2004). A wide variation was seen among the entries for the chroma of tuber flesh color. There was a significant entry by year interaction for chroma among the 138 entries. Significant differences were found between entries for hue (true color), while the year was insignificant.

A wide variation was also seen for total carotenoid content among the subset of 100 entries. There was a significant entry by year interaction. Diploids had higher levels of carotenoid content than tetraploids, about three times more. The diploids that were in the top ten for carotenoid content contained six to thirteen times more carotenoids than Yukon Gold. Problems with instrumentation for the analysis of carotenoid composition may have affected the data in 2011. Higher levels of lutein were seen among the eight entries, indicating it is a major carotenoid. Violaxanthin levels were high among the four diploid entries ranked in the top twenty for total carotenoid content, indicating it may also be a major carotenoid.

There was a high positive correlation between chroma and total carotenoid content. Violaxanthin levels had a high positive correlation with both chroma and total carotenoid content of 2011 samples. For 2012 samples, positive correlations were seen between all carotenoids, total carotenoid content, and chroma. Neoxanthin and chroma had a high positive correlation and so did the lutein and total carotenoid content.

Limonene was quantified in the subset of 12 entries, while a qualitative analysis was done for alpha-copaene, decanal, isovaleraldehyde, and 2-pentanone. Two of the 12 entries were

in the top ten for total carotenoid content and were diploids while the rest were tetraploids. Samples were both microwaved and steamed. Steaming was a better cooking method for the retention of limonene. However, the compounds were more quantifiable in microwaved than steamed samples. The recently named cultivar Masquerade had the highest limonene content for microwaved samples. Limonene was not present in the two entries with high carotenoid content but was a main volatile in the others. Alpha-copaene was a main volatile detected in all twelve entries. The two diploids had the highest peak areas for alpha-copaene. Isovaleraldehyde was also a main volatile, detected in ten of the entries. 2-pentanone was only detected in the three russet varieties for both cooking methods used.

The sensory evaluation included five entries that were included in the volatile compound analysis. Three were tetraploids and two were the diploids with high carotenoid content. The sensory assessment showed that the three tetraploid entries had similar scores and were preferred over the two diploids. Flesh color, texture, taste, flavor, overall acceptability, and an overall rank were the attributes evaluated for both microwaved and steamed samples. There were a total of 98 untrained panelists who completed the sensory evaluation. The bi-color tetraploid, Masquerade, received the highest score for overall acceptability and had high scores for all other attributes for the two cooking methods.

Comparison of the volatile compounds and sensory scores for the five entries indicated differences for limonene content (refer to Figures 3.2 and 3.8). Limonene was not detected in the two diploids with high carotenoid content and they also received the lowest sensory scores. Limonene might be a major volatile contributing to flavor. No relationship was seen between the other four volatile compounds and sensory scores (refer to Figures 3.3 to 3.6 and Figure 3.8).

4.2 Future Directions

The research presented provides insight into the tuber flesh color, carotenoid content, volatile profiles, and sensory assessment of various entries from the Colorado Potato Breeding and Selection Program. The association between tuber flesh chroma and total carotenoid content will allow for the indirect selection for high carotenoid content due to the time and cost of carotenoid analysis. More data should be obtained to determine the major carotenoids present in the entries with high total carotenoid content. This would provide more information about the health benefits of the potato entries.

Further research work needs to be done for the volatile compounds present in the entries with high carotenoid content. Collecting more data and including more entries could help determine if a relationship is present between the two volatiles, limonene and 2-pentanone, and carotenoid content. The two entries with high carotenoid content were not preferred by consumers; however, this study included a limited selection of diploids. More research should be done on diploid entries with high carotenoid levels to find entries with promising flavor characteristics. Increasing carotenoid levels through the use of diploid potato entries will be a target for future breeding efforts. The main focus from this work is to develop cultivars with both greater nutrient levels and enhanced flavor.

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APPENDIX A: GENOTYPIC AND CONTENT INFORMATION

Table A.1: An entry list for the 2011 and 2012 tuber-flesh color and carotenoid content study. A total of 138 entries were chosen for this study, with nine market available potato entries. The study had 103 tetraploids and 35 diploids, with 128 yellow flesh, 3 white flesh and 7 colored flesh potato entries.

