DISSERTATION

ANTIOXIDANT PROPERTIES OF COLORADO GROWN POTATOES

Submitted by

Juma S Al-Obeidani

Department of Horticulture and Landscape Architecture

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JUMA S AL-OBEIDANI ENTITLED ANTIOXIDANT PROPERTIES OF COLORADO GROWN POTATOES BE ACCEPTED AS FULLFILING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Adviser

Department Head

ABSTRACT OF DISSERTATION

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Antioxidants are a group of compounds that provide protection against the harmful effects of free radicals and other reactive oxidants when included in the human diet. Free radicals are chemical compounds that have one or more unpaired electrons and can react with a range of biological molecules such as nucleic acid and protein, resulting in cell damage. Many environmental factors may affect free radical production. There is strong evidence that consuming more fruits and vegetables offers protection against degenerative diseases of aging such as cancer and heart disease. It is generally assumed that antioxidants in fruits and vegetables account for these beneficial effects.

Potato (*Solanum tuberosum* L) a member of the Solanaceae family is a cool season crop and a major world food crop. Potatoes were chosen as a model for this research because preliminary results at Colorado State University demonstrated that potatoes accumulate a variety of secondary plant metabolites including vitamin C (ascorbic acid (AA)) and phenolic compounds which strongly contribute to antioxidant activity. Based on this, the objectives of these studies were to characterize the antioxidant content and radical scavenging capacity for advanced potato selections and various cultivars grown in Colorado, to determine the impact of production environment on antioxidant status, and to evaluate antioxidant heat stability for different potato cooking methods. This was accomplished by examining AA in potatoes using a high-performance liquid chromatographic (HPLC) method. Total phenolic compounds (TP)

were measured using Folin-Ciocalteu reagent, and total antioxidant activity was estimated by the Trolox equivalent antioxidant capacity (TEAC) assay.

AA was examined in four Colorado potato genotypes (Russet Burbank, Chipeta, CO 94183-1R/R (red flesh), and CO 94165-3P/P (purple flesh) grown in the Arkansas Valley (AV), the San Luis Valley (SLV), and at Powder Horn (PH) regions of Colorado. The colored flesh genotypes expressed higher AA than others and the warmer lower elevation site (AV) had more AA content than the cooler higher elevation site (PH). These findings provide evidence of the importance of evaluating antioxidant status of potato clones in different sites.

Among cultivars examined for TP and antioxidant activity (TEAC), the colored flesh genotypes (CO 94183-1R/R and CO 94165-3P/P) had higher TP content than the russet skin (Russet Burbank) and the white flesh genotype (Chipeta). In addition, skin periderm had higher TP content than flesh or skin+flesh. Locations and cultivars examined were different in the TP and TEAC. A linear positive relationship existed between TP and TEAC in the examined genotypes ($r = 0.9663$) indicating that TP content and ABTS radical scavenging capacity were strongly related.

Potato tubers occupy a remarkable position in human diets due to the content of vitamins, minerals, and other bioactive compounds. However, the nutritional value and the quality of processed potatoes decrease significantly due to cooking methods. Sixteen Colorado grown potato cultivars were examined for their antioxidant heat stability during cooking using three different cooking methods (microwave, boil, and bake). AA, TP, and TEAC content decreased significantly after cooking in all cooking methods, with baking being the most destructive method.

Knowledge and findings from this research will assist potato breeders in introducing high quality, well adapted, and more nutritive cultivars. While potatoes provide a major source of antioxidants (AA, phenolic compounds ...etc), their role in human health through dietary interventions and providing consumers with nutritionally high value products, will receive increasing attention. Finally the data presented in this research will assist potato marketing and consumer confidence.

Juma S Al-Obeidani
Department of Horticulture
and Landscape Architecture
Colorado State University
Fort Collins, CO 80523
Spring 2005

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Chapter 1
Literature Review

1.1 Origin and importance of potato

The potato is a member of the Solanaceae family. The cultivated potato (*Solanum tuberosum* L) is closely related to the tomato, pepper, and eggplant. Potatoes originated in the Andes mountains of South America specifically Peru and Bolivia and isolated locations in Central America and Mexico. The Incas domesticated and cultivated the potato in pre-Columbian times. They had been growing and eating potatoes for more than 2,000 years before the Europeans came to the new world. The potato was not only a staple food for the Incas, but they also believed it had the power to heal ailments, such as headaches, broken bones, skin disease, and rheumatism (Love et al., 2004). The Incas used the name *papas* for potatoes in general, but had other, colorful names to describe particular types. One with red flesh was called "weep blood for the Incas". Another, tough type was known as the "human head" and "red mother". The Incas had a tendency to select for unusual colors, and the cultivated types spanned the rainbow ranging from the white and yellow to blue, red, brown, and black. (Love et al., 2004). Food production, processing, and storage systems based on the potato, allowed the Incas society to flourish. It also allowed citizens to pursue others interests, such as engineering, art, law, and science (Love et al., 2003). The potato was well suited to the high sierras of western South America, not only because of growing conditions, but also because it could be used as a staple in the diet for most of the year (Stephen et al., 2003).

Potato as a major world food crop is exceeded only by wheat, rice, and maize in world production for human consumption (Ross, 1986). Potato tubers give an exceptionally high yield per acre, many times that of any grain crop (Burton, 1969), and are used in a wide variety of table, processed, livestock feed, and industrial uses (Feustel and Talburt, 1987). The original home of cultivated potatoes, in the South American Andes, still possesses a wide range of wild potatoes, cultivated potatoes, and hybrids of intermediate potatoes at various ploidy levels. But that is not the major world center of potato culture. Most potatoes are grown in temperate climates or the mountains of tropical areas.

Potatoes are a cool-season crop. Yields are highest when average daytime temperatures are about 21°C. Cool night temperatures are important because they affect the accumulation of carbohydrates and dry matter in the tubers. At lower night temperatures, the respiration process is slowed; less dry matter is burned up resulting in more starch storage in the tuber. The optimum soil temperature for initiating tubers is 16-19°C. Tuber development declines as soil temperatures rise above 20°C and practically stops at soil temperatures above 30°C (Stephen et al., 2003).

1.2 Reproduction and genetics of potato

All commercial cultivars are tetraploids, the result of a natural doubling in chromosome number. Although potatoes will sometimes produce seed balls, the seed cannot be used because it is never true to type. Commercially, the potato crop is established from 'seed' tubers'. The potato has the richest genetic resources of any cultivated plant, and these genetic resources are generally easily incorporated into

cultivars. The variation includes not only wild potato species in Andean South America, but also semi-cultivated plants, local land races, and hybrid swarms of cultivated and wild plants (Ross, 1986). The potato has a series of ploidy levels, based on a haploid number of 12, ranging from diploid (2n=24) to hexaploid (6n=72), and including triploids, tetraploids, and pentaploids (Dodds, 1962). The cultivated potatoes are autotetraploid (4n=48); many wild species are diploid, but may range up to hexaploid. The tetraploid cultivated potatoes are not diploidized, so that there are four interchangeable genes at each locus (Ross, 1986).

The potato "seed" of commerce is not true botanical seed, but rather consists of sections of potato tuber with one or more "eyes", i.e. lateral buds (Everett, 1981). The potatoes of commerce are therefore all reproduced vegetatively, as clones. This necessarily means that once a cultivar is produced, it is genetically stable in perpetuity, barring mutation, clonal variation (Shepard et al., 1980) or some other unusual event. It also means that potato clones are especially susceptible to disease transmission via the tuber sections (Ross, 1986).

True potato seed (TPS), is genuine botanical seed, and once was the province of the plant breeder's art. It is now used sometimes in commercial and garden (Page, 1982; Page, 1985; Park, 1989) culture. There are several advantages to TPS, including prevention of disease transmission, storage and shipment convenience, and reduction of acreage used for seed production (Ross, 1986). Potato plants are notorious for sterility, both male and female (Ross, 1986). This causes difficulties in potato breeding. Most commercial cultivars are sterile (Burbank, 1921). 'Russet Burbank' is a male sterile cultivar. 'Russet Burbank' potatoes will not produce viable pollen that will fertilize

potato flowers and thus spread genetic material. One of the major difficulties associated with traditional potato breeding relates to the tetrasomic nature of inheritance in conjunction with high heterozygosity and severe inbreeding depression in parental clones. These difficulties require large populations of individuals for evaluation as potential clones. In addition, the initial selection for many desirable characters can often be inefficient and/or time consuming. The main objectives of most potato breeding programs involve the improvement of specific processing attributes and resistance to pest and diseases, while maintaining or improving traits such as tuber color, shape and yield (Ross, 1986). Red-fleshed clones appeared in proportions suggesting multigenic control of degree of pigmentation. Red-fleshed and purple-fleshed clones were always accompanied by red and purple skin, respectively (Brown et al., 2003). Purple-fleshed progeny were obtained from red x white crosses where the white-fleshed parent harbored the P pigment gene in juxtaposition with the multiplex recessive state of the I gene (Brown et al., 2003).

1.3 Cultivation and use of potato

A potato tuber, from a botanical prospective is actually a modified stem and has all of the internal structures that are characteristic of stem tissue. Growth of a potato plant occurs in several stages: sprout development, plant establishment, tuber initiation, tuber bulking, and tuber maturation. Timing of these growth stages varies depending upon production, management practices, and environmental factors, such as elevation and temperature, soil type, availability of moisture, cultivars selected, and geographic location (Stephen et al., 2003).

The average consumption of potatoes differs widely between countries. Relevant statistical data are given by the Food and Agriculture organization of the United Nations (Table 1.1).

Potatoes for direct consumption should be cooked before eating because of the indigestibility of non-gelatinized starch and the presence of anti-nutritional proteins. Different methods of preparation result in various amounts of nutrient losses e.g. ascorbic (AA) acid: 13% loss during cooking of unpeeled potatoes vs. 41% loss of peeled potatoes (Weber et al., 1998). Potato starch, which is a mixture of amylose and amylopectin (75:25), has specific properties that are different from starch of other sources. Therefore, several industrial applications prefer potato starch, e.g. coating of papers, sizing of cotton, finishing in the textile industry (Treadway, 1975). Potato starch is also used in the food industry, particularly, in pre-gelatinized or modified form. Additional specific applications for potato starch can be foreseen with the development of potato cultivars containing mainly one or the other starch component.

The popularity of potatoes was largely attributed to the fact they were readily adapted to temperate growing conditions and produced more nutritionally valuable food on smaller amounts of land than most other crops. Potato production changed the political climate of Europe and had become a staple crop in Ireland. The processing industry has had a tremendous influence on where, how, and which potatoes are grown in the United States. Idaho produces the largest quantity of potatoes of any state in America. In 2000, about 168,000 ha of potatoes were planted in Idaho, which accounted for nearly 34% of the nation's fall harvested acreage. The production is not only extensive, but also diverse. Over 30 cultivars were produced in growing regions throughout the state. Production

environments range from the high elevation seed areas of eastern Idaho, with 90 to 100 growing days, to areas of the Treasure Valley in western Idaho which have the warmest mean temperatures and up to 180 frost-free days (Stephen et al., 2003).

Colorado's largest vegetable crop is potatoes (26305 ha), grown in both the San Luis Valley and northeastern plains. Colorado's potato industry includes summer potato harvest, third largest in the nation, and fall crop, which typically ranks Colorado as the fifth largest potato-growing state in the nation (www.usda.gov). Also the key for potato industry is the seed potato industry in the San Luis Valley, where seed potato research and production are recognized around the world. The San Luis Valley is located in south central Colorado and is one of the largest productive areas in the United States at a high elevation. Russet cultivars accounted for 82 % of the 26305 ha of all potatoes planted in the San Luis Valley for the 2004 crop. Yellow Flesh cultivars were planted on 11% of the total acreage, Red cultivars were planted on 5% of the total and the remaining 2% of the total was made up of White and Other cultivars (Colorado Agricultural Statistics Service San Luis Valley Research Center - Colorado State University, Colorado Department of Agriculture).

Potato production in developing countries entered a new, rapidly expanding phase in the 1990s. Output surpassed 100 million tons by mid-decade, up from less than 30 million tons in the early 1960s. For the last ten years, potato production has increased at an annual average rate of 4.5%, and area planted at 2.4%. More remarkable is that as potato output continues to expand, the growth rate for area planted and production continues to accelerate (FAOSTAT, September 1998). As a result, the growth rate in potato production has nearly doubled over the last twenty years. According to the latest

FAO data (FAOSTAT, September 1998), potato production worldwide stands at 293 million tons and covers more than 18 million hectares. The major world producers, in order of production, are U.S.S.R, Poland, United States, East Germany, West Germany, and France (Talburt, 1987). In the United States, potatoes are widely grown, but especially in the States of Idaho, Washington, Oregon, Colorado, North Dakota, Wisconsin, and Maine (Jewell, 1988). China has been the world's fastest growing potato producer since 1993 and accounted for more than 80% of the increase in global potato production from 1990 to 2002 (Wang et al., 2004).

Potatoes account for about half of the world's annual output of all roots and tubers, and since the early 1960s, the increase in area planted in developing countries has been higher than for any other major food crop. Annual world production currently totals 293 million tons on 18 million hectares, with China and India accounting for 22 percent of this total. In less than a generation, most of the world's potatoes will be harvested in Asia, Africa, or Latin America, where the potato is becoming an increasingly important source of food and its cultivation provides rural employment and income for the growing population (Huaman et al., 1999).

1.4 Effects of environmental factors on potato yield and chemical composition

The potato plant is basically a starch factory. In fact, over 90% of the dry weight of the potato tuber is a direct result of photosynthesis by which the plant uses sunlight, carbon dioxide and water to produce sugars. Part of this sugar is broken down and used to keep the plant alive in a process called respiration. As temperature increases, the rate of respiration goes up dramatically, while at the same time photosynthesis declines. The net

result is that at high temperatures there is less sugar available to drive plant and tuber growth. In addition, high temperatures promote vine growth and tendency for more of the sugar to go towards the vines at expense of tuber growth.

Environmental stress will affect the growth, and production, and may alter chemical composition of the tubers. Stressed potatoes often exhibit high levels of internal and external defects, as well as lower specific gravity. High temperatures reduce specific gravity by reducing the total amount of sugar available for transport from leaves to tubers, and by reducing the rate of incorporation of that starch into the tuber tissue. Low specific gravity in the stem-end of the tuber is often associated with high levels of reducing sugars. When processed, these potatoes make sugar-end fries that are very poor quality. Stress that occurs during the early part of tuber bulking causes the highest incidence of sugar ends.

One of the most obvious signs that potatoes have been stressed is an increase in the proportion of misshapen tubers, such as pointed ends, knobs and dumb bells. During stress, the low availability of starch and/or nutrients may temporarily stop tuber growth. When growth resumes, it occurs at the site of most active growth. The end result is malformed tubers.

According to Hizukuri, (1985), the influence of elevated growth temperature on potato starch properties leads to decreased phosphate content. Broad environmental compositional variations for potato starch have been reported, although the amylase content was described as being relatively constant (Hasse et al., 1996). Recent work on 12 field grown cultivars of potato (Yusuph et al., 2003) evaluated how UK adapted cultivars grown in the same location, at the same time, and variation in starch

composition would affect gelatinization characteristics. Clearly there are variations in the physiological parameters reflecting the genetic origins of the different cultivars. The author also concluded individual cultivars responded to growth conditions slightly differently.

Reyes et al., (2004) conducted research to study the influence of the environmental conditions on the content and yield of anthocyanins and total phenolics (TP) in purple- and red-flesh potatoes during tuber development in both Texas and Colorado. They reported that in both locations, TP content (mg/100 g tissue) decreased with tuber growth and maturity, while tuber weight (kg), total yield (ton ha^{-1}) and yields (kg ha^{-1}) increased. Longer days and cooler temperatures in Colorado favored about a 2.5- and 1.4-times higher TP content, respectively, than in Texas-grown tubers. In addition, harvesting potatoes at later maturity stages maximized yields while minimizing the glycoalkaloid content.

Northern vigor in potato is also very important in term of yields and production. Plants grown in northern latitudes produce seed tubers that are more vigorous and higher yielding than those grown in southern latitudes. This superiority was due to reduced levels of seed borne diseases and some inherent physiological characteristics of the seed tuber itself. The physical basis of northern vigor was not known until 1986 when a research project was started at the Department of Horticulture Science University of Saskatchewan, Canada, which demonstrated the advantages of producing seed in cool climates over warm climates.

Ortiz et al., (2004) conducted research to determine the importance of the genotype x environment interaction in true potato seed breeding in three Peruvian

locations, San Ramon (warm mid-altitude slopes), Huancayo (cool Andean highlands), and La Molina (coastal desert). He reported that the genotype x environment interaction affected tuber yield in all breeding materials. For example: mean tuber yield of 4x-2x crosses for tuber yield were different when tested in warm and cool locations. Similarly, two clones derived from 4x-4x crosses were among the best 15 hybrids assessed in the two lower altitude locations.

From 1995 to1997, field trials were conducted to study the influence of environmental conditions of regions with different altitudes, cultivar, year and production methods on total polyphenol content (in Agria and Karin variety) and AA (in seven cultivars after five months of storage) in potato tubers (Hamouz et al., 1999). They reported higher total polyphenolic content (46.25 mg/100 g^{-1}) in potato tubers from traditional potato regions of the Czech Republic with higher altitude (cooler and more humid) in comparison with drier and warmer regions of lower altitudes (43.50 mg/100 g^{-1}). However, cultivar influence was more apparent than locality influence in the TP content. This research did not demonstrate dependence of AA content on different ecological conditions, including higher and lower elevations in regions of the Czech Republic.

Vokal et al., (2003) investigated cooking quality and its components (raw and cooked potato appearance, tuber odor after cooking, tuber flavor, durability of tuber color after cooking, flesh firmness and sloughing) in potato tubers grown in various soil climatic conditions of traditional potato-growing production regions (higher elevations) and in warmer, higher yielding, mainly beet-growing production regions (lower elevations). A decisive influence of year was detected, which affected total cooking

quality, raw and cooked potato appearance, odor and flavor. Influence of cultivars was not unambiguous (influence was observed in decisive characteristics, i.e., total cooking quality cooked potato appearance, odor and flavor).

1.5 Storage and chemical changes in potato

At harvest, potato tubers are dormant and will not sprout. As the period of post-harvest storage is extended, tuber dormancy is broken and sprout growth commences. The loss of tuber dormancy and onset of sprout growth is accompanied by numerous biochemical changes, many of which are detrimental to the nutritional and processing qualities of potatoes (Suttle, 2004).

Potatoes tend to sprout during storage after harvesting, but this problem is generally controlled by low temperature storage of treatment of the tubers with chemicals such as maleic hydrazide (Wills et al., 1989). Since the potato is an important source of AA in the total diet, contributing as much as 34% of the total AA intake (Thomas, 1984), it is important that any preservation method used to extend its shelf-life will not markedly effect AA content (Graham et al., 1997). Some loss of AA has been observed in potatoes soon after x-ray irradiation (Malteseva et al., 1967). On the other hand, several studies have reported a reduction in AA content of potatoes following irradiation and storage (Thomas 1984; & Joshi et al., 1990). Graham et al., (1997) reported that storage and cooking, boiling and baking, reduced total ascorbic acid (TAA); however microwave cooking proved to be more destructive than boiling.

Changes in the anthocyanin concentration of colored potato tubers have been investigated in cultivars with colored skin and colored or white flesh. Lewis et al., (1998)

reported that during cold storage (4°C) the anthocyanin concentration in colored tubers increased, whereas tubers stored at higher temperatures did not show this increase.

Brierley et al., (1997) conducted research to analyze soluble protein and free amino acids in tubers of the cv. Pentland Dell after storage at 5 and 10°C for up to 33 weeks. He found that asparagine and glutamine accumulated during late storage, which coincided with an upturn in proteinase activity.

Sprouted potato tubers lose nutritional value, and their increased content of reducing sugar renders them unsuitable for the French fry and chipping industries. Thus, tuber sprouting in storage is a primary concern of the potato industry, and is currently controlled by application of chemical sprout suppressants. Such suppressants are subject to widespread regulation by governmental agencies (Haines et al., 2003).

The effect of cold storage under light or in dark was studied with new-season-harvested cv. Monalisa potatoes. Flavonols were detected after a lag period of 3 days of cold storage. The content ranged from 6 to 14 mg/100 g of fresh weight depending on the cultivar after 6 days of storage. Flavonol induction was higher in fresh-cut potatoes stored under light than in the dark. However, caffeic acid derivatives were not affected (Tudela et al., 2002).

Rivero et al., (2003) conducted research to evaluate the moisture, starch, amylose, ash, fructose, sucrose, glucose and ascorbic acid content in five cultivars of Tenerife potatoes in order to evaluate their variation during storage at 12°C and 90% humidity. The Negra cultivar presented the worst behavior in storage, while the Bonita and Colorado cultivars were the best. The moisture content remained constant for the first 6 weeks; afterwards a decrease was observed. A progressive decrease of starch (dry basis)

over the time of storage was detected, while an increase of reducing sugars occurred. The amylose/amylopectin ratio increased during the first 6 weeks of storage. The concentration of AA decreased markedly during storage, and after the 20th week, the content had decreased by more than 50%. Aqueous extracts of potato peel waste were freeze-dried. High performance liquid chromatography (HPLC) of the freeze-dried extracts revealed that chlorogenic (50.31%), gallic (41.67%), protocatechuic (7.81%), and caffeic (0.21%) acids were the major phenolics. During 15 days storage of the freeze-dried extract, no degradation of phenolics occurred (Rodriguez et al., 1994).

The storage of potato tubers at low temperatures leads to the accumulation of sugars in a process called "low-temperature sweetening". The sugar contents and the activity of acid invertase over several months in tubers of six Japanese cultivars stored at 4°C or 20°C. At 20°C, few changes in sugar contents took place in any of the tubers. When stored at 4°C, three types of changes were observed among the cultivars: (1) increased levels of reducing sugars during storage; (2) a pattern similar to that of type 1, but with 4- to 6-fold lower levels of reducing sugars throughout storage; and (3) increased sucrose, but not reducing sugars. (Matsuura-Endo et al., 2004).

Linnemann et al., (1984) investigated the changes in the content of L-ascorbic acid (AA) glucose, fructose, sucrose and total glycoalkaloids in potatoes (cv. Bintje) stored at 7, 16, and 28°C. The initial amount of L-AA was 8.2 mg/100 g fresh weight; after 12 weeks storage at 7°C it had increased, while at 16 and 28°C it had decreased.

Atlantic, BelRus, Kennebec, and Superior potato cultivars were evaluated for AA, soluble proteins, and sugar content, at harvest, after 6 weeks of storage at 3°C, and after 2 weeks of reconditioning at 25°C. At harvest AA and soluble protein content varied

among the cultivars, with 'Superior' containing the highest AA (154 mg/100 g dry weight). Cold storage resulted in a drastic reduction (±50%) in AA content in all four cultivars. AA also decreased during reconditioning of tubers, but the reduction was less than during cold storage (James et al., 1995). Potato crisps were stored at 2, 20, 30, and 50°C and the loss of AA was determined over 12 weeks, crisps at 2°C showed little loss of AA, but the rate of the loss increased exponentially as the temperature was increased. The rate of loss at 20°C was much slower than at 30°C where 50% had degraded by 6 weeks whereas at 50°C a loss of 50% occurred after 3 weeks, but the level stabilized at about 25% of the original level. A temperature of 20°C appeared to be the maximum for retention of AA during storage (Wills et al., 1990).

HPLC was used to follow the loss of AA in the processing and serving of fortified mashed potatoes. Cumulative losses of AA were: 56% for adding AA to freshly mashed potatoes; 82% storing the flakes 4.3 months at 25°C, and 96% reconstituting mashed potatoes and holding them 30 min on a steam table (Wang et al., 1992). Changes in AA content of tubers during their growth are a matter of great controversy, but all the available literature on the influence of storage on the AA content indicates a decrease (Shekhar et al., 1978). It has been reported that only 15% of AA remained in potato tubers stored for 8 months at low temperature (Nirmala et al., 1984).

During storage after harvesting, potatoes tend to sprout but this problem is generally controlled by low temperature storage or treatment of the tubers with chemicals such as maleic hydrazide (Wills et al., 1989). Irradiation, storage and cooking, both boiling and baking, all significantly ($P<0.001$) affected TAA, AA and DHAA

concentrations. During storage, TAA, AA and DHAA concentrations were significantly ($P<0.001$) reduced (Graham et al., 1997).

1.6 Nutrition changes from cooking potatoes

Potato tubers occupy a remarkable position in human diet, i.e. 150-400 g and more *per capita* per day, mainly as boiled, fried, mashed or in the form of chips and French fry strips (Rogan et al., 2000).

During microwave processing, food quality is one of the most important consumer concerns. The microwave drying of foodstuff gives rise to complicated chemical conversions and reactions. Such reactions can cause degradation of vitamins, lipid oxidation and browning reactions, with the mechanisms being influenced by factors, such as concentrations, temperature and water activity (a_w) (Bruin et al., 1980). The rate of AA destruction was found to increase with increasing a_w and was more rapidly destroyed in a desorption system due to the decrease in viscosity (Labuza, et al., 1977).

After-cooking darkening (ACD) is one of the most widespread, undesirable characteristics of cultivated potato. After-cooking darkening is caused by the oxidation of the ferri-chlorogenic acid in the boiled or fried potatoes (Wang et al., 2004).

Bu-Contreras (2001) studied the influence of heating conditions (40-80°C) and starch on the storage modulus of 'Russet Burbank' (RB) and 'Yukon Gold' (YG) potatoes. He reported that starch content of 'YG' potatoes was higher than that of 'RB' potatoes, but the raw starch granule size distribution of both cultivars was similar. 'RB' samples had the highest storage modulus values (25% higher than raw tissue) when heated in 60°C water for 35 min. In addition, image analysis revealed that the perimeters

of starch granules in the potato samples increased by about 50% during heating for 30 minutes at 60°C. Heating temperature and time and starch content influenced the storage behavior.

Domestic cooking such as boiling, microwaving, and frying provoked a partial loss of the flavonols, which were retained in the range of 4-16 mg per serving (213 g). Steam-cooking resulted in the highest retention of caffeic acid derivatives and aromatic amino acids compared with the other cooking methods studied. This means that due to the large amount of potatoes consumed in the Western diet, fresh-cut potatoes can be a significant source of health-promoting phenolics (Tudela et al., 2002).

Nourian et al., (2003) studied the kinetic changes in cooking quality (texture and color) of potatoes stored at different temperatures (4-20°C). He reported a progressive softening in cooked potatoes with the passage of storage time. The most and the least changes were observed at the two extreme storage temperatures 4 and 20° C.

Takatsuki et al., (2004) reported that acrylamide concentrations were found in baked potato, asparagus, pumpkin, eggplant and green sprouts. Concentrations of AA in potato, asparagus and green sprouts baked after being precooked by microwave irradiation were higher than those in the products baked without being precooked. On the other hand, precooking by boiling reduced the production of acrylamide by baking to 1/10-1/4. Acrylamide was not found in microwaved or boiled vegetables.

The prime justification for using antioxidants in frying oils is to delay lipid oxidation during frying. Antioxidants also play an important role in the storage of fried foods, as they retard oxidation and prolong the shelf life of the product (Hamama et al., 1991). Che Man and Jaswir (2000) studied the effect of rosemary and sage extracts on the

physicochemical changes in refined, bleached and deodorized palm oil during deep frying. The results indicated that both spices retarded oil deterioration and prolonged the storage life of potato chips.

1.7 Stability and concentration of AA in potato

Humans are unable to synthesize L-ascorbic acid (L-AA, ascorbate, AA), and are thus entirely dependent upon dietary sources to meet needs. In both plant and animal metabolism, the biological functions of L-ascorbic acid are centered on the antioxidant properties of this molecule (Mark et al., 2000). L-AA is stable when dry, but solutions readily oxidize, especially in the presence of trace amounts of copper, iron and alkali. In both plant and animal systems L-AA interacts enzymatically and non-enzymatically with damaging oxygen radicals and their derivatives, so-called reactive oxygen species (ROS). The biological importance of the antioxidant behavior of L-AA is unlike that of other low-molecular-weight antioxidants (α-tocopherol, uric acid, carotenoids, flavonoids, etc), L-AA is able to terminate radical chain reactions by conversion to non-toxic, non-radical products, i.e. DHA and 2, 3-diketogulonic acid. Indeed, one of the most important features of the non-enzymatic antioxidant activity of L-AA, is its involvement in the regeneration of the lipophilic, membrane-associated α -tocopherol (vitamin E, a-chromanoxy), radical (Mark et al., 2000).

AA is important in the human diet as an essential vitamin and as an antioxidant. Potatoes are a valuable source of AA. A single 148 g uncooked potato provides 45% of the average adult daily requirement of AA (Pennington and Wilkening, 1997). This is a

lower value than fruits and vegetables such as bell peppers, cabbage, broccoli, and kiwifruit. But given the high consumption rate for potatoes, 180 g per day in the United State in 1999 (National Potato Council, 2001), potatoes are one of the most important sources of AA in the human diet. Cooking has a direct impact on the AA concentration in consumed food. Analysis of AA in potato after various methods of preparation has been the subject to numerous studies. Published retention values of AA compared to raw potatoes depends on cooking methods and ranges from 54% to 82% for blanched French fries, 53 to 80% for preformed patties, 51% to 81% for baked tubers, 88% for microwaved peeled chunks, 85% for boiled chunks, 25% to 33% for mashed potatoes (Ortiz et al., and Augustin et al., 1979a, 1979b, Wang et al., 1992). Augustin et al., (1975) reported AA concentration of eight potato cultivars ranged from 7.9 to 36/100 g of fresh weight.

In Australia, it is estimated that potatoes provide approximately 25% of AA, while it has been calculated that, in the United Kingdom, with an estimated consumption of 200 g day^{-1}, over 30% of the intake of AA from fruit and vegetables comes from potatoes (Finlay et al., 2002).

1.8 Antioxidants in human health

The effect of food factors on health status has been recognized since antiquity. More recently, epidemiological studies have led to fundamental research for unraveling the chemistry and mechanism of action of dietary phytochemicals and bioactives. Functional foods and natural health products encompass a wide range of food ingredients, with a variety of bioactives responsible for their efficacy in health promotion and disease

prevention. Phenolic and polyphenolic compounds constitute an important class of secondary plant metabolites that act as free radical scavengers and inhibitors of LDL cholesterol oxidation and nucleic acid breakage, among others. Thus, the role of food phenolics and polyphenolics in the prevention of cardiovascular disease and certain types of cancer is well recognized (Shahidi, 2004).

Antioxidants are a group of compounds that provide protection against the harmful effect of free radicals and other reactive oxidants. Free radicals are chemicals that have one or more unpaired electrons and can react with a range of biological molecules such as nucleic acid and protein, resulting in cell damage. Free radicals are generated in our body all the time as a by-product of breathing oxygen, exercising and breaking down food for energy. Antioxidants have an important role in helping to prevent undesirable changes in foods (Lister, 1999).

Reactive oxygen species (ROS) include such compounds as superoxide ($O^{\bullet}_2$). singlet oxygen ($_1O_2$), hydrogen peroxide (H_2O_2), and the highly reactive hydroxyl radical ($OH^{\bullet}$) Chloroplasts, as well as mitochondria and peroxisomes all produce ROS as by products of normal cellular metabolism, but this production is enhanced by a variety of environmental stresses, including drought, starvation, wounding, high salt, high light, exposure to pollutants (ozone), etc. The toxicity of ROS is considered to be due to their ability to initiate radical cascade reactions that lead to the production of the hydroxy radicals and other destructive species, which can cause protein, damage, lipid peroxidation, nucleic acid damage and finally cell death. Aerobic organisms have therefore developed a range of efficient mechanisms to detoxify these species by both enzymatic and non-enzymatic means (Koutroubakis et al., 2004).

There is strong evidence that a diet rich in fruit and vegetables, offers protection against degenerative diseases of ageing such as cancer and heart disease. Various antioxidants, such as carotenoids, flavonoids, and AA and E, present in fruit and vegetables may be responsible for these protective effects (Lister, 1999). Oxidative stress and depletion of antioxidants may play a key role in the pathogenesis of inflammatory bowel disease (IBD)-related intestinal damage (Koutroubakis et al., 2004).

1.9 Antioxidants in potato

The term antioxidant is not defined by any international accepted definition. Chipualt, (1962) defined antioxidants in foods as "substances that in small quantities are able to prevent or greatly retard the oxidation of easily oxidizable materials such as fats". Another widely used definition, which covers all oxidizable substrates, i.e. lipids, proteins, nucleic acid and carbohydrates suggested by (Halliwell et al., 1989) is "any substance that when present in low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substance".

In recent years, the physiological function of foods has received much attention due to the increasing interest in human health and has been studied in vitro and in vivo by many researchers. The antioxidative action, one of the important physiological functions of foods, is supposed to protect living organisms from oxidative damages, resulting in the prevention of various diseases such as cancer, cardiovascular diseases, and diabetes. Antioxidants can minimize or prevent lipid oxidation in food products. Preliminary studies conducted at Colorado State University have shown that potatoes contain important antioxidants properties derived from the phenolic compounds.

Alsaikhan et al., (1995) reported that antioxidant activity in potato compared with that of broccoli, onion, carrot and bell pepper was higher than all except broccoli. He also reported that the activity varied among potato cultivars, but was not related to flesh color or total phenolics. Antioxidant activity was evenly distributed within tuber parts and/or sections, except for skin tissue, which had the greatest antioxidant activity and TP content. TP varied among cultivars, with some containing two fold higher concentrations than other cultivars. He concluded that phenolic content differences were genotype dependent and not related to flesh color.

Oxygen radical absorbance capacity (ORAC) and ferrous reducing ability (FRAP) of plasma revealed that the antioxidant levels in the red or purple-fleshed potatoes were two to three times higher than white-fleshed potato (Brown et al., 2003). TP increased to 60% with a parallel 85% increase in antioxidant capacity due to wounding stress in purple flesh potatoes (Reyes et al., 2003).

Determination of free phenolic compounds in potato samples was optimized using a high-performance liquid chromatographic (HPLC) method. This method was applied to samples of four cultivars of potatoes harvested in Tenerife (Canary Islands). The free phenolic compounds found in the potato samples were (+)-catechin, chlorogenic acid, caffeic acid, p-coumaric acid, and ferulic acid. Potato samples belonging to Colorado cultivar andigena, had mean concentrations of total phenolic compounds and chlorogenic acid higher than those found for Kerr's Pink and Cara cultivars, ssp. tuberosum, and for Negra cultivar, S. x chaucha (Mendez et al., 2004).

Increased consumption of fruits and vegetables containing high levels of phytochemicals has been recommended to prevent chronic diseases related to oxidative

stress in the human body. Chu et al., (2002) reported broccoli as the highest in TP content, followed by spinach, yellow onion, red pepper, carrot, cabbage, potato, lettuce, celery, and cucumber. Red pepper had the highest total antioxidant activity, followed by broccoli, carrot, spinach, cabbage, yellow onion, celery, potato, lettuce, and cucumber.

Freeze-dried aqueous extract of potato peel powder showed strong inhibitory activity toward lipid peroxidation of rat liver homogenate induced by the $FeCl_2$-H_2O_2 system. Furthermore, potato peel powder exhibited a strong concentration-dependent inhibition of deoxyribose oxidation. Potato peel powder also showed a considerable antioxidant activity in the DPPH radical assay system. The multiple antioxidant activity of potato peel powder was evident as it showed strong reducing power, superoxide scavenging ability and also ferrous ion chelating potency. The data obtained in the in vitro models clearly establish the antioxidant potency of freeze-dried extract of potato peel. Considering that potato peels are discarded as waste and not effectively utilized, these in vitro results suggest the possibility that potato peel waste could be effectively employed as an ingredient in health or functional food, to alleviate oxidative stress (Singh et al., 2004).

Khraisheh et al., (2004) conducted research to evaluate the quality and structural changes in potatoes during microwave and convective drying. He reported that AA deterioration demonstrated first-order kinetic behavior, and was further found to depend on air temperature, microwave power and moisture content. Love et al., (2004) investigated 75 clones from 12 North American potato-breeding programs grown in Idaho, Oregon, and Washington in 1999 and 2000. They reported differences among

clones and clone by environment interaction. Concentration of ascorbic acid of the clones was continuously distributed over a range of 11.5 to 29.8 mg/100 g.

Zarzecka et al., (2003) investigated the effect of herbicide applications on the content of AA and glycoalkaloids in potato tubers. They found that the application of herbicides to potato fields caused an increase in the content of AA in comparison with the control.

Orsak et al., (2001) reported that potato tubers contained in average 300-680 mg AA/kg^{-1} dry matter. UV-C and gamma -irradiation decreased AA content in dry matter of potato tubers (Orsak et al., 2001).

Wills et al., (1990) reported that potato crisps stored at 2°C had little loss of AA, but the loss rate increased exponentially at the temperature was increased. The rate loss at 20°C is much slower that at 30°C where 50% had degraded by 6 weeks where as at 50°C a loss of 50% occurred after 3 weeks. A temperature of 20°C appeared to be the maximum for retention of AA during storage.

1.10 Analytical methods to characterize antioxidants in potatoes

Due to the wide application of phenolic antioxidants in the food, pharmaceutical, and chemical industries, various methods have been developed to define structure-activity relationships for antioxidants. Among the methods that have been developed to estimate the radical-scavenging activity, assays based on the scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) are the most popular ones. In addition, TP using Folin-Ciocalteu reagent adapted from the method of Spanos and Wrolstad (1990).