Clone/Cultivar:		
07S018	BDC724-3-1R/Y	CO07021-2R/Y
07S019	BDC724-3-2W/Y	CO07030-1RU/Y
07S020	BDC741-4-1R/Y	CO07039-3RU/Y
4X91E22	BDC741-4-2R/Y	CO07041-4RU/Y
AC03534-2R/Y	BDC741-4-3W/Y	CO07044-2W/Y
AC05175-3P/Y	BDC741-4-4R/Y	CO07044-3RU/Y
AC05175-9PW/Y	BDC747-1-1W/Y	CO07105-4RU/Y
AC06259-5W/Y	BDC758-2-1R/Y	CO07109-1W/Y
AC06358-1W/Y	BDC758-2-2RW/Y	CO07114-2RW/Y
AC06358-2W/Y	BDC758-2-3R/RY	CO07119-1W/Y
AC06725-1W/Y	BDC758-2-4R/Y	CO07131-1RW/Y
AC06887-4W/Y	BDC758-2-5W/Y	CO07131-2W/Y
AC06908-1W/Y	BDC758-2-6R/Y	CO07150-1W/Y
AC07315-1W/Y	BDC758-2-7W/Y	CO07150-2W/Y
AC97521-1R/Y	CO00412-5W/Y	CO07153-3RW/Y
AC99330-1P/Y	CO01399-10P/Y	CO07249-1RU/Y
ATC00293 -1W/Y	CO03060-2W/Y	CO07323-2R/Y
ATC02263-1W/Y	CO03341-1R/Y	CO07329-1P/Y
ATC05175-1PW/Y	CO03392-6RU/Y	CO07329-5P/Y
ATC05175-2RW/Y	CO04013-1W/Y	CO07370-1W/Y
ATC06258-1R/Y	CO04021-2R/Y	CO08390-1P/P
ATC06258-8R/Y	CO04022-1R/Y	CO97232-1R/Y
ATC06277-2P/Y	CO04023-3R/Y	CO97232-2R/Y
ATC06277-3P/Y	CO04029-3RW/Y	CO97233-3R/Y
ATX9132-2Y	CO04029-5W/Y	CO97237-4RU/Y
BDC691-2-2R/Y	CO04067-10W/Y	CO97237-5RU/Y
BDC691-2-3W/Y	CO04067-8R/Y	CO99045-1W/Y
BDC694-3-1W/Y	CO04099-3W/Y	PA4X137-12
BDC694-3-2W/Y	CO04099-4W/Y	POR00PG4-1
BDC694-3-3W/Y	CO04117-5PW/Y	POR02PG37-2
BDC696-1-1R/Y	CO04155-2R/Y	POR04PG11-2
BDC696-1-2R/Y	CO04159-3R/Y	VC0967-2R/Y
BDC696-1-3W/Y	CO04188-4R/Y	VC0967-2R/Y
BDC699-1-1R/Y	CO05028-8R/RY	VC1002-3W/Y
BDC699-1-2W/Y	CO05030-5W/Y	VC1009-1W/Y
BDC701-4-1R/Y	CO05035-1PW/Y	YDH4X.36*
BDC701-4-2W/Y	CO05035-5PW/Y	Agria
BDC701-4-3W/Y	CO05035-7PW/Y	Chipeta
BDC701-4-4W/Y	CO05035-8PW/Y	Inka Gold
BDC702-1-1R/RY	CO05037-2R/Y	Masquerade
BDC702-1-2R/Y	CO05037-3W/Y	Purple Majesty

BDC704-1-1W/Y	CO05085-5R/RY	Rio Grande Russet
BDC712-1-1R/Y	CO05100-1W/Y	Rose Valley
BDC712-1-2R/Y	CO05122-1W/Y	Russet Nugget
BDC715-1-1R/Y	CO05239-1R/Y	Sierra Gold
BDC715-1-2R/Y	CO06257-2R/Y	Yukon Gold

* Not included in 2012

Table A.2: Color rating for yellow and white flesh potato entries (n=131) grown in 2011. Entries were rated using a standard color chart on a scale of 1-5, with 1 being white and 5 being dark yellow. Standard color chart used in the Western Regional Potato Variety Trials.