Quantification of TP content by the Folin-Ciocolteu method is based on the number of phenolic groups or on other potential oxidizable groups present in compounds in the sample (Singleton et al., 1999). TP content is often used to explain antioxidant activity; however, it is unlikely to account for antioxidant capacity and activity (Amarine et al., 1980). More detailed identification of phenolic compounds in foods and diets would facilitate the quantification of antioxidant capacity of discussion of the potential antioxidant capacity. It is necessary to extract polyphenolic compounds effectively when antioxidant capacities are measured. In general, ethanol or methanol solutions containing some water are more efficient in the extraction of polyphenolic compounds than water, pure ethanol or methanol (Katsube et al., 2004). TP in an extract or liquid samples are measured using Folin-Ciocalteu reagent, adapted the method of Spanos and Wrolstad (1990). This assay is based on the color reaction of phenolics with a phosphomolybdic-phosphotungstic acid reagent (e.g. Folin-Ciocalteu reagent).

The electron transfer antioxidant capacity is commonly quantified by the TEAC (Trolox Equivalent Antioxidant Capacity) assay and the FRAP (Ferric Reducing Antioxidant Power) assay. The TEAC assay relies on the reduction of the colored cation radical of 2,2'-azionobis(3-ethylbenothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) and the antioxidant capacity is quantified as the concentration (in mM) of the water soluble-vitamin E analogue, Trolox, that produce the same effect as the test sample (Re et al., 1999). The FRAP assay measures antioxidant capacity by the reduction of the ferric tripyridyltriazine complex to the blue ferrous complex (Benzie et al., 1999). However, the reducing capacity measured does not necessarily correlate with actual antioxidant capacity (Frankel et al., 2000).

The so-called $ABTS^{\bullet+}$ assay is a relatively recent one, which involves a more drastic radical, chemically produced, and is often used for screening complex antioxidant mixtures such as plant extracts, beverages, and biological fluids. The excellent spectral characteristics, the solubility in both organic and aqueous media, and the stability in a wide pH range raised the interest in the use of $ABTS^{\bullet+}$ for the estimation of the antioxidant activity of pure compounds, both lipophilic and water-soluble (Nenadis, et al., 2004).

The $ABTS^{\bullet+}$ assay has been applied in beverages and foods as well as in biological fluids. The radical cation of the $ABTS^{\bullet+}$ has a characteristic absorption spectrum, with maxima at 414, 645, 734 and 815 nm. The ABTS assay was originally designed as an inhibition assay in which antioxidants are added to the medium before the radical is formed. This strategy has the drawback that antioxidants in the sample may interact with reagents, as occurs with the polyphenol quercetin, the activity of which is overestimated (Villano et al., 2004). For this reason, Cano et al., (1998) developed a post-addition assay in which the $ABTS.^{+}$ radical is generated first; then, the sample is added, which decreases the radical concentration. Some advantages of this method are that high temperatures are not required to generate $ABTS^{\bullet+}$ radicals, the commercial peroxidase used does not need previous purifications, and the reaction is recorded at the optimal wavelength of 414 nm, which in turns yields better detection limits.

$ABTS^{\bullet+}$ is stable at room temperature but is unstable above 35°C and/or at pH values of above 7.5. Nevertheless, the most important factor in its stability is the $ABTS/ABTS^{+}$ concentration ratio in the medium. The radical reacts with the antioxidant,

L-AA, with a high rate constant, the stoichiometry of the reaction being 1 mol of L-AA per 2 mol of $ABTS^{\cdot+}$ reduced (Cano et al., 1998).

Antioxidant activity also depends on the time at which the decrease in absorbance is measured. Some authors use a fixed time point (Pellegrini et al., 2001), (Gil et al., 2000) and (Buratti et al., 2001) and others monitor the reaction kinetics (George et al., 1997), (Berg et al., 2000), (Marino et al., 2001) and (Jesus et al., 2002). In both cases there is a lack of consensus about the optimal time. Several authors have obtained different figures (expressed as Trolox equivalent antioxidant capacity (TEAC)) for antioxidant activity depending on the time of absorbance measurement.

1.11 Importance of this research

Potatoes provide a proven source of good-quality protein and energy. They are also an important source of vitamins and minerals such as calcium, potassium, and phosphorus, and their value in the human diet is often underestimated or ignored, particularly as a source of AA. There is an increasing interest in the role of various antioxidants in our diet, in fruit and vegetables, and also in the various environmental factors that can affect these levels (Finlay et al., 2003). The cultivated potato is one of the most important world food crops and demands continued genetic improvement to meet the needs of a changing world. Even though potato breeding has been a cumbersome task due to inherent biological factors (Douches, 1996), considerable progress has been made in development of many new and superior cultivars.

Long-term storage of potatoes is an important aspect of marketing potatoes for processing. Maintenance of low levels of reducing sugars in the potato tuber is important

for acceptable processing qualities and different nutrition values. Different methods of cooking potatoes will affect the chemical composition of the tissue. Therefore it is important to study the best cooking methods and temperatures that will preserve the nutrition benefits of potatoes for human health.

There is limited published data on the extent of variation with regard to antioxidant content and its stability within potatoes cultivars. Therefore the main objectives of this research are:

1. Characterize the antioxidants and radical scavenging capacity in various potato cultivars in different Colorado locations..
2. Determine the effects of genotype and production environment interaction on antioxidant content.
3. Evaluate antioxidant heat stability for different cooking methods and selected cultivars.
4. Characterize AA content in selected potato cultivars.

The findings from this research will play an important role to assure introduction of high quality and well adapted new cultivars for Colorado producers and will add important information that focuses on human health. The data from this research will assist marketing and processing of potatoes. It will improve breeding opportunities and will determine the optimum environmental and storage conditions that can maximize health benefits of new cultivars.

Table 1.1 Average potato consumption in 1998 (FAO, 2001).

	Potato consumption (kg/Cap/Year)
World	30
Africa	11
Asia	19
Europe	94
EU	78
North America	63
South America	31
Developing countries	17
Developed countries	75

Chapter 2

Effect of Production Location on Ascorbic Acid Content of Selected Colorado Grown Potato Cultivars

2.1 Abstract

Vitamin C (Ascorbic acid, AA) is a potent antioxidant. It is also important in energy production, essential for collagen and bone formation, and a powerful immune booster. Potatoes are a very good source of AA and provide up to 25% of the daily requirement in some European diets. Only limited data has been published on AA content, its stability, and effect of production environment in potato cultivars. This study examined the availability of AA in four Colorado potato genotypes: Russet Burbank (RB), Chipeta (Ch), CO 94183-1R/R, and CO 94195-3P/P grown at 3 Colorado locations: Arkansas Valley (AV), San Luis Valley (SLV), and Powder Horn (PH). The data were subjected to 2-way ANOVA. Results showed differences due to interaction between locations and cultivars ($P<0.0001$, $F=5.824$). AA content among locations ($P<0.0001$, $F=111.2$), and cultivars/tissues were different ($P<0.0001$, $F=6.922$). The highest AA content was detected in 'CO 94183-1R/R' grown in the AV location (42.01 mg/100gfw in skin+flesh tissue), followed by 'Chipeta' grown in SLV (23.65 mg/100gfw in skin+flesh) and in 'CO 94165-1P/P' grown in PH (22.14 mg/100gfw in flesh). AA content was lowest in 'RB' in AV for skin+flesh tissue (23.57 mg/100gfw), SLV (16.40 mg/100gfw in flesh tissue), PH (12.89 mg/100gfw in skin+flesh tissue).

The evidence presented in this research suggests that maximum AA content in potatoes can be attained by selecting and developing potato genotypes that are best suited for different climatic production regions. Potato breeders can expect to find differences among genetic selections and to produce new cultivars that are well adapted to specific environments and climates. However, more research is also needed to study the maximum expression and stability of AA among diverse production environments.

2.2 Introduction

Potato is the fourth largest food crop after wheat, corn and rice and is produced in nearly all countries in the world. Cultivation differs widely among climatic regions, and the use of irrigation, permits fresh potatoes to be marketed throughout the year. Supply is usually plentiful in fall and limited in spring (Will et al., 1991). 'Russet Burbank' is one of the major cultivars used for French fries in the North American potato producing areas of Canada and USA (Coffin et al., 1997).

AA is an essential component of the human diet and most of our daily requirement is derived from fruits and vegetables (Smirnoff et al., 2001). Van et al., (1990) estimated that potatoes supply about one-fourth of the daily human requirement for AA, and that 100 gm of freshly harvested potato can meet 40% of the daily human requirement of AA.

AA has been reported to play multiple roles in nutrition, human health, and food chemistry. Dietary deficiency of AA causes the human disease scurvy due to formation of abnormal collagen resulting in skin and gum lesions and fragility of blood vessels. AA is widely reported to protect both plants and animals against oxidative stress induced by

potentially toxic as well as cancer and teriosclerosis-inducing reactive oxygen species (ROS) including hydroxyl radicals, superoxide anions, singlet oxygen, and hydrogen peroxide The recommended dietary allowance (RDA) of AA ranges from 30 to 120 mg (Sook-Han et al., 2004).

AA is found in all plants in practically every compartment of the cell (Arrigoni et al., 2000). There has been a resurgence of interest in AA due to the increasing evidence of the importance of AA in both redox-associated and developmental processes and also to the recent determination of a complete biosynthetic pathway in plants (Wheeler et al., 1998). The broad distribution of AA implicates a role of this compound in a wide range of physiological phenomena. Best studied among these functions is its role in redox processes during photosynthesis, environment-induced oxidative stress (ozone, UV, high light, SO_2, etc.), and during wound- and pathogen-induced oxidative processes (Davey et al., 2000).

Changes in AA content of tubers during their growth are a matter of controversy. But all the available literature on the influence of storage on AA content indicates a decrease. It has been reported that only 15% of AA remained in potato tubers stored for 8 months at low temperature (Kumari et al., 1990). The reported content of AA in potatoes ranges from 8 to 30 mg/100 g of fresh weight (Sook Han et al., 2004). Changes in AA content of tubers during their growth are a matter of considerable controversy. But all literature on the influence of storage on AA content indicates substantial losses occur over storage time (Shaker et al., 1978). James et al., (1995) reported that AA losses could vary by up to 50% under different storage temperatures and durations. There is limited published data on the extent of variation with regard to antioxidant content and its

stability within potato germplasm. A review of the nutritional status of potatoes reported a range of variation for AA content from 15 to 25mg/100 g fresh weight (Storey et al., 1992). AA content ranged from 84 to 145 mg/100 g dry weight basis has been reported (Augustin, 1975). However, AA in fresh foods, including potatoes, is not stable, with levels decreasing with post-harvest storage time to 30-60% of the original content within the first 2 months of storage and subsequently stabilizing at about 25% of the original level (William, 1951). Love et al. (2004) reported AA concentration range of 11.5 to 29.8 mg/100 gfw in 72 clones from 12 North American potato-breeding programs. Augustin et al., (1978) reported AA concentration of 8 cultivars ranged from 7.9 to 36.1 mg/ 100gfw.

In a research report submitted to the San Luis Valley Colorado Potato Research Committee (Stushnoff, 2003) reported AA content dropped from 31.86 to 16.76 mg/100gfw for 'Russet Burbank', 27.87 to 15.9 mg/100gfw for 'Chipeta', 29.47 to 19.63 mg/100gfw for 'CO 94165-3P/P', and 26.28 to 17.10 mg/100gfw for 'CO 94183-1R/R' after four months storage. For the purpose of this research the same four cultivars were analyzed for AA content grown at 3 Colorado locations.

Because of the significance of AA as a quality index for fresh and processed food, as a vitamin factor and as an antioxidant, a quick and routine method of its estimation is still of great interest. HPLC analytical methods are now replacing the traditional titrimetric and colorimetric methods based on the reducing properties of AA (Rizzolo et al., 1984). However HPLC methods can be compromised by interference due to other reducing substances or end point determination, especially with colored samples. This new instrumental technique is now becoming a good alternative to other modern

analytical methods such as GC (Schlack, 1974), polargraphy (Jadhar et al., 1975), enzymatic techniques (Kelly et al., 1981) and microfluorimetry (AOAC, 1975).

Different procedures of HPLC techniques have been developed over the last few years using HPLC, namely on both weak (Mai-Huong, 1980; Dennison et al., 1981; Geigert et al., 1981) and strong (Pachla et al., 1976; Marsili et al., 1981; Busling et al., 1982) ion-exchange columns and on reversed phase even ion-paired columns (Moledina et al., 1981).

According to previous observations, AA was easily separated within a very short time on a column packed with an NH_2-bonded phase acting as a weak anion-exchanger, eluting either with phosphate or acetate buffer solutions (Giangiacamo et al., 1983). However, such stationary phases showed a slight solubility in the aqueous mobile phases, resulting in a reduced efficiency and stability. Therefore, a porous silica-based strong exchanger that is more stable and can withstand buffer salt changes over a wide range of pH and high temperature without suffering a loss of column efficiency should be used.

Potato tubers contain L-ascorbic acid and dehydroascorbic acid (Keijbets et al., 1991), both of which can be determined either separately or as a combined value by the reduction of dehydroascorbic to AA prior to analysis (Sinden et al., 1978). In the potato, dehydroascorbic acid accounts for considerably less than 10% of the total ascorbate content of the tubers, even after prolonged storage, and indeed the value for dehydroascorbate frequently falls within the standard deviation value for AA content (Keijbets et al., 1991). Consequently, the total ascorbate content of tubers is an excellent approximation of the AA value of potatoes. The objective of this research is to

characterize the effect of the production locations on the AA content in different Colorado grown genotypes.

2.3 **Materials and methods**

2.3.1 Experimental procedure and environmental characteristics

For the purpose of this study four potato cultivars (Russet Burbank (RB) (russet skin/white flesh), Chipeta (Ch) (white skin/white flesh), CO 94183-1R/R (red skin/red flesh), and CO 94165-3P/P (purple skin/ purple flesh) were grown in 2002 and 2003 at three Colorado locations. These are Arkansas Valley (AV), Powder Horn (PH), and San Luis Valley (SLV). AV is located in southeast Colorado (1274 m), with loamy soils, hot days, warm nights, high plains, a very long season, and limited regional potato production. PH is located in southwest Colorado (2339 m). It has the highest elevation, cool days, cool nights, a short production season, and is considered a specialized potato production site. SLV is considered as the number one potato production site in Colorado. It is located in south central Colorado. The valley is a high mountain location (2348 m) with sandy soil, warm days, cool nights, moderate season, and an ideal climate for irrigated potato production.

Climatologic weather data were collected for May to August for 2002 and 2003 seasons using Colorado climate website (CoAgMet) and National Climatic Data Center (NCDC). Rocky Ford station weather data were used to estimate conditions for the AV site. Similarly Gunnison station 3 weather data were used to estimate conditions at PH. These weather stations were considered the closest estimate of conditions at the production sites where no weather stations were present.

2.3.2 Chemicals

AA (A5960) was purchased from Sigma-Aldrich (St. Louis, Mo. 63103). Phosphoric acid used in the column washing process (PX0996-6, EM science, Darmstadt, Germany) was of HPLC grade quality. Phosphate buffered saline (PBS) solutions for sample extraction and standards preparations were made up of 36 mM KH_2PO_4 (7100-03 Mallinckrodt, Paris, Ky.) and 36 mM Na_2HPO_4 (4062-01 Mallinckrodt Barker Inc. Phillipsburg, N.J. 08865). Methanol (MX 0475P-1) of the HPLC grade was purchased from EMD (Gibbstown, N.J. 08027).

2.3.3 HPLC analysis

AA was detected using a Hewlett-Packard 1050 HPLC (Palo Alto, Calif. 94304) equipped with a UV-VIS detector. The separation was run at 1.0 ml/min at 35°C with a column heater using water- soluble vitamin analysis and an Inertsil C_4 column. AA was eluted using a gradient consisting of 100% 50 mM phosphoric acid (pH 2.0) for 5 minutes followed by 85% methanol for 7 minutes at a flow rate of 1 ml/min and the effluent was monitored at 254 nm.

2.3.4 Standard curve preparation

Standard solutions of AA were prepared fresh each day. 10 mg of AA and 100 mg of DTT (dithiothreitol) were weighed and both dissolved in 10 ml of 100% methanol. Five standard curve dilutions were made (Table 2.1) and put in HPLC amber vials and stored on ice.

2.3.5 Sample preparation

Three tubers/cultivar (3 replicates) from each location were selected and the middle portion of each tuber was cut into 3 thin slices, representing skin w/flesh, and flesh only. Slices were freeze-dried using a Virtis Genesis 25 LL freeze dryer (Gardiner, N.Y. 12525). 300 mg of DTT (dithiothreitol) were weighed daily and dissolved in 10 ml 100% methanol (extraction solution). 100 mg of the selected ground freeze-dried potato powder was weighed and put in a 1.7 ml Eppendorf tube. 300 µl of the extraction solution (methanol w/DTT) was added to each tube and vortexed for 15 seconds until a paste formed. 700 µl of 36 mM PBS solution was added and then vortexed for 15 seconds after which the tubes were rotated in the dark for 15 minutes at 4°C. The Eppendorf tubes were centrifuged for 5 minutes @ 4000 RPM at 4°C. The top clear supernatant was poured into clean Eppendorf tubes and kept cool. This process was repeated before syringe filtration. Supernatants from both stages were syringe filtered with 45uM nylon Acrodisks into amber HPLC vials.

2.3.6 Quantification

Quantification was accomplished by comparing integrated chromatographic peak areas from the test samples to peak areas of known amounts of standard AA.

2.3.7 AA recovery and HPLC method validation

Potato extracts were analyzed before and after addition of known amounts of AA. Recovery from 3 potato cultivars in 3 different locations was assessed by spiking with 50 µl and 150 µl at concentrations of 0.05 mg/ml and 0.15 mg/ml respectively (n=6). Then

calculating % Recovery = (S-U) x 100/SP, where S is the spiked sample, U (unspiked sample), and SP (spike standard). The colored (purple/purple) genotype had the highest recovery of 120% in the lower concentration (0.05 mg/ml) and the lowest recovery of 85% in the higher concentration (0.15 mg/ml). Therefore, The validity of the method is supported by the good symmetry of the ascorbic acid peaks, lack of baseline noise in the chromatograms, linear concentration response of integrated peak areas, and >90% recoveries of AA from spiked potato extracts. Table 2.5 shows % recovery for different potato samples and the AA mean retention time ± standard deviation.

2.3.8 Statistical analysis

The collected data were first organized using a spread sheet. The data were then analyzed using GraphPad Prism version 4.00 software (San Diego, Calif., 2003). Two-Way analyses of variance (ANOVA) were carried out and a Bonferroni posttest was used to compare the replicate means. Pearson correlation was conducted to examine the relationship between AA content and environmental factors using GraphPad Prism version 4.00 software (San Diego, Calif., 2003).

2. 4 Results

2.4.1 Linearity of standard curves

The HPLC-UV detection method responded linearly over the concentration of 0.01, 0.05, 0.1, 0.15, and 0.2 mg/ml of AA (Figure 2.3). A linear relationship between peak areas and concentration was obtained using Pearson correlation (r =0.9998) (Fig 2.3). The symmetrical AA peak eluted at ~2.6 min (Fig 2.4).

2.4.2 Effect of location x genotype interaction in AA content

The data collected across years, locations, and genotypes were subjected to 2-way Analysis of variance (ANOVA). The results in Table 2.2 show differences for interaction between locations and genotypes ($P<0.0001$, $F=5.824$) and contributed to 17.26% of the total variance (Fig 2.1).

2.4.3 Effect of locations in the AA content

The dominant effect in AA was due mainly to locations (Table 2.2). There were differences between locations ($P<0.0001$, $F=111.2$) and contributed to 47% of the variation. The means represented in (Table 2.3) illustrate the major significant items identified within the analysis of variance. There was no difference in the amount of AA produced among the 3 sites ($P>0.05$) for the genotypes (CO 94165-3P/P & Ch) for skin+flesh tissue. AA content was the lowest in 'RB' in AV for skin+flesh tissue (23.57 mg/100gfw), SLV (16.40 mg/100gfw in 'RB' flesh tissue), PH (12.89 mg/100gfw in CO 94183-1R/R skin+flesh tissue). While the highest AA content in AV. was is 'CO 94183-1R/R' (42.01 mg/100gfw in skin+flesh tissue), 'Chipeta' (23.65 mg/100gfw in skin+flesh) in SLV, and in 'CO 94165-3P/P' 23.71 mg/100gfw in skin+ flesh tissue for PH. The colored flesh genotype CO 94183-1R/R was different ($p< 0.001$) for all three sites for both tissues (Table 2.3). The other colored genotype CO 94165-3P/P was different ($P<0.001$) in AV vs SLV, and AV vs PH, but not different between SLV and PH (Fig 2.2).

2.4.4 Effect of genotypes in the AA content

Table 2.2 indicates significant differences between genotypes regardless of the tissue used ($P<0.0001$, $F= 6.922$), however they contribute only 10.26% of the total variation.

2.4.5 AA correlation with environmental factors

Pearson correlations (r) were tested for AA contents with tuber weight, heat unit degree days, number of days above the maximum temp. (30°C) and number of days below the minimum (10° C) using GraphPad Prism version 4.00 software (San Diego, Calif.). AA content was not correlated to tuber weight ($r =0.3666$, $P=0.2411$), but it was positively correlated to heat unit degree days ($r =0.7721$, $P=0.0033$), and the number of days above 30°C ($r =0.7602$, $P=0.0041$). AA content was negatively correlated with days below 10°C ($r = -0.6312$, $P=0.0277$) (Table 2.6).

2.5 Discussion

The variance structure illustrated in (Table 2.2) provides critical insights into possible behavior of AA according to the environment and genotypes chosen. Table (2.3) shows that higher AA was obtained from AV followed by SLV and PH respectively. Storey et al., (1992) reported AA content in potatoes ranged from 15 to 25 g/100gfw, however in our study the range was 12.89 to 42.01 mg/100gfw. This 3.0 fold variation from the lowest to the highest value affords the consumer the option of choosing high AA potato cultivars (CO 94183-1R/R and CO 94165-3P/P). Sook Han et al., (2004) reported about 300 g of these cultivars provides the high RDA requirement of

~120 mg of AA. This amount will, however, be reduced after the fresh potatoes are subjected to home-processing conditions to make them edible. Orsak et al., (2001) reported potato tubers had a very high average of 300-680 mg/100gfw of AA, but provided no explanation for these extraordinarily high values. Our values in this research were much lower than those reported, but are consistent with the data reported by Storey et al., (1992) with a higher content in colored genotypes. Because climatic conditions were not defined in the other two reports, and our data show differences among locations we recommend future studies carefully monitor climatic data.

AV with the lower elevation (1274 m), higher accumulative degree days (2791° C), and warm nights among other locations produced the highest AA content 42.01 mg/100gfw in the CO 94183-1R/R genotype. SLV considered the ideal location for growing potatoes in Colorado with higher elevation (2348 m), warm days, and cool nights, came second after AV Finally PH with about the same elevation as SLV (2339 m) but with cool days and nights produced the lowest AA content (12.89 mg/100gfw) in CO 94183-1R/R (Fig 2.5).

Marilyn et al., (1994) reported that potatoes grow well in cool environments. The rate of shoot growth is the highest in the range of 20 to 25°C, whereas maximum tuber yields are produced at lower temperature range of 10 to 20°C. Despite this relatively low temperature optimum for tuber production, potato cultivars are sensitive to cold temperature. For example, the plants exhibit reduced shoot growth at 9°C, do not grow at 6°C and are killed by freezing at -3°C. Table 2.4 shows that the high temperatures and more days above the maximum temperature (heat stress) in both cultivation years for AV might be the reason for the elevated AA content in all genotypes. On the other hand, the

lower temperatures and more days below the minimum (cold stress) in PH might result in the lower AA contents. In addition SLV with more moderate climate (warm days & cool nights) results in less variation of AA content among all genotypes. AA is known to be sensitive to air, heat, and water and can be easily denatured or destroyed by prolonged storage, overcooking, and processing of potatoes (Finlay et al., 2003). Therefore, the storage time after harvest and before processing in the freeze drier may also be another reason for decreasing the AA content in the tubers. In our study tubers were freeze dried as soon as possible after harvest, thus storage should not be a factor influencing differences in AA content.

The high contribution of the locations suggests that selection and breeding genotypes within a specific environment will probably be effective. In addition, genotype x environment interaction indicates that multiple evaluations over different genotypes will be needed to maximize AA. These results may aid potato breeders in selecting new genotypes. This information will also help guide a search for new genotypes that provide maximum health benefits for human consumption, especially cultivars with high AA and other antioxidants.

All potatoes are a good source of complex carbohydrates, potassium, and AA. These colored flesh cultivars are seen as new types of potato for a previously untapped market. In general the colored (CO 94165-3P/P & CO 94183-1R/R) cultivars were higher in AA content (Table 2.3). All results presented in this research were consistent with the preliminary research reported by Stushnoff, (2003), in that all colored genotypes produced more of AA than other white or yellow fleshed genotypes. It's also important to

note that tubers from 2002 were larger than those from 2003. Smaller tubers (size effect) might result in the less AA levels compared to those of larger size.

Table 2.1 Composition of solutions for preparation of the ascorbic acid standard curve.

Concentration (mg/ml)	µl of Ascorbic acid w/Dithiothreitol	µl of 36 mM Phosphate buffered saline
0.01	10	990
0.05	50	950
0.10	100	900
0.15	150	850
0.20	200	800

Table 2.2 Summary of analysis of variance (ANOVA) for ascorbic acid in locations, cultivars, and interaction.

Effect	DF	SS	MS	P-Value	F-Value	% Variation
Interaction (L x C/T)	14	1822	130.1	P<0.0001***	5.824	17.26
Location (L)	2	4967	2484	P<0.0001***	111.2	47.07
Cultivars/Tissue (C/T)	7	1083	154.7	P<0.0001***	6.922	10.26

*** Significant (P<0.0001).

Table 2.3 Means±SE for ascorbic acid content (mg/100gfw) for genotype and locations.

Cultivars/tissue	Arkansas Valley	San Luis	Powder Horn
Russet Burbank (b)	23.57±3.1 ^{a**}	16.73±3.1 ^{ab}	14.22±3.1 ^{b**}
Chipeta (b)	29.22±3.1 ^{a**}	23.65±3.1 ^{ab}	19.06±3.1 ^{b**}
CO 94165-3P/P (b)	30.86±4.4 ^{a}	23.57±4.4 ^{a}	23.71±4.4 ^{a}
CO 94183-1R/R (b)	42.01±2.9 ^{a***}	19.51±2.9 ^{b***}	12.89±2.9 ^{b***}
Russet Burbank (f)	24.36±2.8 ^{a*}	16.40±2.8 ^{b*}	15.87±2.8 ^{b*}
Chipeta (f)	26.89±2.8 ^{a}	22.42±2.8 ^{a}	19.74±2.8 ^{a}
CO 94165-3P/P (f)	33.67±3.8 ^{a***}	19.90±3.8 ^{b***}	22.14±3.8 ^{b***}
CO 94183-1R/R (f)	39.11±2.7 ^{a***}	19.41 b±2.7 ^{b***}	13.90±2.7 ^{b***}

[a] Numbers with the same letter within each row are not significantly different ($P>0.05$) by Bonferroni posttest. * = significant ($P<0.05$), **= significant ($P<0.01$), ***= significant ($P<0.001$). The letter (b) represents the skin+flesh tissue and the letter (f) represents the flesh tissue only.

Table 2.4 Number of days recorded above 30°C and below 10°C in 2002 and 2003.

Location	Number of days above the maximum temp. (30°C)	Number of days below the minimum temp. (10°C)
Gunnison (PH)	1 (2003)	30 (2002)
San Luis valley	6 (2002) & 6 (2003)	7 (2002) & 7 (2003)
Rocky Ford (AV)	60 (2002) & 79 (2003)	2 (2002) & 1 (2003)

Table 2.5 Recovery of ascorbic acid from potato extracts spiked with ascorbic acid.

Sample	%Recovery Ascorbic acid spike 0.05 mg/ml	Mean retention time ±SD	%Recovery Ascorbic acid spike 0.15 mg/ml	Mean retention time ±SD
Chipeta w/s (PH)	107	2.594±0.019	98	2.566±0.002
Russet Burbank w/s (SLV)	103	2.582±0.006	107	2.568±0.012
CO 94165-3P/P w/s (AV)	120	2.586±0.008	85	2.571±0.004

w/s represents potato tuber flesh tissue samples with skin, (PH) Powder Horn, (SLV) San Luis Valley, and (AV) Arkansas Valley.

Table 2.6 Pearson correlation coefficients for Ascorbic acid with tuber weight, and indicators of heat unit exposure for the 3 locations.

Parameter	Tuber weight	Heat unit degree days	Number of days above 30°C	Number of days below 10°C
Pearson r	0.3666	0.7721	0.7602	- 0.6312
P-value	0.2411 ^{NS}	0.0033 **	0.0041 **	0.0277 *
R^2	0.1344	0.5961	0.5779	0.3984

^{NS}=non significant (P>0.05), **=significant (P<0.05), *= significant (P<0.05).

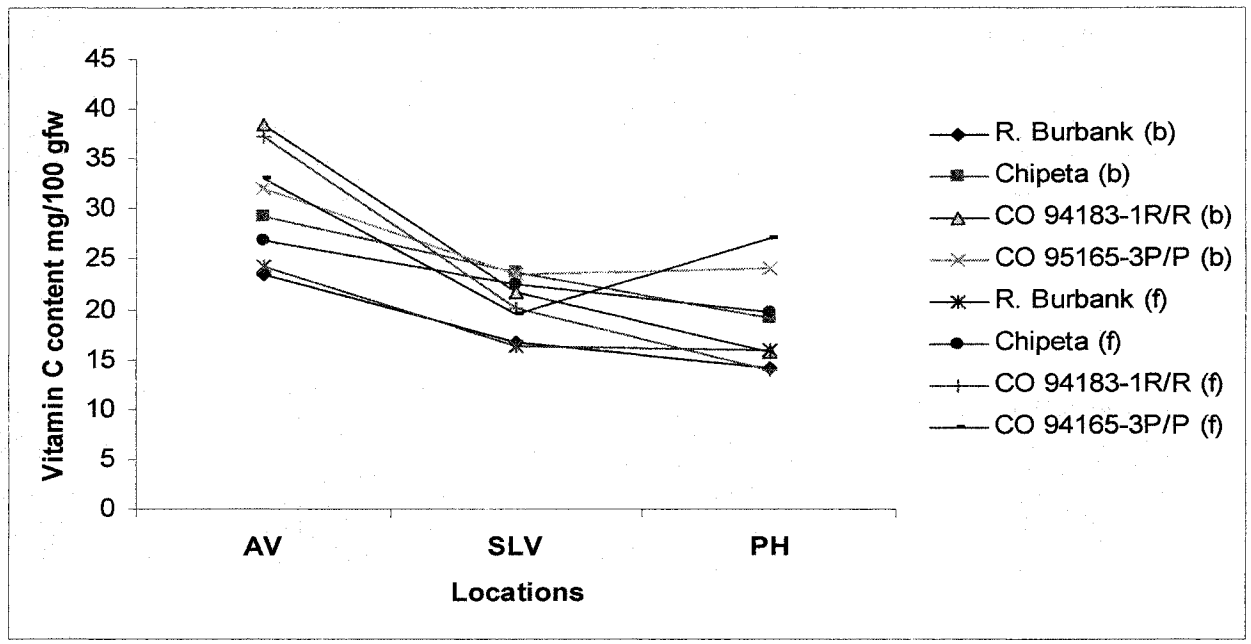

Figure 2.1 Ascorbic acid content (mean ± SE) showing interaction between genotypes x locations. The letter (b) represents tissue with both skin+flesh & the letter (f) represents the tissue flesh only.

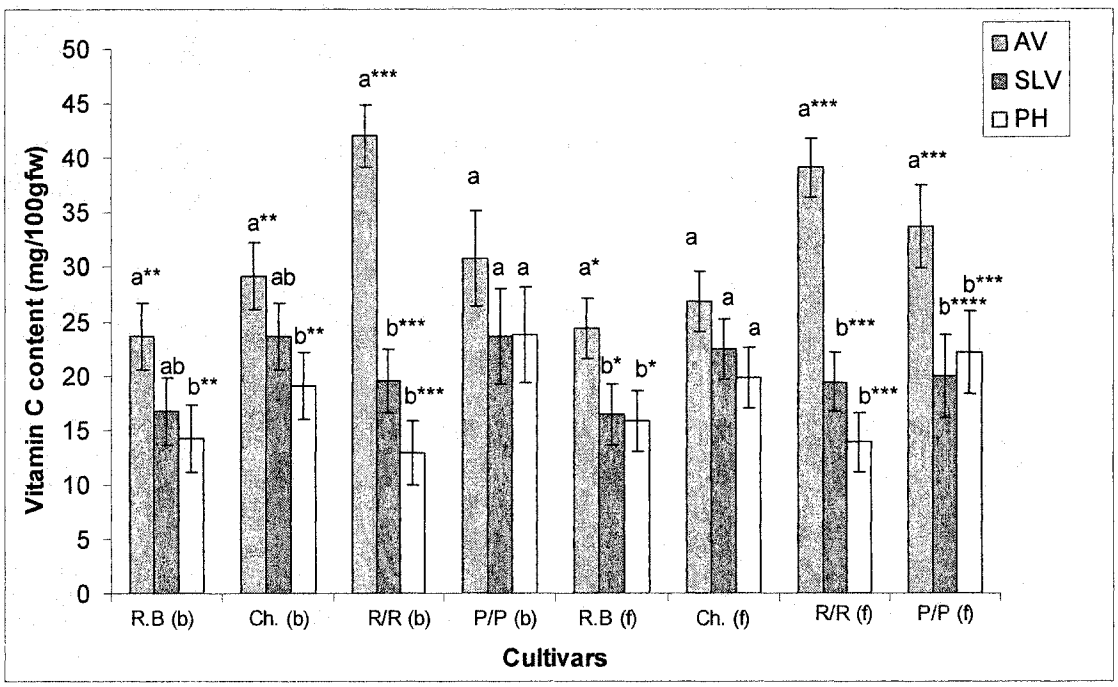

Figure 2.2 Mean ± SE for ascorbic acid content (mg/100mgfw) for three locations (AV, SLV, & PH). R.B represents 'Russet Burbank', Ch represents 'Chipeta', R/R represents 'CO 94183-1RR' red/red, and P/P represents 'CO 94165-3P/P' purple/purple. The letter (b) represents tissue with both skin+flesh & the letter (f) represents the tissue flesh only. [a] Locations with the same letter for each cultivar are not significantly different ($P>0.05$), * significant ($P< 0.05$), ** significant ($P<0.01$), *** significant ($P<0.001$) by Bonferroni post test.

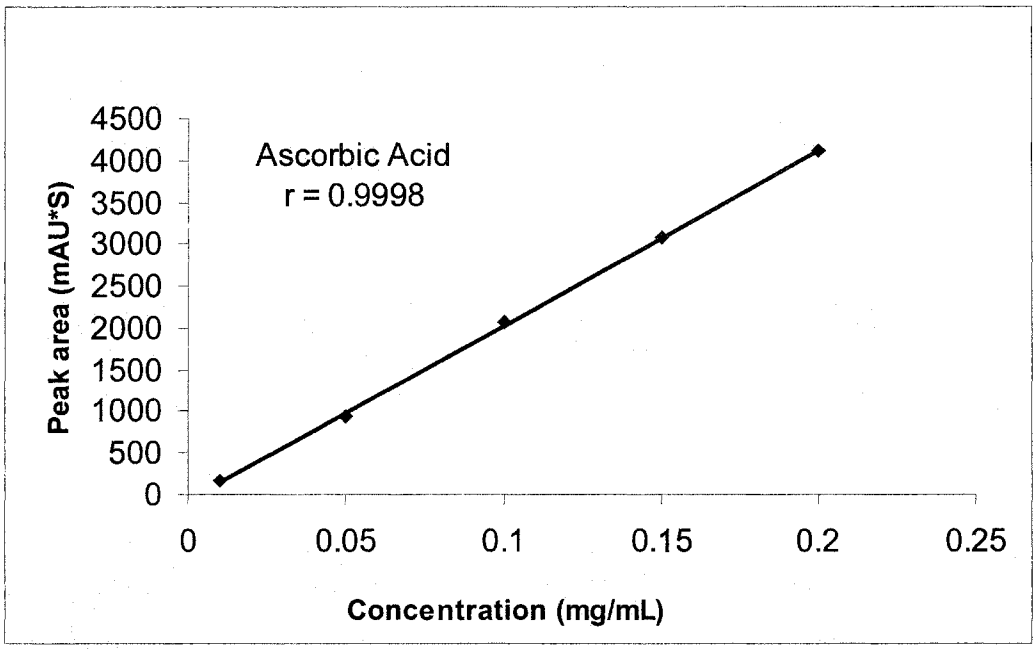

Figure 2.3 HPLC analysis of ascorbic acid: linear relationship between the concentration of AA standards and integrated peaks on HPLC chromatograms.