Clone/Cultivar	Rating	Clone/Cultivar	Rating	Clone/Cultivar	Rating
07S018	4	BDC715-1-2R/Y	4	CO07021-2R/Y	4
07S019	4	BDC724-3-1R/Y	5	CO07030-1RU/Y	4
07S020	3	BDC724-3-2W/Y	4	CO07039-3RU/Y	3
4X91E22	5	BDC741-4-1R/Y	5	CO07041-4RU/Y	3
AC03534-2R/Y	3	BDC741-4-2R/Y	5	CO07044-2W/Y	4
AC05175-3P/Y	3	BDC741-4-3W/Y	5	CO07044-3RU/Y	4
AC05175-9PW/Y	4	BDC741-4-4R/Y	5	CO07105-4RU/Y	3
AC06259-5W/Y	4	BDC747-1-1W/Y	5	CO07109-1W/Y	4
AC06358-1W/Y	3	BDC758-2-1R/Y	5	CO07114-2RW/Y	3
AC06358-2W/Y	4	BDC758-2-2RW/Y	5	CO07119-1W/Y	4
AC06725-1W/Y	3	BDC758-2-4R/Y	4	CO07131-1RW/Y	5
AC06887-4W/Y	4	BDC758-2-5W/Y	4	CO07131-2W/Y	4
AC06908-1W/Y	4	BDC758-2-6R/Y	5	CO07150-1W/Y	4
AC07315-1W/Y	4	BDC758-2-7W/Y	4	CO07150-2W/Y	4
AC97521-1R/Y	4	CO00412-5W/Y	3	CO07153-3RW/Y	4
AC99330-1P/Y	4	CO01399-10P/Y	4	CO07249-1RU/Y	3
ATC00293 -1W/Y	3	CO03060-2W/Y	4	CO07323-2R/Y	3
ATC02263-1W/Y	4	CO03341-1R/Y	3	CO07329-1P/Y	4
ATC05175-1PW/Y	4	CO03392-6RU/Y	3	CO07329-5P/Y	3
ATC05175-2RW/Y	3	CO04013-1W/Y	3	CO07370-1W/Y	4
ATC06258-1R/Y	3	CO04021-2R/Y	4	CO97232-1R/Y	4
ATC06258-8R/Y	3	CO04022-1R/Y	4	CO97232-2R/Y	3
ATC06277-2P/Y	4	CO04023-3R/Y	4	CO97233-3R/Y	4
ATC06277-3P/Y	4	CO04029-3RW/Y	3	CO97237-4RU/Y	3
ATX9132-2Y	4	CO04029-5W/Y	4	CO97237-5RU/Y	4
BDC691-2-2R/Y	4	CO04067-10W/Y	4	CO99045-1W/Y	4
BDC691-2-3W/Y	4	CO04067-8R/Y	4	PA4X137-12	5
BDC694-3-1W/Y	4	CO04099-3W/Y	4	POR00PG4-1	4
BDC694-3-2W/Y	4	CO04099-4W/Y	3	POR02PG37-2	3
BDC694-3-3W/Y	5	CO04117-5PW/Y	4	VC0967-2R/Y	3
BDC696-1-1R/Y	3	CO04155-2R/Y	3	VC0967-2R/Y	3
BDC696-1-2R/Y	4	CO04159-3R/Y	3	VC1002-3W/Y	3
BDC696-1-3W/Y	4	CO04188-4R/Y	4	VC1009-1W/Y	3
BDC699-1-1R/Y	5	CO05030-5W/Y	4	YDH4X.36	4
BDC699-1-2W/Y	5	CO05035-1PW/Y	3	Agria	4
BDC701-4-1R/Y	4	CO05035-5PW/Y	3	Chipeta	2
BDC701-4-2W/Y	5	CO05035-7PW/Y	3	Inka Gold	4
BDC701-4-3W/Y	5	CO05035-8PW/Y	3	Masquerade	3
BDC701-4-4W/Y	5	CO05037-2R/Y	3	Rio Grande Russet	1

BDC702-1-2R/Y	4	CO05037-3W/Y	4	Rose Valley	4
BDC704-1-1W/Y	5	CO05100-1W/Y	3	Russet Nugget	2
BDC712-1-1R/Y	5	CO05122-1W/Y	4	Sierra Gold	3
BDC712-1-2R/Y	4	CO05239-1R/Y	3	Yukon Gold	3
BDC715-1-1R/Y	4	CO06257-2R/Y	3		

Table A.3: Color rating for yellow and white flesh potato entries (n=131) grown in 2012. Entries were rated using a scale of 0-3 with 0 being white and 3 being dark yellow.