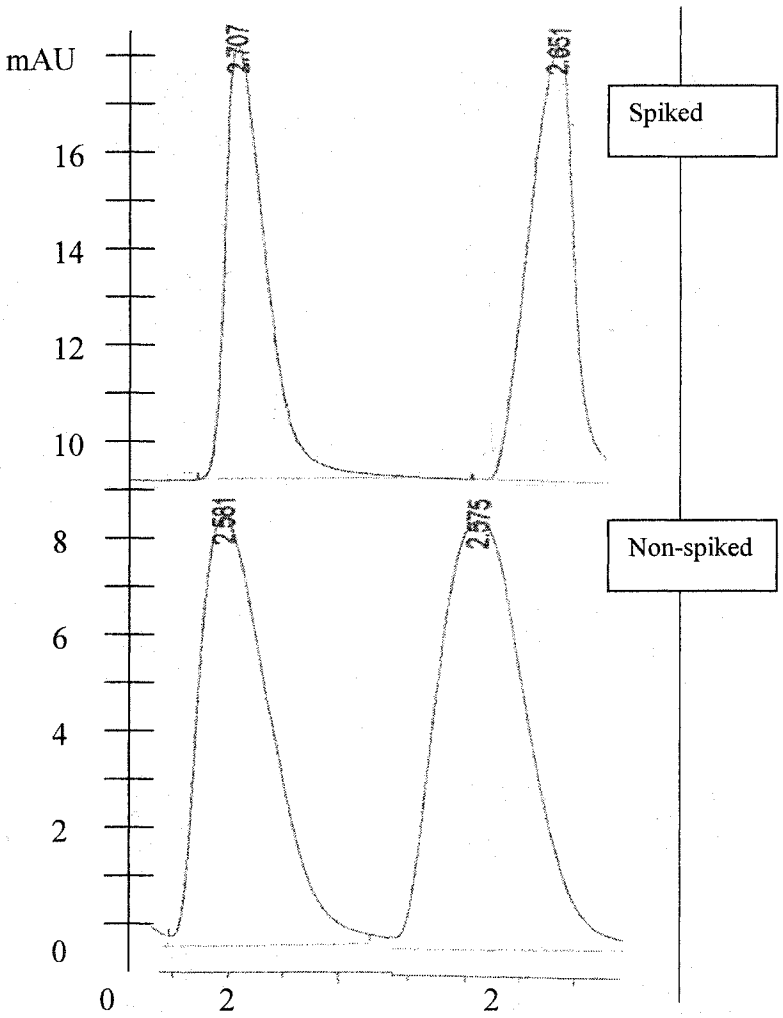

Figure 2.4 Representative peaks for ascorbic acid chromatograms in a 'Russet Burbank' potato extract before and after spiking with ascorbic acid at 0.05 (left) and 0.15 mg/ml (right).

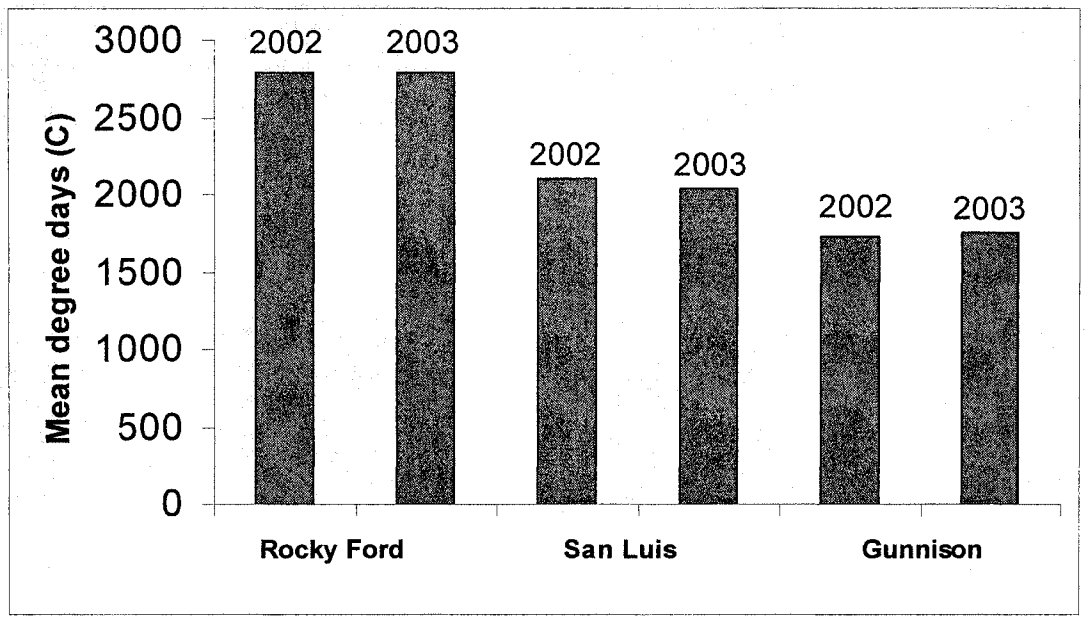

Figure 2.5 Mean cumulative degree days (C) for years 2002 & 2003 (May-August). Arkansas Valley and Powder Horn are represented by climatological data from Rocky Ford & Gunnison respectively. Data were calculated from CoAgMet & NCDC. websites.

Chapter 3

Effect of Production Location on Antioxidant Properties of Selected Colorado Grown Potato Cultivars

3.1 Abstract

Fruits and vegetables in the diet have been found in epidemiology studies to be protective against several diseases. Epidemiological evidence suggests that phenolic compounds have been associated with reduced incidences of heart disease by acting as antioxidants. Potato (*Solanum tuberosum* L) provides a good source of phenolic compounds, but not much work has been done to analyze total phenolic (TP) and antioxidant activities in different genotypes grown in different environmental sites. TP in seven potato cultivars cultivated in four locations were determined spectrophotometrically based on the Folin-Ciocalteu procedure and calculated as gallic acid equivalents (GAE). TP content in the selected cultivars ranged from 2.12 to 39.16 mg GAE/g dw. Among all cultivars analyzed, high TP were found in the skin tissue. The red and purple-fleshed cultivars had the highest TP compared to russet, white or yellow skinned and white-fleshed cultivars. Cultivars were highly different ($P<0.0001$, $F=4.16$) and contributed up to 35% of the variation. Cultivation sites also contributed significantly to differences ($P<0.0001$, $F=11.12$), but with less variation (14.4%). Cultivars x location interactions were not significantly different ($P>0.05$, $F=0.53$) and contributed only 13.6% of the total variations. Radical scavenging capacity was measured using a modified

3-ethylbenothiazoline-6-sulfonic acid (ABTS) assay. Hydroxyl radical scavenging capacity was measured spectrophotometrically and results are expressed as Trolox Equivalent Antioxidant Capacity (TEAC). TEAC ranged from 0.47 to 4.31 μmol TE/g dw in 'RB' and 'CO 94153-3P/P' in SLV. The colored cultivars CO 94165-3P/P and CO 94183-1R/R had the highest TEAC among all other cultivars tested. A linear positive relationship existed between TP and antioxidant activity in the tested potatoes (r =0.9663) concluding that 93% of antioxidants activity is due to the phenolic compounds. The overall objective of this research is to study the phenolic content and antioxidant properties of several Colorado potatoe cultivars grown in different locations.

3.2 Introduction

Potato (*Solanum tuberosum* L) is a major crop in the United States and other parts of the world. Potatoes are among the top five vegetables beside tomato, corn, pinto bean, and onion in daily consumption and account for 70% of the daily TP consumption (Joe et al., 1998). Epidemiological studies have pointed out that regular consumption of fruits and vegetables imparts health benefits. The health benefits of fruit and vegetable consumption seems to be related to antioxidant phenolic metabolites. Therefore, there has been a renewed interest in the evaluation of the phenolic content of fruits and vegetables. Potato is one of the main vegetables consumed in European and American diets (Tudela et al., 2002). In recent years, the physiological functionality of foods has received much attention due to the increasing interest in human health and has been studied in vitro and in vivo by many researchers. The antioxidative action, one of the important physiological functions of foods, is supposed to protect living organisms from oxidative damage,

resulting in the prevention of various diseases such as cancer, cardiovascular diseases, and diabetes (Azuma et al., 1999).

There has been increasing interest in the consumption of natural colorants and antioxidants due to evidence of their positive influence on human health. Major antioxidants present in plant tissues are phenolic compounds, vitamin C (ascorbic acid, AA), vitamin E, and carotenoids. Antioxidants interfere with oxidation processes through chain-breaking reaction processes (primary antioxidants) or through scavenging of free radicals (secondary antioxidants). Phenolic compounds can act as both types of antioxidants. Several other properties such as antibacterial, antiviral, anti-inflammatory, antiallergic, antimutagenic, and anticarcinogenic activities are also associated with phenolic compounds. All of these health-related properties stimulate the search for new phenolics-rich plant sources or the need of development of strategies to increase the content of phenolic compounds in plant tissue. For example, purple-and-red-flesh potatoes offer a valuable novel source of natural colorants and antioxidants, both associated with their phenolic compounds (Reyes et al., 2003).

Phenolic compounds act as antioxidants by virtue of the free radical scavenging properties of their constituent hydroxyl groups, allowing them to act as reducing agents, hydrogen-or electron-donating agents or singlet oxygen scavengers (Paganga et al., 1998). Natural antioxidants can be phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids as well as AA (Velioglu et al., 1998). At present there is overwhelming evidence to indicate that free radicals cause oxidative damage to lipids,

proteins, and nucleic acids. Low plasma levels of antioxidant vitamins have been associated with an increased risk of subsequent cancer mortality (Wang et al., 1996).

Marja et al., (1999) conducted research to evaluate the antioxidant activity in 92 plant extracts. They reported that potato 'Rosamunda' peels contain 4.3 mg/gdw of TP whereas, 'Matilda' peels contain 4.2 mg/gdw of TP. However, (Velioglu et al., 1998) reported that purple potato contains 781 mg/100gdw of TP. Potatoes ranked after spinach, yellow onion, red pepper, carrot, and cabbage in TP content (Chu et al., 2002). Lister, (2003) reported that potato contains a large variation in composition of some antioxidants depending on color (in most cases the stronger the color the higher the antioxidants). Lister (2003) reported that potatoes contain traces of carotenoids, 24-128 mg/100g phenolics, 10 mg/100 g AA, and trace amount of Vitamin E and rated the overall antioxidant activity in potato to be moderate to good.

There are many assays that can be used to measure antioxidant activity in fruits and vegetables. Wang et al., (1996) used the oxygen radical absorbance capacity (ORAC) to measure the total antioxidant capacity in 12 fruits and 5 commercial fruit juices. Whereas Kim et al., (2004), Paganga et al., (1998), and Reyes et al., (2003) used DPPH (2,2-diphenyl-1-picrylhdrazyl), and ABTS (2,2-azinobis (3-ethylbenothiazoline-6-sulfuric acid) assays to measure total antioxidant capacity of arginine-conjugated linoleic acid (CLA), and the Trolox equivalent antioxidant capacity (TEAC) assay developed by (Winston et al., 1998) to quantify the total antioxidant activity. Reyes et al., (2004) measured TP and anthocyanins content in purple and red flesh potatoes using Folin-Ciocalteu (F-C) phenol reagent assay developed by Swain et al., (1959). The Trolox equivalent antioxidant capacity (TEAC) assay relies on the reduction of the colored

cation radical of 2,2'-azonobis(3-ethylbenothiazoline-6-sulfonic) ($ABTS^{\bullet+}$) (Re et al., 1999).

Determination of free phenolic compounds in potato samples was optimized using a high-performance liquid chromatographic (HPLC) method with on-line diode array detection (Mendez et al., 2004, Fernando et al., 2003). Measurement of radical scavenging activity using discoloration of 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH radical scavenging assay) has been widely used due to its stability, simplicity, and reproducibility. However, physiological evaluation of antioxidant potential is problematic. It has been posed that the measurement of low density lipoprotein antioxidant activity (LDL oxidation assay) is more physiopathologically important and more informative for screening antioxidant activity to prevent atherosclerosis than other methods such as the Folin-Ciocalteu assay and the DPPH radical scavenging assay (Takuya et al., 2004). Potato samples of the cultivar Colorado, ssp. andigena, had mean concentrations of TP compounds and chlorogenic acid higher than those found for Kerr's Pink and Cara cultivars, ssp. tuberosum, and for Negra cultivar, S. x chaucha. In contrast, p-coumaric acid was not detected in any potato samples of the Colorado cultivar (Mendez et al., 2004).

The purpose of this research was to evaluate selected Colorado grown potato genotypes as a source of natural antioxidants and phenolic compounds grown in different environments. An additional goal was to explore a possible relationship between phenolic content and antioxidant activity. Therefore, TP and TEAC assays were conducted to evaluate antioxidant capacity.

3.3 Materials and methods

3.3.1 Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (01301 HU) and F-C (F-9252) were purchased from Sigma-Aldrich (Milwaukee, WI.). Gallic acid (3,4,5-trihydroxybenzoic acid) (G-7384) and manganese oxide (MnO_2) were purchased from Sigma Chemical (St. Louis, Mo.). Sodium carbonate ($NaCO_3$) (S 263-1) was purchased from Fisher Chemical (Fair Lawn, N.J.). Acetone (UN 1090- HPLC grade) was purchased from Burdick & Jackson (Muskegon, Mich.). The chemical reagent ABTS (2,2'-azionobis-3-ethylbenothiazoline-6-sulfonic acid) was purchased from CALBIOCHEM (Darmstadt, Germany).

3.3.2 Plant materials

Seven potato cultivars (Russet Burbank, Chipeta, CO 94183-1R/R, CO 94165-3 P/P, Yukon Gold, Russet Norkotah, and Russet Nugget) were grown at 4 major cultivation sites in Colorado (Arkansas Valley (AV), San Luis Valley (SLV), Powder Horn (PH), and Delta. Three tubers were selected from each cultivar (3 replicates) and the three middle slices were chosen to represent flesh with skin, flesh, and skin only. Slices were freeze dried using a Virtis Genesis 25 LL freeze dryer (Gardiner, N.Y. 12525).

3.3.3 Sample preparation for TP and radical scavenging assays

600 mg samples of freeze-dried plant material of each cultivar were ground and extracted in 10 ml of 80% acetone. Sample extracts were rotated at 4 °C in the dark at 20

RPM for 2 hours using a Lab quake rotator (model 412110 Barnstead/Thermolyne, Dubuque, Ia.) and centrifuged at 3800 RPM for 10 minutes (Biofuge^{TM} 17R model 2752 Heraeus Sepatech Gmbh, West Germany). One ml of supernatant was centrifuged to dryness at 45°C using a Vacufuge^{TM} "Eppendorf AG" (Westbury, N.Y.) and stored at -80°C for future experiments. Each sample (3 replicates) was dissolved in 1 ml acetone prior to analysis for total phenolics and radical scavenging capacity.

3.3.4 TP compound analysis

TP content was measured using Folin-Ciocalteu (F-C) reagent, adapted from the method of Spanos and Wrolstad (1990) and Lister and Wilson (2000). A colored solution was created by incubating a mixture of 50 µL 80% acetone extract, 2.5 mL 10% F-C in H_2O, 2.0 mL 7.5% Na_2CO_3 in H_2O at 45° C for 15 min. Each sample was assayed in triplicate (Stushnoff et al., 2003). Absorbance was related to a gallic acid standard curve measured at 765 nm using a spectrophotometer/microplate reader (SPECTRAmax PLUS384 UV-vis spectrophotometer, Molecular Devices, Sunnyvale, Calif.) and Pro version 3.1.2 software. Results were compared to a gallic acid standard curve and calculated as gallic acid equivalents (GAE) in mg g^{-1} (Table 3.1).

3.3.5 Measurement of radical scavenging capacity

Radical scavenging capacity was measured using a modified ABTS assay (Miller and Rice-Evans 1996; Lister and Wilson (2000). A 0.5 mM solution of ABTS was prepared according to the number of samples to be tested (Table 3.2). Approximately 1g MnO_2 was used to oxidize 5 mM ABTS in 5 mM saline buffer (pH=7.4) to create a green

colored solution of activated $ABTS^{+*}$ as the reactive oxygen species (ROS) that was diluted to absorbance of 0.700 at 734 nm at 30°C using a spectrophotometer/microplate reader (SPECTRAmax PLUS384 UV-vis spectrophotometer, Molecular Devices, Sunnyvale, Calif.). The solution was filtered through a Buchner funnel under vacuum using a Whatman 70mm circle filter paper, (Whatman International Ltd., Maidstone, England) and finally with a 0.2µm syringe-end filter. 200 ml phosphate buffered saline (PBS) was prepared by diluting 25mM of the stock at 200 ml/L of water (Table 3.2). 1.0 ml of ABTS was added to each 100µL standard/sample in 150mm glass tube and allowed to react for exactly one- minute when the two fluids were mixed. The antioxidant quenching capacity was spectrophotometrically measured as deactivation of the activated $ABTS^{+*}$ ROS in solution compared to a standard, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, a water soluble vitamin E analogue). Hydroxyl radical scavenging capacity was measured at 734 nm and expressed as Trolox Equivalent Antioxidant Capacity (TEAC) (µmol g^{-1} dw). TEAC is defined as the concentration in mM of the tested compound, or natural concentration in the case of potato extract (Stushnoff et al., 2003).

3.3.6 Statistical analysis

Three replicates of each sample were used for statistical analysis. Results from 2 years (2002 & 2003) were subjected to analysis of variance (ANOVA) using GraphPad Prism 4 software (GraphPad Software, Inc. San Diego, Calif. 92130). Correlation between TEAC and total phenolic content was conducted using Pearson correlation (r) obtained from GraphPad Prism 4.00 (San Diego, Calif.).

3.4 Results

3.4.1 TP analysis

TP content varied in different genotypes and ranged from 2.12 to 39.16 mg GAE/g dw for 'YG' in PH to 'CO 94165-3P/P' in AV respectively. In general all skin tissue in all genotypes produced the highest TP compared to other tissues (skin+flesh & flesh only), whereas the flesh tissue had the lowest TP in all locations. The skin tissue in 'CO 94165-3P/P' had the highest TP (39.16 mg GAE/ g dw) from AV followed by SLV (34.13 mg GAE/ g dw), and 19.12 mg GAE/ g dw in Delta, with the lowest in flesh tissue (5.49 mg GAE/ g dw) from PH. The CO 94193-1R/R cultivar produced the highest TP (29.06 mg GAE/ g dw) in AV. and 25. 12 mg GAE/ g dw in SLV, followed by PH and Delta (22.57 and 14.50 mg GAE/ g dw) respectively. 'Russet Norkotah' had the highest TP in skin tissue from AV (25.57 mg GAE/ g dw), followed by SLV (24.19 mg GAE/ g dw), Delta (16.57 mg GAE/ g dw) 15.35 mg GAE/ g dw in PH, and the lowest in the flesh tissue in Delta (4.34 mg GAE/ g dw). 'Russet Burbank' skin produced the highest TP in the skin tissue at AV (23.78 mg GAE/ g dw) about the same in both SLV and PH (~15.00 mg GAE/ g dw) and no data was available from Delta. The 'Chipeta' genotype produced the highest TP in the skin tissue at AV (17.39 mg GAE/ g dw) and the about the same TP in SLV and PH (~13.00 mg GAE/ g dw) and the lowest in the flesh tissue at Delta (2.78 mg GAE/ g dw). Interestingly, 'Russet Nugget' skin had the highest TP from Delta in skin tissue (11.87 mg GAE/ g dw), followed by PH (8.03 mg GAE/ g dw) and about the same in AV and SLV (~6.50 mg GAE/ g dw), yet the flesh tissue had the lowest TP in Delta (2.50 mg GAE/ g dw). Finally 'Yukon Gold' skin produced the

highest TP from AV (23.80 mg GAE/ g dw) and the lowest in flesh from PH (2.12 mg GAE/ g dw) (Table 3.4).

The contribution to variance for cultivars (C), locations (L), and the C x L interaction within the tested genotypes is found in Table 3.6. The largest contribution to variance based on the ANOVA model was from the cultivars with 35.82% of the total variation ($P<0.0001$, $F=4.16$). However, a locations effect was observed ($P<0.0001$, $P=11.12$) although the amount of variation was only 14.4%. Finally, the C x L interaction was not observed ($P>0.05$, $F=0.53$) and contributed only 13.6% of the total variation.

3.4.2 Radical scavenging capacity

Antioxidant capacities of 5 potato genotypes grown at 3 cultivation sites in Colorado are shown in Table 3.5. TEAC values ranged from 0.47 to 4.31 μM Trolox equivalents in 'Russet Burbank' at SLV and 'CO 94165-3P/P' at PH respectively. In general, the 'CO 94165-3P/P' genotype had the highest TEAC, followed by 'CO94183-1 R/R', 'Chipeta', 'Russet Burbank', and 'Yukon Gold'. Therefore, the colored genotypes expressed the highest TEAC followed by the russet skin and white/yellow cultivars. Among the 2 colored cultivars examined, the 'CO94165-3P/P' had the highest TEAC of 4.31 μmol /g dw from PH, followed by 2.83 μmol TE/g dw from SLV and 2.15 μmol TE/g dw from AV whereas 'CO 94183-1R/R' had more TAA in AV (1.99 μmol TE/g dw) followed by 1.7 μmol TE/g dw in PH and finally 1.61 μmol TE/g dw in SLV. On the other hand, among the normal uncolored cultivars, 'Yukon Gold' and 'Chipeta' had the lowest TEAC in PH (~0.6 μmol TE/g dw) and SLV (~0.7 μmol TE/g dw) respectively.

However, 'Russet Burbank' had the highest TEAC (1.19 μmol TE/g dw) in AV and about the same (~0.5 μmol TEAC/g dw) in both SLV and PH.

Table 3.6 shows the results from ANOVA analysis and the total variance for cultivars (C), locations (L), and C x L interaction. As in TP, TEAC of cultivars was different ($P<0.01$, $F=7.55$) and contributed to 60.8% of total variation. However, a location effect, and the C x L interaction were not observed different ($P>0.05$, $F=0.49$ & 0.44) and contributed only 1.97 & 7.04% of the variation respectively. The relationship between TEAC and TP contents in the genotypes examined had a correlation coefficient, $r= 0.9663$ (Fig. 3.1).

3.5 Discussion

Joe et al. (1998) reported that potato had the lowest phenolic content (1.2 μmol/g dw) among 23 vegetables tested, but did not specify which cultivars or tissues, nor the growing conditions from which samples were taken. Yet potato had the second best antioxidant quality of all vegetables based on total phenols. Potatoes contain up to 0.53 mg of chlorogenic acid/kg of wet weight (Friedman, 1997). However, the HPLC assay of potato found no flavonoids at all in potatoes (Justenes et al., 1997), which is not surprising because they did not measure chlorogenic acid, the major phenol present in potatoes. Thus, TP content from our research is higher than previously published work, and variation was more due to cultivars than locations, even though locations had highly significant difference (Table 3.6).

Marja et al., (1999) reported that extracts of the peel of purple skinned potato cultivar Rosamunda showed remarkable antioxidants activity, and the yellow skinned

potato cultivar Matilda had lower TP content and antioxidant activity than Rosamunda. However, the difference in antioxidant activity between potato cultivars may result partly from the presence of anthocyanins. Our data from this research showed that skin tissue in general had the highest TP content in almost all colored and white/yellow cultivars compared to other tissues. However our values for TP contents were higher than reported by (Marja et al., 1999) 4.3 and 2.5 mg/gdw in 67 and 80 mg of dry extract respectively and much lower than reported by (Velioglu et al., 1998) 781 mg/100gdw in purple potatoes. We can conclude that antioxidant studies with potato must specify, the cultivar, type of tissue and if possible growing conditions, all of which can influence phenolics content and antioxidant capacity.

In the previous chapter, potatoes expressed more AA in warmer (AV) climate than in cooler locations (PH). In general the data from this work showed that more TP was produced in AV followed by SLV (warm days, cool nights) than in PH (cool days, cool nights) with some slight variation in SLV and PH in some cultivars. However locations were not different and most of the variation arises from contributions of the cultivars. Interestingly, Reyes et al., (2004) reported that Colorado genotypes had higher anthocyanin content compared to the same genotypes grown in Texas. However, for TP content, these differences were not observed. He also reported that 'CO 94165-3P/P' contained 155 mg/100g total phenolics when grown in Colorado and 123 mg/100gfw when grown in Texas. In addition, 'CO 94183-1R/R' contained 128 mg/100gfw grown in Colorado and 123 mg/100gfw when grown in Texas. The accumulation of phenolic compounds in plant tissues may be induced by different abiotic stresses. Low temperature

stresses have been reported to influence the biosynthesis of phenolic compounds (Reyes et al., 2004).

Reyes et al., (2004) reported that both cooler temperatures and longer days (higher solar radiation) at the Colorado planting sites could have produced higher anthocyanin content compared to Texas. This is true in our research when comparing all 3-selected sites in Colorado with all variation in climatic conditions.

The TP content of the potato cultivars investigated in this study varied from 4.70 to 29.29 mg GAE/g dw when examining the skin+flesh tissue only (Table 3.5). The correlation coefficient (r= 0.9663) suggests a strong relationship between antioxidant capacity and gallic acid equivalent total phenolics content, suggesting that phenolics are the main source of antioxidants responsible for antioxidant capacity. In addition, this also suggests that only a small proportion of the capacity is due to the presence of other antioxidant secondary compounds such as carotenoids and vitamins. From the curve's slope we determined that phenolic compounds have a specific antioxidant capacity of 0.11 μM of Trolox/g of potato. This is contradicted with what AlSaikan et al., (1995) reported that antioxidant activity varied among cultivars, but was not related to TP content.

Phenolic compounds are the major secondary metabolites of plant metabolism, and their biosynthesis involves the induction of phenylalanine ammonia-lyase, the first and the rate-limiting enzyme in the phenyl-propanoid metabolism. These compounds have a wide range of structural classes and biological functions; however, within a single tissue, not all of their pathways maybe expressed (Rhodes, 1985). The phenolic compounds accumulate in potatoes and other plant tissues, as protection against

mechanical bruising and injury by predators (Friedman, 1997). Increased phenolic content contributes to stress resistance by forming oxidation compounds (polymeric products) that are more toxic to pathogens, thus helping in the healing process (Shahidi et al., 1995).

The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to acts as reducing agents, hydrogen donors, and singlet oxygen quenchers (Rice-Evans, et al., 1995).

Table 3.1 Gallic acid proportions for total phenolics standard solutions.

Gallic acid (μg/ml)	Volume of Gallic acid stock	Volume of H_2O
0	0	500
10	10	490
20	20	480
40	40	460
60	60	440
80	80	420
100	100	400

Table 3.2 Amount of ABTS solutions required according to number of samples to be tested.

Water (ml)	ABTS (mg)
5	13.7
10	27.4
15	41.1
20	54.8
25	68.5
40	109.6
50	137.0

Table 3.3 Solution proportions required to prepare Trolox standard curve dilutions.

Trolox (μm)	Volume of 0.5 mM stock (μl)	Volume water (μl)
0	0	100
5	11	89
10	22	78
15	33	67
20	44	56

Table 3.4 Means for total phenolics (mg GAE/g dw) for 7 genotypes grown at 4 locations in Colorado in 2002 and 2003.

Cultivar/Location		Total Phenolic (mg GAE/g dw)		
		Skin +flesh	Flesh	Skin
Russet Burbank	AV	13.5 [a]	8.8 [a]	23.78 [a]
	SLV	14.36 [a]	10.4 [a]	15.63 [a]
	PH	9.52 [a]	8.51 [a]	15.38 [a]
	Delta	N/A	N/A	N/A
Chipeta	AV	12.65 [a]	5.87 [a]	17.39 [a]
	SLV	6.47 [a]	6.12 [a]	13.37 [a]
	PH	7.70 [a]	5.94 [a]	12.69 [a]
	Delta	3.97 [a]	2.78 [a]	4.09 [a]
CO 94183-1R/R	AV.	21.59 [a]	18.21 [a]	29.06 [a]
	SLV	19.62 [a]	17.83 [a]	25.12 [a]
	PH	22.08 [a]	19.38 [a]	22.57 [a]
	Delta	11.25 [a]	8.76 [a]	14.50 [a]
CO 94165-3P/P	AV	27.16 [a]	19.53 [a]	39.16 [a*]
	SLV	29.29 [a]	26.69 [a]	34.13 [ab]
	PH	16.25 [a]	13.20 [a]	5.49 [ab]
	Delta	16.12 [a]	11.77 [a]	19.12 [b*]
Yukon Gold	Ark. V	12.58 [a]	10.23 [a]	23.80 [a]
	SLV	4.70 [a]	5.43 [a]	3.52 [a]
	PH	2.33 [a]	2.12 [a]	5.31 [a]
	Delta	N/A	N/A	N/A
Russet Norkotah	AV	23.98 [a]	12.78 [a]	25.57 [a]
	SLV	13.59 [a]	7.92 [a]	24.19 [a]
	PH	6.69 [a]	4.34 [a]	15.35 [a]
	Delta	6.17 [a]	3.44 [a]	16.57 [a]
Russet Nugget	AV	7.04 [a]	3.60 [a]	6.85 [a]
	SLV	7.69 [a]	5.57 [a]	6.66 [a]
	PH	4.49 [a]	2.66 [a]	8.03 [a]
	Delta	4.54 [a]	2.50 [a]	11.87 [a]

[a] Numbers within each column for each cultivar is non significant difference ($P>0.05$), *significant ($P<0.05$). [N/A] data not available for corresponding location.

Table 3.5 Means of total phenolics (TP) content and antioxidant capacity (TEAC) for skin and flesh tissues from 5 potato genotypes grown at 3 sites in Colorado in 2002 & 2003. TP data are expressed as milligrams of gallic acid (GAE) equivalents per gram dry weight. TEAC data are expressed as micromoles of Trolox equivalents per gram dry weight.

Cultivar	Location	Total phenolics (mg GAE/g dw)	Antioxidant capacity (μmol TEAC/g dw)
Russet Burbank	AV	13.5	1.19
	SLV	14.36	0.47
	PH	9.52	0.51
Chipeta	AV.	12.65	1.40
	SLV	6.47	0.75
	PH	7.70	0.62
Yukon Gold	AV	12.58	0.71
	SLV	4.70	0.67
	PH	2.33	---
CO 94183-1R/R	AV	21.59	1.99
	SLV	19.62	1.61
	PH	22.08	1.70
CO 94165-3P/P	AV	27.16	2.15
	SLV	29.29	2.83
	PH	16.25	4.31

Table 3.6 Summary of analysis of variance for total phenolics and antioxidant capacity.

Effect	Total Phenolic (mg GAE/g dw)					Antioxidant activity (μmol TE/g dw)				
	DF	SS	MS	% Variation	F-value	DF	SS	MS	% Variation	F-value
Location (L)	3	3163	1054	14.36	11.12^{***}	2	0.90	0.45	1.97	0.49^{NS}
Cultivars (C)	20	7889	394.4	35.82	4.16^{***}	4	27.9	6.97	60.80	7.55^{**}
(L x C)	60	3006	50.10	13.65	0.53^{NS}	8	3.23	0.40	7.04	0.44^{NS}

^{NS} = Not significant P>0.05, ** significant (P <0.01), *** significant (P < 0.0001).

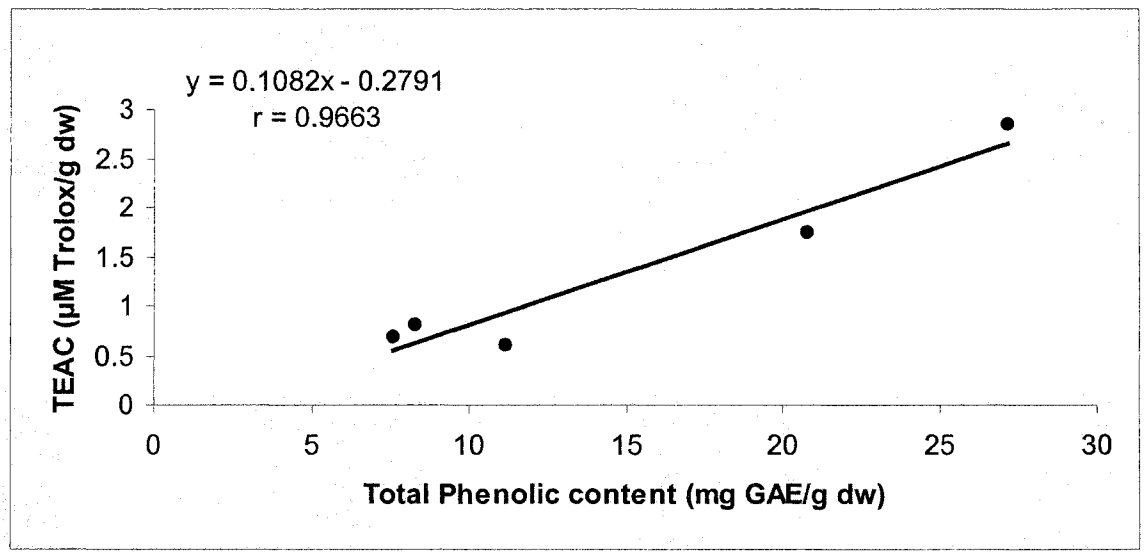

Figure 3.1 Linear correlation of Trolox equivalent antioxidant capacity (TEAC) (*Y*) versus the total phenolic content (*X*) of 5 potato genotypes (Russet Burbank, Chipeta, Yukon Gold, CO 94183 R/R, and CO 94165 P/P) from 3 locations in Colorado (Arkansas valley, San Luis valley, and Powder Horn, determined by Pearson correlation using GraphPad Prism 4.00

Chapter 4

Effects of Cooking on Antioxidant Heat Stability in Colorado Grown Potato Cultivars

4.1 Abstract

Potatoes constitute one of the main vegetables consumed in European and American diets. They provide an important source of vitamin C (Ascorbic acid, AA), minerals, phenolic compounds and antioxidants, that are directly related to human health through their protection against oxidative and free radical processes. During cooking, a number of changes occur in the potato tuber. The nature and magnitude of these changes influence the quality of cooked potatoes. Sixteen potato cultivars grown in the San Luis Valley, the main potato production site in Colorado, were examined for their AA total phenolics (TP), and antioxidant activity (TEAC) after cooking by microwaving, boiling, and baking.

AA in raw tubers ranged from 17.67 to 29.27 mg/100gfw, while in microwaved samples AA content ranged from 9.78 to 17.87 mg/100gfw, and from 9.60 to 16.65 mg/100gfw in boiled samples. Baked samples had the lowest AA content compared to other cooking methods and ranged from 8.70 to 15.2 mg/100gfw. The highest AA content was detected in 'All Blue' in cooked and uncooked samples.

Differences among cooking methods were detected (P<0.0001, F=32.60), but differences in AA among cultivars were not observed (P= 0>0.05, F=1.591) nor was the interaction between cooking methods and cultivars (P>0.05, F=0.2892). Of all cultivars, 'Russet Burbank' lost the most AA when micro-waved (58%), boiled (56%), and baked (62%), while 'Keystone Russet' lost the least AA when micowaved (11%), followed by boiled and baked 'Freemont Russet' at 23% and 25% respectively.

Differences among cultivars (P<0.0001, F=4.062) and cooking methods (P<0.01, F=5.028) were observed for TP content. TP content for raw samples ranged from 5.77 to 28.84 mg GAE/gdw or 115.4 to 576.8 mg GAE /100 g fresh weight. Microwaved samples ranged from 4.38 to 13.35 mg GAE/gdw, boiled samples ranged from 5.54 to 13.00 mg GAE/gdw and baked samples ranged from 5.29 to 14.14 mg GAE/gdw.

Trolox equivalent antioxidant capacity based on ABTS radical scavenging capacity ranged from 0.2 to 2.84 μmol TE/gdw for raw samples, from 0.17 to 2.22 μmol TE/gdw for micro-waved samples, from 0.25 to 3.06 μmol TE/gdw for boiled samples and from 0.24 to 1.40 μmol TE/gdw for baked samples. TEAC and TP contents in the raw and microwaved samples were closely related with a Pearson correlation coefficient of (r= 0.8722, P<0.0001), (r=0.8346, P<0.0001) respectively. However the relationship in boiled and baked samples had lower coefficients (r=0.6254, P=0.0032) (r=0.5462, P=0.0127) respectively.

Cooking methods did not alter TEAC (P>0.05, F=0.4555), but they did produce variation (P<0.01, F=5.028) in TP. However, in both assays cultivars were different (P<0.0001, F=4.549 & 4.062 respectively), and the interaction for both assays (TEAC & TP) was not different (P>0.05, F=0.3410 & 0.6691 respectively).

The % loss in TP ranged from 36% to 52% in microwaved samples, 21% to 53% in boiled, and 25% to 33% in baked. Percent loss in TEAC ranged from 21% to 32% in micowaved samples, 0% to 3% in boiled samples, and 0% to 50% in baked samples.

The main objective of this study was to define the content of the AA, TP, and TEAC in widely consumed Colorado potato cultivars after subjecting them to different cooking methods. The data will provide information on antioxidant phytochemical stability of Colorado grown cultivars when subjected to different coking methods.

4.2 Introduction

Potato tubers occupy a remarkable position in the human diet, i.e., 150–400 g and more per capita per day, mainly as boiled, fried, mashed or in the form of chips and French fry strips. Potato products are considered to be a significant component of dietary profiles due to the content of selected vitamins, minerals and other bioactive compounds (Rogan et al., 2000). Nowadays, many food companies offer almost ready-to-eat processed potato products (i.e., blanched French fry strips, powdered, noodles or chips), which are consumed widely across the world. Such products are ready to eat prior frying, microwave heating, cooking or boiling. Plant tissue subjected to these simple culinary processes undergoes physicochemical changes, which impact texture formation and texture properties of the end-use product (Wioletta et al., 2004).

Potato is one of the main vegetables consumed in European and American diets. It is always consumed after cooking (boiling, frying, baking, etc). Previous studies indicate that potato tubers contain caffeic acid derivatives (chlorogenic acid) as the main phenolic constituents (Brandl et al., 1984). An increase in the content of chlorogenic acid and

other phenolic compounds was observed in light-exposed sliced potatoes stored for nine days (Laanest, et al., 1995). Steam cooking resulted in the highest retention of caffeic acid derivatives and aromatic amino acids compared with the other cooking methods studied (Tudela et al., 2002).

Potatoes and other foods that have a high content of the amino acid asparagine and a high accumulation of reducing sugars are subject to the formation of acrylamide upon frying. Results showed that both cultivar and modified frying systems could play an important role in reducing acrylamide formation in fried potatoes. As the frying temperature decreased from 180 °C to 165 °C, acrylamide content in potato chips dropped by 51% during traditional frying and by 63% as the temperature decreased from 140 °C to 125 °C in vacuum frying. Increased frying time increased acrylamide formation during traditional frying for all temperatures and frying methods analyzed. However, the effect on acrylamide concentration was greater for traditional frying than vacuum frying (Granda et al., 2004).