Clone/Cultivar	Rating	Clone/Cultivar	Rating	Clone/Cultivar	Rating
07S018	3	BDC715-1-2R/Y	3	CO07021-2RU/Y	2
07S019	3	BDC724-3-1R/Y	3	CO07030-1RU/Y	1
07S020	3	BDC724-3-2W/Y	2	CO07039-3RU/Y	1
4X91E22	2	BDC741-4-1R/Y	3	CO07041-4RU/Y	1
AC03534-2R/Y	1	BDC741-4-2R/Y	3	CO07044-2W/Y	1
AC05175-3P/Y	1	BDC741-4-3W/Y	3	CO07044-3RU/Y	2
AC05175-9PW/Y	1	BDC741-4-4R/Y	3	CO07105-4RU/Y	1
AC06259-5R/Y	1	BDC747-1-1W/Y	3	CO07109-1W/Y	2
AC06358-1W/Y	2	BDC758-2-1R/Y	3	CO07114-2RW/Y	2
AC06358-2W	2	BDC758-2-2RW/Y	2	CO07119-1W/Y	2
AC06725-1W/Y	1	BDC758-2-4R/Y	3	CO07131-1RW/Y	3
AC06887-4W/Y	2	BDC758-2-5W/Y	2	CO07131-2W/Y	3
AC06908-1W/Y	2	BDC758-2-6R/Y	3	CO07150-1W/Y	2
AC07315-1W/Y	2	BDC758-2-7W/Y	2	CO07150-2W/Y	2
AC97521-1R/Y	2	CO00412-5W/Y	3	CO07153-3RW/Y	2
AC99330-1P/Y	3	CO01399-10P/Y	1	CO07249-1RU/Y	2
ATC00293 -1W/Y	3	CO03060-2W/Y	3	CO07323-2R/Y	2
ATC02263-1W/Y	2	CO03341-1R/Y	2	CO07329-1P/Y	1
ATC05175-1PW/Y	1	CO03392-6RU/Y	1	CO07329-5P/Y	2
ATC05175-2RW/Y	1	CO04013-1W/Y	2	CO07370-1W/Y	2
ATC06258-1R/Y	1	CO04021-2R/Y	2	CO97232-1R/Y	3
ATC06258-8R/Y	1	CO04022-1R/Y	3	CO97232-2R/Y	3
ATC06277-2P/Y	3	CO04023-3R/Y	3	CO97233-3R/Y	3
ATC06277-3P/Y	2	CO04029-3RW/Y	2	CO97237-4RU/Y	2
ATX9132-2Y	3	CO04029-5W/Y	2	CO97237-5RU/Y	2
BDC691-2-2R/Y	1	CO04067-10W/Y	2	CO99045-1W/Y	3
BDC691-2-3W/Y	1	CO04067-8R/Y	2	PA4X137-12	3
BDC694-3-1W/Y	3	CO04099-3W/Y	2	POR00PG4-1	3
BDC694-3-2W/Y	2	CO04099-4W/Y	2	POR02PG37-2	3
BDC694-3-3W/Y	3	CO04117-5PW/Y	3	VC0967-2R/Y	2
BDC696-1-1R/Y	2	CO04155-2R/Y	3	VC0967-2R/Y	2
BDC696-1-2R/Y	2	CO04159-3R/Y	2	VC1002-3W/Y	3
BDC696-1-3W/Y	2	CO04188-4R/Y	2	VC1009-1W/Y	3
BDC699-1-1R/Y	2	CO05030-5W/Y	2	YDH4X.36*	
BDC699-1-2W/Y	3	CO05035-1PW/Y	1	Agria	3
BDC701-4-1R/Y	3	CO05035-5PW/Y	2	Chipeta	0
BDC701-4-2W/Y	2	CO05035-7PW/Y	2	Inka Gold	3
BDC701-4-3W/Y	3	CO05035-8PW/Y	2	Masquerade	2
BDC701-4-4W/Y	2	CO05037-2R/Y	1	Rio Grande Russet	0
BDC702-1-2R/Y	2	CO05037-3W/Y	2	Rose Valley	1
BDC704-1-1W/Y	2	CO05100-1W/Y	2	Russet Nugget	0

BDC712-1-1R/Y	2	CO05122-1W/Y	2	Sierra Gold	2
BDC712-1-2R/Y	2	CO05239-1R/Y	2	Yukon Gold	1
BDC715-1-1R/Y	3	CO06257-2R/Y	2		

*Not included in 2012

Table A.4: Chroma and hue values for potato entries (n=138) for the 2011 and 2012 study.