Cooking ($P<0.001$) reduced TAA, AA and DHAA, with microwaving proving to be more destructive than boiling, although it was not as significant for DHAA compared to TAA and AA. When irradiated potatoes were cooked, TAA, AA and DHAA concentrations were reduced, as was the case for non-irradiated potatoes. However, it is noteworthy that cooking did not decrease the AA content of irradiated potatoes to any greater extent than that of their non-irradiated counterparts (Graham et al., 1997).

Jaswir et al., (2000), suggested the use of natural antioxidants could improve the sensory acceptability of potato chips during repeated deep fat frying. Natural antioxidants

($P < 0.05$) lowered the rate of oxidation of oil during deep fat frying and contributed to measured sensory acceptability of fried potato chips.

The effect of peeling on the total phenols, total glycoalkaloids (TGA), discoloration, and flavor of 50-80g sized cooked potatoes was studied. Three methods of cooking were studied; (1) boiling in distilled water; (2) boiling in 16% NaCl solution; and (3) steaming. In all three methods, potatoes cooked without the peel were lower in phenolic and TGA content, discolored less, and were less bitter than potatoes cooked with the peel. During cooking phenols migrated from the peel into both the cortex and internal tissues of the potato. Glycoalkaloids were less mobile than phenols and migrated only into the cortex. The movement of phenols and TGA into the cortex increased both discoloration and bitterness in potatoes cooked with the peel (Mondy et al., 1988).

HPLC was used to analyze the content of ascorbic acid (AA) in tubers of four Korean potato cultivars (Chaju, Sumi, Deso, and Dejima). In a series of baked, boiled, braised, fried, microwaved, pressure-cooked, and sautéed potato slices from the Dejima cultivar and in 14 commercial Korean and 14 processed potato foods sold in the United States (chips, snacks, mashed potatoes, fries), the AA content for the four cultivars ranged from 16 to 46 mg/100 g of fresh weight. The distribution of AA in each of the eight potato slices (sticks, plugs) cut horizontally from the stem end of the 'Dejima' potato ranged from 6.8 to 19.3% of the total. The corresponding distribution in seven sticks cut vertically was much narrower, ranging from 11.7 to 17.5% of the total. Losses of AA in water (pH 5.2) were greater than in 5% metaphosphoric acid (pH 1.0). Less degradation occurred in water solutions of the vitamin stored at 1°C than at 25°C. Losses of AA observed during home-processing of three cultivars with low (Dejima, 16 mg/100

gfw), intermediate (Sumi, 32 mg/100 gfw), and high (Chaju, 42 mg/100 gfw) AA contents were as follows: boiling in water, 77-88%; boiling in water containing 1-3% NaCl, 61-79%; frying in oil, 55-79%; sautéing, 61-67%; pressure-cooking in water, 56-60%; braising, 50-63%; baking, 33-51%; and microwaving, 21-33%. The content of the Korean foods ranged from trace amounts to 25 mg/100 g and that of the U.S. foods from 0.4 to 46 mg/100 g. These results permit optimization of the AA content of the diet by (a) using high-AA potato cultivars such as 'Chaju', (b) selecting sticks cut horizontally for frying, (c) baking or microwaving rather than boiling or frying, and (d) selecting commercial potato foods with high AA content (Han et al., 2004).

During cooking a number of changes occur in the potato tuber. The nature and magnitude of these changes influence the quality of cooked potatoes. For instance, some cooking conditions may cause excessive quality loss due to the breakdown of the cellular material such as pectin. Starch present in the potato tubers also plays an important role in the cooking quality because it can absorb water and swell creating internal pressures that can lead to cell separation, reduced cohesiveness and softening (Ibarz et al., 1999, Lozano et al., 1997, Maskan, 2001). The objective of this research is to determine the effect of different cooking methods on the stability of AA, TP, and TEAC content.

4.3 Material and methods

4.3.1 Potato tuber preparation and storage

Freshly harvested potato tubers were obtained from different cultivars grown in San Luis Valley in 2002 and 2003. These cultivars include: All Blue, Centennial Russet, Chipeta, Freemont Russet, Durango Red, CO 94183-1R/R, CO 94165-3P/P, Cherry Red,

Keystone Russet, Russet Burbank, Russet Norkotah-S8, Sangre-S10, Silverton Russet, and Yukon Gold. Uniform, medium-sized tubers (200-400 g) were washed in running water to remove surface soil and dried at room temperature.

4.3.2 Cooking procedure

Whole tubers were microwaved individually for five minutes at the maximum setting in a 700 watt General Electric microwave. Boiled tubers were cooked in halves, unpeeled for 30 minutes at full boil. Baked tubers were cooked wrapped in aluminum foil for 60 minutes at 170 °C. Thermocouples inserted approximately into the tuber center indicated the microwaved tubers attained a temperature of 94-96 °C, the boiled samples were 96 °C and the baked tubers were 160-170 °C. After cooling to room temperature three thin slices (3-5mm) were cut radially from the center of each tuber, skewered onto toothpicks to ensure separation during freeze drying and quickly frozen at –80 °C in weighing trays. Samples were placed into freezing trays of a Virtis freeze dryer with a shelf temperature set at –45 °C. The freeze drying regime was: 24 hours at each of –45 °C, -10 °C, 18 °C and 28 °C. Dry samples were ground and screened with a 100 mesh sieve to ensure uniform particle size prior to extraction.

4.3.3 Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (01301 HU) and F-C (F-9252) were purchased from Sigma-Aldrich (Milwaukee, WI.). Gallic acid (3,4,5-trihydroxybenzoic acid) (G-7384) and manganese oxide (MnO_2) were purchased from Sigma Chemical (St. Louis, Mo.). Sodium carbonate ($NaCO_3$) (S 263-1) were

purchased from Fisher Chemical (Fair Lawn, N.J.). Acetone (UN 1090- HPLC grade) was purchased from Burdick & Jackson (Muskegon, MI 49442). The chemical reagent ABTS was purchased from CALBIOCHEM (Darmstadt, Germany). Ascorbic acid (A5960) was purchased from Sigma-Aldrich (St. Louis, Mo.). Phosphoric acid used in the washing process (PX0996-6, EM science, Darmstadt, Germany) was of HPLC grade quality. Phosphate buffered saline (PBS) solutions for sample extraction and standards preparations were made up of 36 mM KH_2PO_4 (7100-03 Mallinckrodt, Paris, Ky.) and 36 mM Na_2HPO_4 (4062-01 Mallinckrodt Barker Inc. Phillipsburg, N.J.). Methanol (MX 0475P-1) of the HPLC grade was purchased from EMD (Gibbstown, N.J.).

4.3.4 HPLC analysis

AA was detected using a Hewlett-Packard 1050 HPLC (Palo Alto, Calif.) equipped with a UV-VIS detector. The separation was run at 1.0 ml/min at 35°C with a column heater using water- soluble vitamin analysis and an Inertsil C4 column. AA was eluted using a gradient consisting of 100% 50 mM phosphoric acid (pH 2.0) for 5 minutes followed by 85% methanol for 7 minutes at a flow rate of 1 ml/min and the effluent was monitored at 254 nm. Standard solutions of AA were prepared fresh each day. 10 mg of AA and 100 mg of DTT (dithiothreitol) were weighed and both dissolved in 10 ml of 100% methanol. Five standard curve dilutions were made and put in HPLC amber vials and stored on ice.

4.3.5 Sample preparation for TP and antioxidant activity measurements

For both assays, 100 mg freeze-dried potato powder was ground and dissolved in 10 mL 80% acetone and vortexed for 20 seconds. Sample extracts were rotated in the dark for 1 hr at 20 RPM and centrifuged at 3800 RPM for 10 min. One milliliter of supernatant was centrifuged to dryness at 45°C using a Vacufuge™ (West Germany).

4.3.6 Determination of radical scavenging activity and TP

Radical scavenging capacity was measured in nine replicate wells with a modified ABTS assay (Miller and Rice-Evans, 1996; Wilson et al., 2000). MnO_2 was used to oxidize 5 mM ABTS saline buffer (pH=7.4) to create a solution of green activated $ABTS^{+*}$ as the reactive oxygen species (ROS) that was diluted to absorbance of 0.7 at 734 nm at 30°C using a SPECTRAmax-PLUS384, UV-vis 96 well microplate spectrophotometer. The antioxidant capacity were measured against a Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard curve and expressed as TEAC ($\mu mol\ g^{-1}$ DW).

TP content was measured spectrophotometrically using a method based on Folin-Ciocalteu reagent (Spanos & Wrolstad, 1990; Lister and Wilson, 2001). To 50 µL of each sample (3 replicates), 2.5 ml 1/10 dilution of Folin-Ciocalteu reagent and 2 ml 7.5% of Na_2CO_3 were added and incubated at 45°C for 15 min. The absorbance was measured at 765 nm using SPECTRAmax-PLUS384, UV-vis spectrophotometer. Results were expressed as milligrams gallic acid equivalent per gram of dry weight (mg GAE/g dw).

4.3.7 Statistical analysis

The collected data were first organized in Microsoft Office Excel 2003. The data were then analyzed using GraphPad Prism version 4.00 software (San Diego, Calif., 2003). Two-Way analyses of variance (ANOVA) were carried out and a Bonferroni post-test was used to compare the replicate means. One-way ANOVA was conducted to between AA content retained and cooking methods. Correlations using Pearson (r) were obtained using GraphPad Prism 4.00 (San Diego, Calif.).

4.4 Results

4.4.1 AA content

Table 4.1 shows the mean AA content in different cultivars for uncooked, microwaved, boiled, and baked samples. AA in raw tubers ranged from 17.16 to 29.27 mg/100gfw with the lowest in 'Keystone Russet' and the highest in 'All Blue' and. AA content in microwaved samples ranged from 9.78 to 17.87 mg/100gfw, with the lowest in 'Russet Burbank' and the highest in 'All Blue'. Furthermore, AA for boiled samples ranged from 9.60 mg/100g fw in 'Cherry Red' to 16.65 mg/100g fw in 'All Blue'. Finally, baked samples had the lowest AA content compared to other cooking methods and ranged from 8.60 mg/100g fw for 'Cherry red' to 15.84 mg/100g fw for 'Freemont Russet'. Microwaved samples of 'All Blue', 'Centennial Russet', 'Cherry Red', 'Keystone Russet', 'Russet Norkotah-S3 & 8', 'Sangre-S10', 'Silverton Russet', and 'Yukon Gold' had more AA content than boiled and baked samples respectively.

There was no differences due to an interaction between the cooking methods and the cultivars ($P>0.05$, $F=0.2892$) and contributed only 6.32% of the variation (Table 4.2).

However, cooking methods were different (P<0.0001, F=32.60) and contributed to 50.88% of the variation. Cultivars were not different (P= 0.1084, F=1.591) and contributed only 11.59% of the variation. Bonferroni mean separation post-tests indicated mostly that neither different cooking methods nor cultivars were different (P>0.05). However, there was a significant difference (P<0.05) between raw and baked samples of 'All Blue', 'Silverton Russet', 'Russet Burbank' (Table 4.1).

4.4.2 AA content retained after cooking

AA content retained after cooking was calculated relative to the raw content. AA content retained in micro-waved samples ranged from 1.91 mg/100g fw in 'Keystone Russet' to 13.58 mg/100g fw in 'CO 94165-3P/P', whereas in boiled samples AA retained ranged form 5.67 mg/100g fw in 'Freemont Russet' to ~12.00 mg/100g fw in 'All Blue', 'Russet Burbank' and 'Silverton Russet', and in baked samples ranged from 5.31 to ~14.00 (mg/100g fw) for the same cultivars as in boiled samples, respectively (Table 4.3).

'Russet Burbank' had the highest % AA loss when micro-waved (58%), boiled (56%), and baked (62%) while 'Keystone Russet' had the lowest when micowaved (11%), followed by Freemont Russet (23%) in boiled, and baked (25%) (Table 4.4)

One-way ANOVA for retained AA content was different between cooking methods (microwave, boil, bake) compared to uncooked (raw) (P<0.001), and between microwave vs bake (P<0.01) for all cultivars except for 'Yukon Gold'. However, there was no difference between microwave vs boil and boil vs bake (P>0.05) (Table 4.3).

4.4.3 TP content

TP contents for raw samples ranged from 5.77 mg GAE/g dw in 'Freemont Russet' to 28.84 mg GAE/g dw in 'CO 94183-1R/R' (Table 4.5). Microwaved samples had less TP content and ranged from 4.38 to 13.35 mg GAE/gdw in 'Yukon Gold' and 'All Blue' respectively. Conversely, TP content in boiled samples ranged from 5.54 to 13.0 mg GAE/gdw in 'Chipeta' and 'All Blue' respectively. TP in baked samples ranged from 5.29 mg GAE/g dw in 'Chipeta' to 14.14 mg GAE/g dw in 'CO 94165-3P/P'.

Cultivar differences were detected ($P<0.0001$, $F=4.062$) and contributed to 36.07% of the total variation. Differences in cooking methods were also significant ($P=0.01$, $F=5.028$). A significant interaction between cultivars and cooking methods ($P=0.9283$, $F=0.6691$) was not observed (Table 4.7).

4.4.5 ABTS antioxidant radical scavenging capacity

The TEAC for raw samples ranged from 0.2 to 2.84 μmol TE/g dw in 'Freemont Russet' and 'All Blue' respectively (Table 4.5). TEAC for microwaved samples ranged from 0.17 μmol TE/g dw in 'Russet Burbank' to 2.22 μmol TE/g dw in 'All Blue', whereas TEAC for boiled samples ranged from 0.25 μmol TE/g dw in 'Cherry Red' and 'Russet Burbank' to 3.06 μmol TE/g dw in All Blue. Finally, TEAC for baked samples ranged from 0.24 μmol TE/gdw in 'Cherry Red' to 1.4 μmol TE/g dw in 'All Blue'. Table 4.3 shows that 'All Blue' had the highest activity among all cultivars tested, and 'Freemont Russet', 'Russet Burbank', and 'Cherry Red' were the lowest.

The ANOVA statistical summary for the antioxidant activity is presented in (Table 4.8). Cultivars were different ($P<0.0001$, $F=4.549$) and contributed 45.55% of the

variation. Neither interaction nor cooking methods were different. The relationship between TEAC and TP contents in the raw and microwaved samples examined had a correlation coefficient of r= 0.8722 and r=0.8346 respectively, however boiled, and baked samples had lower correlation coefficients r=0.6254 and r=0.5462, respectively (Fig 4.1, 4.2, 4.3, and 4.4).

4.4.6 TP and antioxidant activity content retained after cooking

Table 4.6 shows the content of TP and antioxidant activity retained after cooking. There was a sharp drop of both TP and TEAC activity for almost all cooked cultivars. The content of TP retained in the microwaved samples ranged from 0.00 to 17.16 mg/100g fw in 'Keystone Russet' and 'CO 94183-1R/R' respectively. In boiled samples the content retained ranged from 0.46 mg/100g fw in 'Cherry Red' to 14.82(mg/100g fw) for 'All Blue'. TP content retained in baked samples ranged from 1.79 to 15.24 (mg/100g fw) in 'Chipeta' and 'All Blue' respectively. On the other hand, the content of TEAC activity retained ranged from 0.11 to 1.91 μmol TE/g dw, while boiled samples ranged from 0.005 to 1.8 μmol TE/g dw, and baked from 0.05 to 1.76 μmol TE/g dw. The minus sign in Table 4.6 indicates cultivars in which total phenolics and TEAC activity were higher after cooking compared to uncooked samples (raw).

4.5 Discussion

Potatoes provide one of the main vegetables consumed in American diets. They are always consumed after cooking (boiling, frying, baking, microwaving, etc.). AA content differed depending upon different cooking methods. The uncooked (raw) potato

samples contain 1.5-2 times more AA content than cooked samples (micro-waved, boiled, and baked). However, the AA content in the cooked samples still represents important dietary quantities of AA. Significant losses of AA can occur during any post-harvest storage period and losses can also occur during preparation and cooking of foods, partly due to oxidation and partly due to leaching into the water used for cooking (Fox et al., 1995). Davey et al., (2000) suggested that the milder the cooking and the lower the temperature, the better the retention of AA. Graham et al., (1997) reported irradiation, storage and cooking, both boiling and baking, affected TAA, AA, and DHAA concentrations. However, microwaving was more destructive than boiling.

Love et al., (2004) reported that published retention values for AA-as compared to raw potatoes- depending on the cooking method was from 54% to 82% for blanched French fries, 53% to 80% for preformed patties, 51% to 81% for baked tubers, 88% for microwaved peeled chunks, 85% for boiled chunks, and 25% to 33% for mashed potatoes. In this research the amount of AA retained in microwaved samples ranged from 11 to 58%, in boiled samples from 27 to 56%, and in baked samples from 25 to 62%, which are lower than those published. However, it is very important to take into consideration that AA in fresh food, including potatoes, is not stable. Finlay et al., (2003) reported that the AA levels decrease with time of post-harvest up to 30-60% of the original within the first 2 months of storage and subsequently stabilize at 25% of the original level.

Degradation of AA occurs during processing with absolute losses in the order of 30% (Wills et al., 1990), although the concentration of AA in potato crisps chips can be higher than in fresh potato due to compensating substantial reduction in water during

frying. Will et al., (1990) reported the level of AA in fresh Sebago potatoes ranged from 22-36 mg/100 g. Preparation of mashed potatoes had the greatest effect with about a 50% decrease in AA but roasting, and baking also caused substantial decreases.

The major factors that catalyze the oxidative degradation of the AA are pH, oxygen, and the trace metals copper and iron. Degradation involves oxidation of AA to dehydroascorbic acid followed by hydrolysis to 2,3-diketogulonic acid and further oxidation, dehydration, polymerization, and reaction with amino acids and proteins to generate up to 50 nutritionally inactive products (Han et al., (2004). Friedman, (1997) reported that polyphenol oxidase (PPO) catalyzed the enzymatic browning of AA, may also contribute to storage- and processing induced chemical modification of the vitamin. Low water content and the presence of air in the cooking atmosphere increased the rate of destruction of AA during home processing (Burg et al., 1995).

AA is particularly sensitive to processing conditions. Temperature, as well as pH, water content, presence of oxidizing substances, oxygen, and the presence of metallic and biological catalysts influence its destruction rate. All these factors can play in one-way or another an important role in AA loss and degradation even though the cultivation site (San Luis Valley) was the same. However the cultivation practice in both years may vary. Burg et al., (1995) reported that AA losses take place in some processing and cooking procedures, like blanching in water, by diffusion of the AA inside the potato, followed by leaching into the blanching fluid. Graham et al., (1997) reported that cooking reduced AA and with microwaving proving to be more destructive than boiling. Our data in Table 4.3 were consistent with results reported earlier by Graham et al., (1997). However, the variation in the % AA loss in the cooking methods can be explained by the difference due

to cooking time and temperature. Burge et al., (1995) reported that the dissipation of microwave energy directly in the bulk of the foodstuff allows a rapid increase of the temperature and consequently the shortest cooking time. The condensation of steam at the surface induces a high heat flux toward the foodstuff. Mareschi et al., (1983) reported that unpeeled potatoes lost less AA during cooking than did peeled potatoes, which raises a question of commercial acceptability by customers.