	2011		2012	
Clone/Cultivar	Chroma	Hue	Chroma	Hue
07S018	25.4	90.9	28.4	90.9
07S019	23.9	90.3	22.0	92.2
07S020	24.3	92.4	27.3	91.5
4X91E22	34.2	83.8	31.1	81.6
AC03534-2R/Y	14.2	93.2	15.3	93.9
AC05175-3P/Y	21.1	91.0	20.9	91.4
AC05175-9PW/Y	23.2	88.9	22.9	89.7
AC06259-5W/Y	24.9	92.5	19.3	93.4
AC06358-1W/Y	23.0	93.5	25.8	92.8
AC06358-2W/Y	28.8	89.5	27.8	91.5
AC06725-1W/Y	25.5	92.5	24.1	91.9
AC06887-4W/Y	25.3	90.7	26.7	91.4
AC06908-1W/Y	26.9	91.7	25.9	91.8
AC07315-1W/Y	27.4	91.5	28.5	91.2
AC97521-1R/Y	23.1	93.7	20.7	92.1
AC99330-1P/Y	22.3	92.8	27.7	91.3
ATC00293 -1W/Y	17.3	93.3	23.2	92.2
ATC02263-1W/Y	25.2	92.0	27.1	91.6
ATC05175-1PW/Y	20.5	88.9	22.9	90.2
ATC05175-2RW/Y	21.0	89.1	24.4	100.7
ATC06258-1R/Y	21.5	90.3	22.3	93.0
ATC06258-8R/Y	20.1	91.0	19.6	92.8
ATC06277-2P/Y	29.7	88.4	32.3	88.9
ATC06277-3P/Y	27.1	92.5	31.0	92.1
ATX9132-2Y	20.9	93.3	28.6	80.8
BDC691-2-2R/Y	29.0	90.2	34.9	90.9
BDC691-2-3W/Y	31.9	91.8	34.0	91.2
BDC694-3-1W/Y	32.4	85.9	33.8	85.3
BDC694-3-2W/Y	28.3	92.1	33.7	91.2
BDC694-3-3W/Y	41.0	79.8	40.6	82.2
BDC696-1-1R/Y	35.6	89.9	31.6	91.0
BDC696-1-2R/Y	35.6	90.8	33.9	92.5
BDC696-1-3W/Y	34.0	92.0	33.5	91.7
BDC699-1-1R/Y	39.6	82.6	37.2	82.5
BDC699-1-2W/Y	37.7	74.6	33.5	77.3
BDC701-4-1R/Y	42.0	81.5	36.3	81.9
BDC701-4-2W/Y	39.7	82.1	36.6	81.4
BDC701-4-3W/Y	36.2	81.8	35.9	79.9
BDC701-4-4W/Y	36.2	81.5	34.8	80.4
BDC702-1-1R/RY	24.5	32.1	21.8	38.3
BDC702-1-2R/Y	43.4	80.8	34.8	79.6
BDC704-1-1W/Y	37.4	78.0	35.4	79.9

BDC712-1-1R/Y	37.3	81.3	36.8	82.3
BDC712-1-2R/Y	36.6	84.1	33.7	84.4
BDC715-1-1R/Y	43.2	76.5	32.2	66.8
BDC715-1-2R/Y	42.6	82.7	34.3	83.9
BDC724-3-1R/Y	36.8	82.8	35.9	78.9
BDC724-3-2W/Y	35.4	82.3	37.7	82.4
BDC741-4-1R/Y	36.3	82.7	39.3	79.3
BDC741-4-2R/Y	37.9	79.3	34.3	78.7
BDC741-4-3W/Y	45.7	81.1	36.5	81.7
BDC741-4-4R/Y	36.7	79.5	33.4	80.5
BDC747-1-1W/Y	34.8	79.5	34.3	79.6
BDC758-2-1R/Y	42.8	79.0	38.9	78.7
BDC758-2-2RW/Y	38.6	80.4	34.8	80.5
BDC758-2-3R/RY	28.7	64.8	26.1	55.9
BDC758-2-4R/Y	37.4	81.4	36.4	80.6
BDC758-2-5W/Y	37.1	85.7	38.2	89.7
BDC758-2-6R/Y	39.9	80.6	38.2	78.4
BDC758-2-7W/Y	40.5	82.8	39.5	82.9
CO00412-5W/Y	22.9	93.7	22.8	92.0
CO01399-10P/Y	20.1	93.7	20.5	91.9
CO03060-2W/Y	25.8	92.7	22.3	93.1
CO03341-1R/Y	17.6	93.1	19.7	93.7
CO03392-6RU/Y	17.7	91.5	14.9	92.9
CO04013-1W/Y	20.2	92.7	20.5	89.9
CO04021-2R/Y	20.7	93.1	16.5	92.6
CO04022-1R/Y	24.2	92.0	18.6	93.5
CO04023-3R/Y	22.1	91.0	19.3	92.8
CO04029-3RW/Y	17.3	89.4	17.7	94.1
CO04029-5W/Y	16.4	89.7	16.4	95.2
CO04067-10W/Y	22.2	92.0	18.7	90.8
CO04067-8R/Y	21.5	91.1	22.7	90.9
CO04099-3W/Y	26.1	90.8	23.8	90.2
CO04099-4W/Y	26.1	91.7	20.8	91.1
CO04117-5PW/Y	22.5	93.6	23.1	92.6
CO04155-2R/Y	20.0	94.3	21.8	93.3
CO04159-3R/Y	19.6	91.1	18.4	93.2
CO04188-4R/Y	20.3	94.6	19.5	92.3
CO05028-8R/RY	20.4	61.0	21.0	56.9
CO05030-5W/Y	22.0	92.3	21.8	93.3
CO05035-1PW/Y	18.7	94.1	19.3	93.9
CO05035-5PW/Y	21.6	92.7	27.2	92.1
CO05035-7PW/Y	18.7	94.5	23.0	94.1
CO05035-8PW/Y	20.6	93.6	21.3	93.4
CO05037-2R/Y	21.4	92.9	22.9	91.0
CO05037-3W/Y	22.9	90.9	22.5	92.8
CO05085-5R/RY	21.3	9.6	19.5	37.5
CO05100-1W/Y	14.3	93.6	18.2	93.4
CO05122-1W/Y	16.4	93.1	16.1	92.6
CO05239-1R/Y	23.0	91.2	20.3	92.0
CO06257-2R/Y	20.1	92.7	22.2	93.1
CO07021-2R/Y	28.5	93.2	28.2	93.4

CO07030-1RU/Y	21.0	91.6	18.9	93.3
CO07039-3RU/Y	20.8	90.2	17.1	94.0
CO07041-4RU/Y	19.8	89.8	15.3	94.3
CO07044-2W/Y	25.3	92.7	27.6	91.3
CO07044-3RU/Y	31.0	91.1	31.3	101.1
CO07105-4RU/Y	19.2	88.2	18.9	93.2
CO07109-1W/Y	25.7	90.4	27.4	91.0
CO07114-2RW/Y	25.2	89.6	23.3	91.3
CO07119-1W/Y	26.6	91.4	26.9	90.7
CO07131-1RW/Y	35.7	80.1	40.7	80.1
CO07131-2W/Y	27.2	81.2	31.1	78.8
CO07150-1W/Y	29.3	90.8	30.8	89.8
CO07150-2W/Y	24.1	91.7	26.3	90.3
CO07153-3RW/Y	22.4	90.8	25.6	91.8
CO07249-1RU/Y	21.4	93.2	23.1	92.9
CO07323-2R/Y	17.6	91.0	18.7	91.3
CO07329-1P/Y	25.1	86.5	23.9	91.3
CO07329-5P/Y	20.4	92.3	19.3	92.1
CO07370-1W/Y	17.1	90.1	24.9	93.6
CO08390-1P/P	8.5	327.9	6.2	327.1
CO97232-1R/Y	20.4	92.2	21.7	91.2
CO97232-2R/Y	13.9	93.8	18.5	91.4
CO97233-3R/Y	25.2	92.7	22.3	92.5
CO97237-4RU/Y	21.8	90.4	27.4	92.1
CO97237-5RU/Y	27.6	89.7	27.5	89.2
CO99045-1W/Y	22.8	93.2	25.2	92.0
PA4X137-12	35.7	80.8	38.2	81.4
POR00PG4-1	32.6	87.3	31.1	91.2
POR02PG37-2	26.1	91.7	23.8	91.4
POR04PG11-2	19.3	63.1	17.1	49.8
VC0967-2R/Y	16.8	92.9	17.5	91.4
VC0967-2R/Y	18.0	92.2	16.6	92.8
VC1002-3W/Y	24.4	93.6	29.3	91.5
VC1009-1W/Y	25.9	91.2	24.1	91.2
YDH4X.36*	25.3	82.7		
Agria	24.5	91.4	23.2	93.4
Chipeta	12.7	89.6	13.6	91.8
Inka Gold	26.1	89.6	27.5	92.9
Masquerade	25.6	92.6	25.9	91.1
Purple Majesty	9.8	306.6	7.4	337.8
Rio Grande Russet	8.6	90.4	10.8	90.4
Rose Valley	20.5	88.1	18.3	92.8
Russet Nugget	15.1	91.4	16.6	92.3
Sierra Gold	20.4	91.0	17.9	93.1
Yukon Gold	17.2	94.2	20.9	92.9

* Not included in 2012

Table A.5: Total carotenoid content for potato entries (n=100) in the 2011 and 2012 study.

	Total Carotenoid Concentration			
	(µg of LE[a]/100gfw)		(µg of LE[a]/100gdw)	
Clone/Cultivar	2011	2012	2011	2012
07S018	563	541	2803	2786
07S019	363	360	2068	1838
07S020	381	394	2013	2061
4X91E22	1590	988	8011	5927
AC05175-9PW/Y	281	390	1734	1882
AC06259-5W/Y	446	215	2828	1149
AC06358-1W/Y	271	429	1478	2295
AC06358-2W	537	502	3059	2567
AC06887-4W/Y	493	454	2653	2306
AC06908-1W/Y	484	628	2511	2942
AC07315-1W/Y	565	355	2342	2531
AC97521-1R/Y	435	271	2013	1456
AC99330-1P/Y	510	422	2387	2023
ATC02263-1W/Y	393	409	1896	2072
ATC06277-2P/Y	404	575	1798	2360
ATC06277-3P/Y	464	501	2213	2595
ATX9132-2Y	342	865	2043	4688
BDC691-2-2R/Y	538	540	2986	2377
BDC691-2-3W/Y	572	639	2725	2971
BDC694-3-1W/Y	1054	780	4743	3770
BDC694-3-2W/Y	787	822	3626	3431
BDC694-3-3W/Y	1643	1743	6674	7126
BDC696-1-1R/Y	661	609	2813	2439
BDC696-1-2R/Y	635	787	2946	3642
BDC696-1-3W/Y	609	702	2725	2574
BDC699-1-1R/Y	1720	1781	6610	6968
BDC699-1-2W/Y	2083	1510	7739	5444
BDC701-4-1R/Y	1666	1791	6025	7284
BDC701-4-2W/Y	1612	1961	6352	7714
BDC701-4-3W/Y	1510	2037	6314	8392
BDC701-4-4W/Y	1347	1908	5784	7555
BDC702-1-1R/RY	1985	1483	9574	7514
BDC702-1-2R/Y	1077	1635	4940	5812
BDC704-1-1W/Y	953	1396	3118	4414
BDC712-1-1R/Y	1593	1131	6061	4585
BDC712-1-2R/Y	1265	1130	5355	4561
BDC715-1-1R/Y	1761	2741	6333	9667
BDC715-1-2R/Y	1211	901	4135	3012
BDC724-3-1R/Y	2022	1689	8814	6746
BDC724-3-2W/Y	1391	1792	5602	6614
BDC741-4-1R/Y	1598	2370	5492	7762
BDC741-4-2R/Y	1358	1999	4977	7348
BDC741-4-3W/Y	1358	1867	6108	7985
BDC741-4-4R/Y	1483	1377	6075	5680
BDC747-1-1W/Y	940	1538	3482	5592
BDC758-2-1R/Y	1514	2297	5476	7093

BDC758-2-2RW/Y	974	1869	3571	6417
BDC758-2-3R/RY	1573	2299	6240	9185
BDC758-2-4R/Y	1407	1460	5101	5543
BDC758-2-5W/Y	696	987	2444	3296
BDC758-2-6R/Y	1267	1999	4403	6074
BDC758-2-7W/Y	1703	1705	6865	6474
CO00412-5W/Y	497	414	2249	1805
CO01399-10P/Y	503	280	2382	1530
CO03060-2W/Y	417	293	2172	1686
CO04022-1R/Y	373	247	2239	1420
CO04099-3W/Y	637	393	2790	1813
CO04099-4W/Y	503	424	2124	1923
CO04117-5PW/Y	489	339	2660	2000
CO05030-5W/Y	328	333	1677	1608
CO05037-3W/Y	409	257	2223	1397
CO05085-5R/RY	399	378	2128	1880
CO07021-2R/Y	450	464	2105	2189
CO07030-1RU/Y	233	248	985	1054
CO07044-2W/Y	439	479	1892	2040
CO07044-3RU/Y	778	567	3335	2501
CO07105-4RU/Y	242	234	1119	1146
CO07109-1W/Y	499	500	2458	2453
CO07114-2RW/Y	316	260	1705	1353
CO07119-1W/Y	588	457	3101	2484
CO07131-1RW/Y	1093	1046	5436	4948
CO07131-2W/Y	1277	835	6597	4219
CO07150-1W/Y	550	658	2489	2927
CO07150-2W/Y	560	481	2982	2451
CO07153-3RW/Y	455	369	1907	1674
CO07249-1RU/Y	432	255	1972	1119
CO07329-1P/Y	277	230	1388	1244
CO08390-1P/P	127	16	767	84
CO97233-3R/Y	440	308	2100	1686
CO97237-4RU/Y	319	341	1429	1560
CO97237-5RU/Y	415	347	1709	1430
CO99045-1W/Y	291	377	1408	1733
PA4X137-12	1991	1556	10422	9009
POR00PG4-1	640	785	3198	3958
POR02PG37-2	471	283	2132	1371
POR04PG11-2	459	435	2347	2299
VC0967-2R/Y	297	279	1589	1456
VC1002-3W/Y	487	553	2179	2524
VC1009-1W/Y	432	409	2272	2161
YDH4X.36*	1710		9695	
Agria	462	508	2725	2480
Chipeta	161	197	726	1037
Inca Gold	508	488	2633	2872
Masquerade	366	393	1651	2009
Purple Majesty	148	155	670	725
Rio Grande Russet	126	143	653	790
Rose Valley	231	501	1255	2180

Russet Nugget	284	190	1091	876
Sierra Gold	257	297	1275	1332
Yukon Gold	166	250	785	1139

[a] LE = Lutein Equivalent
* Not included in 2012

Table A.6: An entry list for the 2012 volatile flavor compound analysis. A total of 12 potato entries were chosen for this study, with three market available entries and nine advanced entries.

Clone/Cultivar	Female Parent	Male Parent
ATX9132-2Y	A8611-10	A86102-6
BDC701-4-3W/Y		
BDC758-2-1R/Y		
CO05030-5W/Y	Masquerade	BC0894-2W
CO07044-3RU/Y	AC00550-4RU	PA4X137-12
CO07109-1W/Y	CO99338-3RU/Y	PA4X137-12
CO07119-1W/Y	CO00278-4R	PA4X137-12
Mercury Russet	AC93047-1	Silverton Russet
Inka Gold	89S104-4	Mi Peru
Masquerade	Inka Gold	A91846-5R
Russet Nugget	Krantz	AND71609-1
Yukon Gold	W5279-4	Norgleam

Table A.7: An entry list for the 2012 sensory evaluation. A total of 5 potato entries were chosen for this study, one market available entry used as a check, two tetraploids and two diploids. Steamed and microwaved cooking methods were used.

Clone/Cultivar:	
Yukon Gold	BDC701-4-3W/Y
Masquerade	BDC758-2-1R/Y
CO05030-5W/Y	

APPENDIX B: ANOVA TABLES

Table B.1: ANOVA table for comparison of chroma of 138 potato entries grown in 2011 and 2012. R^2 for the model is 0.97 and CV value is 6.7.

Source	DF	Type I SS	MS	F-value	Pr>F
Variety	137	45814.6582	334.4136	111.64	<.0001
Year	1	12.5930	12.5930	4.20	0.0408
Variety*Year	135	1576.2700	11.6761	3.90	<.0001
Replication	2	5.2637	2.6319	0.88	0.4159
Error	549	1644.4666	2.9954		

Table B.2: ANOVA table for comparison of hue of 138 potato entries grown in 2011 and 2012. R^2 for the model is 0.98 and CV value is 5.96.

Source	DF	Type I SS	MS	F-value	Pr>F
Variety	137	746050.8917	5445.6269	184.80	<.0001
Year	1	51.2966	51.2966	1.74	0.1876
Variety*Year	135	4210.1611	31.1864	1.06	0.3276
Replication	2	18.3652	9.1826	0.31	0.7324
Error	549	16177.9403	29.4680		

Table B.3: ANOVA table for comparison of total carotenoid content of 100 potato entries grown in 2011 and 2012. R^2 for the model is 0.96 and CV value is 19.2.

Source	DF	Type I SS	MS	F-value	Pr>F
Variety	99	196556646.9	1985420.7	85.19	<.0001
Year	1	368765.2	368765.2	15.82	<.0001
Variety*Year	98	13861401.2	141442.9	6.07	<.0001
Error	398	9275345.1	23304.9		

LIST OF ABBREVIATIONS

cwt	hundredweight or centum weight
USPB	United States Potato Board
lbs	pounds
µg	micrograms
g	grams
kg	kilograms
FW	fresh weight
AMD	age-related macular degeneration
RNA	ribonucleic acid
SDE	simultaneous distillation and extraction
SAFE	solvent-assisted flavor evaporation
SPME	solid-phase microextraction
5'-GMP	guanosine-5'-monophosphate
5'-AMP	adenosine 5'-monophosphate
5'-IMP	inosine 5'-monophosphate
MSG	monosodium glutamate
PCA	principal component analysis
GC/MS	gas chromatography/mass spectrometry
CSU	Colorado State University
SLVRC	San Luis Valley Research Center
USDA-ARS	United States Department of Agriculture-Agricultural Research Service
LE	lutein equivalent
ZE	zeaxanthin equivalent

HPLC	high-performance liquid chromatography
UPLC	ultra-high performance liquid chromatography
mg	milligram
mL	milliliter
µL	microliter
nm	nanometer
MTBE	methyl tert-butyl ether
SD	standard deviation
SAS	statistical analysis system
YMC	YMC Co., Ltd.
LSD	least significant difference
DAD	diode array detector
BEH	ethylene bridged hybrid
PDMS	polydimethylsiloxane