Finally it's important to mention that the stability and retention of AA is not only dependent on the cooking methods, but also on the sample moisture content. However the mechanism by which water controls the reaction is complex (Lee et al., 1975). Water content can affect the dilution of AA, as the moisture content increases AA concentration is lowered which in turn induce a relatively reduced degradation rate.

Our results presented in Table 4.4 showed a difference in the cooking methods used in this research, which agrees with reports by Tudela et al., (2002). Previous studies indicate that potato tubers contain caffeic and derivatives (chlorogenic acid) as the main phenolic constituents (Brandl et al., 1984) and the main biosynthesis of these compounds is affected by external factors such as bruising and wounding (Freidman, 1997). Tudela et al., (2002) also reported that chlorogenic acid constituted up to 70% of the TP content of fresh potato, whereas only 24% was preserved after frying. Friedman, (1997) previously reported that oven-baked, French-fried, and mashed potato contained no chlorogenic acid, whereas boiled and microwaved potato retained 35% and 55% respectively. Comparing our findings to those previously published it is clear that both TP content and antioxidant activity drops due to cooking and the losses vary according to the cultivars and the cooking method used. The amount of loss in the colored cultivars such as 'All

Blue', 'CO 94183-1R/R' and 'CO 94165-3P/P' was higher that that in the white/yellow flesh or russet skin cultivars. The differences observed in phenolic compounds lost and reduced antioxidant activity could be due to both temperature and time of cooking as well as the original content of antioxidants in the cultivar. From results presented in Table 4.3 the variation in Content of TP and antioxidant activity in the raw samples before cooking varied among cultivars and was related to degradation in the content after cooking. In addition, the colored cultivars originally had more TP and TEAC content than the russetted skin or white/yellow cultivars.

These compounds, although in small amounts, can be significant from a dietary point of view. Most cooking treatments preserved at least 50% of total phenolic content available, thus a reasonable amount of the phenolic derivatives are ingested per serving. In addition, although cooking can decrease the TP and antioxidants activity contents of some the potatoes, in others cooking can exert an overall positive effect on phenolic compound bioavailability. Cooking has been shown to have a positive effect on other compounds such as lycopene release from the food matrix in the gastrointestinal tract and their further absorption in the intestine (Van den Berg et al. 2000) and ellagic acid in strawberry (Zafrilla et al. 2001).

The data presented in Table 4.6 show the content of TP and antioxidant activities retained after cooking and provide important information from the commercial point of view. Cooking methods reduced the nutritive value of the potatoes and have a direct impact on the antioxidant health benefits in consumed potatoes. The colored cultivar CO 94183-1R/R, CO 94183-1R/R and All Blue are promising cultivars for commercial use in

microwave, boil and oven-bake cooking due to the lower % loss in both TP and antioxidant activity.

Table 4.1 Means of ascorbic acid content (mg/100gfw) for selected cultivars grown in San Luis valley in 2002 and 2003.

Cultivar	Cooking Method			
	Raw	Microwave	Boil	Bake
All Blue	29.27 [a*]	17.87 [ab]	16.65 [ab]	15.2 [b*]
Centennial Russet	24.78 [a]	15.15 [a]	14.55 [a]	12.3 [a]
Chipeta	21.89 [a]	13.55 [a]	13.2 [a]	11.5 [a]
Freemont Russet	21.15 [a]	12.25 [a]	15.48 [a]	15.84 [a]
Durango Red	21.59 [a]	11.75 [a]	13.85 [a]	13.85 [a]
CO 94183-1R/R	21.69 [a]	12.95 [a]	14.91 [a]	10.85 [a]
CO 94165-3 P/P	24.55 [a]	10.97 [a]	11.35 [a]	10.7 [a]
Cherry Red	17.67 [a]	11.1 [a]	9.6 [a]	8.6 [a]
Keystone Russet	17.16 [a]	15.25 [a]	13.25 [a]	11.4 [a]
Russet Burbank	23.08 [a*]	9.78 [ab]	10.2 [ab]	8.7 [b*]
Russet Norkotah-3	17.71 [a]	12.3 [a]	10.65 [a]	10 [a]
Russet Norkotah-8	21.2 [a]	15.8 [a]	14.2 [a]	12.35 [a]
Sangre-S10	22.84 [a]	16.1 [a]	13.65 [a]	12.85 [a]
Silverton Russet	27.12 [a*]	16.3 [ab]	14.35 [ab]	12.5 [b*]
Yukon Gold	23.65 [a]	15.45 [a]	13.95 [a]	12.5 [a]

[a] Numbers with the same letter within each row are not significantly different ($P > 0.05$) by Bonferroni post test, * significant ($P<0.05$).

Table 4.2 Summary of two-way analysis of variance for ascorbic acid and cooking methods.

Effect	Df	SS	MS	% Variation	F-value
Cooking methods	3	2018	672.7	50.88	32.60***
Cultivars	14	459.5	32.82	11.59	1.591 NS
Interaction	42	250.6	5.967	6.32	0.2892 NS

NS = Not significant (P=>0.05), *** significant (P < 0.0001).

Table 4.3 Means of ascorbic acid content retained in samples after cooking.

Cultivars	Amount AA retained after cooking (mg/100gfw)			
	Raw	Microwave	Boil	Bake
All Blue	29.27 [a]	11.4 [bc]	12.62 [bc]	14.07 [abc]
Centennial Russet	24.78 [a]	9.63 [bc]	10.23 [bc]	12.48 [abc]
Chipeta	21.89 [a]	8.34 [bc]	8.69 [bc]	10.39 [abc]
Freemont Russet	21.15 [a]	8.9 [bc]	5.67 [bc]	5.31 [abc]
Durango Red	21.59 [a]	9.84 [bc]	7.74 [bc]	7.74 [abc]
CO 94183-1R/R	21.69 [a]	8.74 [bc]	6.78 [bc]	10.84 [abc]
CO 94165-3 P/P	24.55 [a]	13.58 [bc]	13.2 [bc]	13.85 [abc]
Cherry Red	17.67 [a]	6.57 [bc]	8.07 [bc]	9.07 [abc]
Keystone Russet	17.16 [a]	1.91 [bc]	3.91 [bc]	5.76 [abc]
Russet Burbank	23.08 [a]	13.31 [bc]	12.88 [bc]	14.38 [abc]
Russet Norkotah-S3	17.71 [a]	5.4 [bc]	7.06 [bc]	7.71 [abc]
Russet Norkotah-S8	21.2 [a]	5.4 [bc]	7.00 [bc]	8.85 [abc]
Sangre-S10	22.84 [a]	6.74 [bc]	9.19 [bc]	9.99 [abc]
Silverton Russet	27.12 [a]	10.82 [bc]	12.77 [bc]	14.62 [abc]
Yukon Gold	23.65 [a]	8.2 [b]	9.7 [b]	11.15 [b]

[a] Numbers with the same letter within row are not significantly different ($P>0.05$), different letters are significant $P<0.001$using one-way ANOVA and Bonferroni posttest.

Table 4.4 % ascorbic acid loss after cooking.

Cultivar	% Loss in microwave	% Loss in boiling	% Loss in baking
All Blue	39	43	48
Centennial Russet	39	41	50
Chipeta	38	40	47
Freemont Russet	42	27	25
Durango Red	46	36	36
CO 94183-1R/R	40	31	50
CO 94165-3P/P	55	54	56
Cherry Red	37	46	51
Keystone Russet	11	23	34
Russet Burbank	58	56	62
Russet Norkotah-S3	31	40	44
Russet Norkotah-S8	25	33	42
Sangre-S10	30	40	44
Silverton Russet	40	47	54
Yukon Gold	35	41	47

Table 4.5 Means of total phenolic content (mg GAE/g dw) and antioxidant activity (μmol TE/g dw) for selected cultivars grown in 2002 and 2003 in the San Luis valley.

Cultivar	Total phenolic content (mg GAE/gdw)				Antioxidant activity (μmol TE/gdw)			
	Raw	Microwave	Boil	Bake	Raw	Microwave	Boil	Bake
Rio Grande	15.19 [a]	12.38 [a]	11 [a]	8.24 [a]	1.50 [a]	1.19 [a]	1.45 [a]	0.87 [a]
All Blue	27.82 [a*]	13.35 [ab]	13 [ab]	12.58 [b*]	2.84 [a]	2.22 [a]	3.06 [a]	1.40 [a]
Centennial Russet	7.56 [a]	7.61 [a]	6.24 [a]	5.47 [a]	0.91 [a]	0.64 [a]	0.75 [a]	0.79 [a]
Chipeta	7.08 [a]	5.11 [a]	5.54 [a]	5.29 [a]	0.23 [a]	0.32 [a]	0.26 [a]	0.26 [a]
Freemont Russet	5.77 [a]	6.28 [a]	6.09 [a]	7.14 [a]	0.2 [a]	0.27 [a]	0.25 [a]	0.30 [a]
Durango Red	12.79 [a]	11.28 [a]	8.97 [a]	9.31 [a]	1.57 [a]	1.70 [a]	1.07 [a]	1.15 [a]
CO 94183-1R/R	28.84 [a*]	11.68 [b*]	10.75 [b*]	11.49 [b*]	2.63 [a]	0.72 [a]	0.82 [a]	0.87 [a]
CO 94165-3P/P	21.23 [a]	12.54 [a]	9.66 [a]	14.14 [a]	1.41 [a]	0.74 [a]	0.59 [a]	0.83 [a]
Cherry Red	6.89 [a]	6.66 [a]	6.43 [a]	7.12 [a]	0.23 [a]	0.27 [a]	0.25 [a]	0.24 [a]
Keystone Russet	7.82 [a]	7.82 [a]	6.72 [a]	9.95 [a]	0.99 [a]	0.88 [a]	0.52 [a]	1.18 [a]
Russet Burbank	7.92 [a]	4.50 [a]	6.46 [a]	5.37 [a]	0.25 [a]	0.17 [a]	0.25 [a]	0.19 [a]
Russet Norkotah-S3	10.45 [a]	9.55 [a]	11.43 [a]	9.78 [a]	0.35 [a]	0.47 [a]	0.47 [a]	0.43 [a]
Russet Norkotah-S8	12.03 [a]	11.29 [a]	7.25 [a]	6.94 [a]	0.34 [a]	0.53 [a]	0.48 [a]	0.35 [a]
Russet Nugget	7.57 [a]	8.4 [a]	8.62 [a]	8.37 [a]	0.41 [a]	0.52 [a]	0.40 [a]	0.36 [a]
Sangre-S10	11.59 [a]	9.93 [a]	7.23 [a]	9.1 [a]	0.29 [a]	0.30 [a]	0.35 [a]	0.36 [a]
Silverton Russet	9.28 [a]	6.41 [a]	7.89 [a]	6.06 [a]	0.22 [a]	0.27 [a]	0.31 [a]	0.28 [a]
Yukon Gold	6.86 [a]	4.38 [a]	5.61 [a]	5.32 [a]	1.05 [a]	0.55 [a]	0.76 [a]	0.68 [a]

[a] Numbers with the same letter within each row for total phenolic and antioxidant activity are not significantly different ($P > 0.05$), * significant $P<0.05$, **$P<0.01$ by Bonferroni posttests.

Table 4.6 Content of total phenolics and antioxidant activity retained after cooking.

Cultivars	Amount of total phenolics (mg GAE/gdw) retained				Amount of antioxidant activity (μmol TE/gdw) retained			
	Raw	Microwave	Boil	Bake	Raw	Microwave	Boil	Bake
Rio Grande	15.19	2.81	4.19	6.955	1.495	0.31	0.05	0.625
All Blue	27.82	14.47	14.82	15.24	2.835	0.62	-0.22	1.435
Centennial Russet	7.575	-0.03	1.335	2.11	0.905	0.265	0.16	0.12
Chipeta	7.075	1.965	1.54	1.79	0.225	-0.095	-0.03	-0.035
Freemont Russet	5.77	-0.51	-0.32	-1.37	0.2	-0.07	-0.05	-0.095
Durango Red	12.79	1.51	3.825	3.485	1.57	-0.13	0.505	0.42
CO 94183-1R/R	28.84	17.16	18.09	17.35	2.63	1.91	1.815	1.76
CO 94165-3P/P	21.23	8.69	11.58	7.09	1.41	0.67	0.82	0.585
Cherry Red	6.885	0.23	0.46	-0.24	0.23	-0.04	0.02	-0.01
Keystone Russet	7.815	0	1.1	-2.13	0.99	0.11	0.475	-0.185
Russet Burbank	7.915	3.42	1.46	2.545	0.25	0.085	0.005	0.065
Russet Norkotah-S3	10.45	0.9	-0.98	0.67	0.345	-0.125	-0.13	-0.085
Russet Norkotah-S8	12.03	0.74	4.785	5.095	0.335	-0.19	-0.14	-0.015
Russet Nugget	7.57	-0.83	-1.05	-0.8	0.41	-0.105	0.015	0.05
Sangre-S10	11.59	1.665	4.365	2.49	0.29	-0.01	-0.06	-0.065
Silverton Russet	9.275	2.865	1.385	3.215	0.22	-0.045	-0.09	-0.055
Yukon Gold	6.855	2.475	1.25	1.535	1.05	0.505	0.29	0.37

^{NS} Not significant ($P>0.05$), ** significant ($P<0.01$), *** significant ($P<0.001$) by Bonferroni posttests. (-) minus sign represents more TP and TEAC in cooked samples than in the raw.

Table 4.7 Summary of analysis of variance for total phenolics.

Effect	Df	SS	MS	% Variation	F-value
Cooking methods	3	362.0	120.7	8.37	5.028 **
Cultivars	16	1560	97.49	36.07	4.062 ***
Interaction	48	770.8	16.06	17.82	0.6691 NS

NS = Not significant (P=>0.05), ** significant (P<0.01), *** significant (P < 0.0001).

Table 4.8 Summary of analysis of variance for antioxidant activity.

Effect	Df	SS	MS	% Variation	F-value
Cooking methods	3	1.549	0.5162	1.65	0.4555 ^{NS}
Cultivars	16	42.66	2.666	45.55	4.549 ***
Interaction	45	9.593	0.1999	10.24	0.3410 ^{NS}

^{NS} = Not significant (P=>0.05), ** significant (P<0.01), *** significant (P < 0.0001).

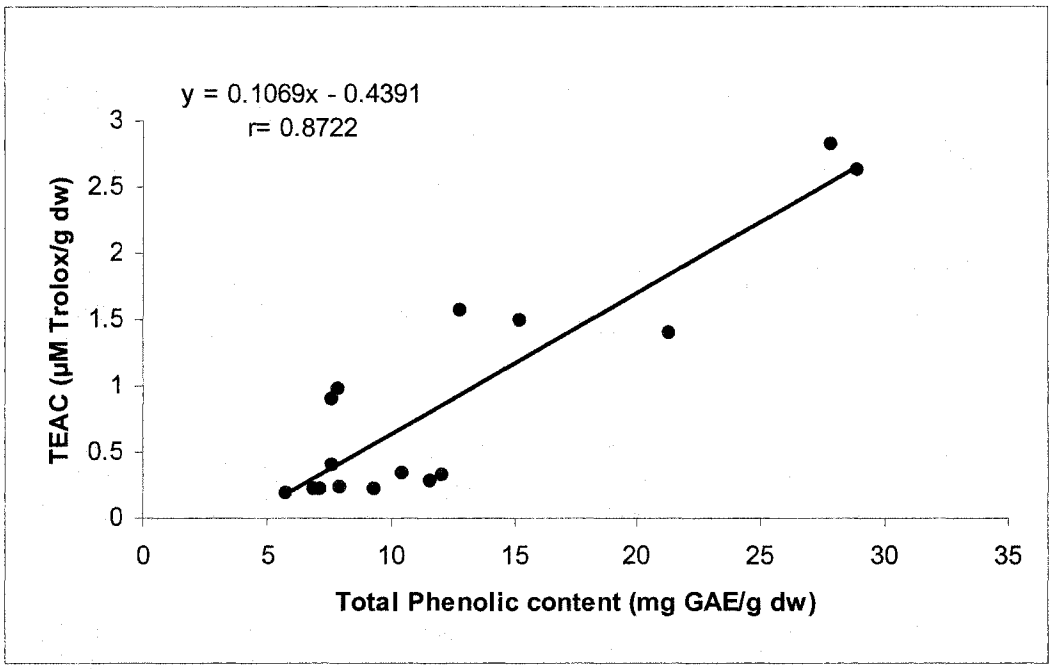

Fig 4.1 Linear correlation of Trolox equivalent antioxidants capacity (TEAC) (*Y*) and total phenolic content (*X*) of raw samples of 17 potato cultivars grown in San Luis valley, Colorado.

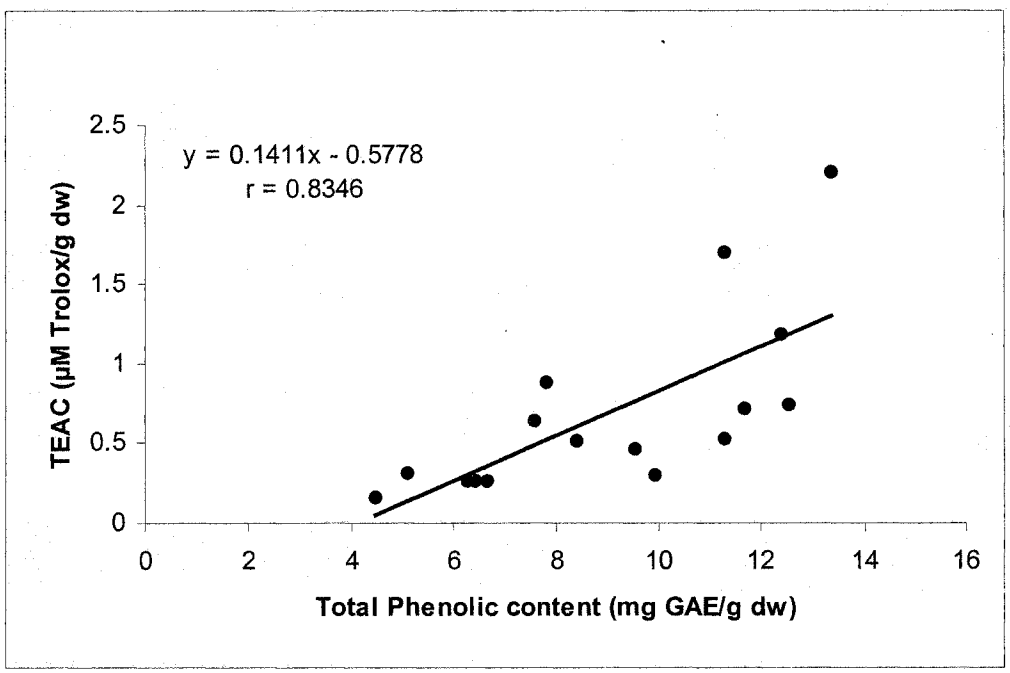

Fig 4.2 Linear correlation between TEAC and TP for microwaved samples of 17 potato cultivars grown in San Luis valley, Colorado.

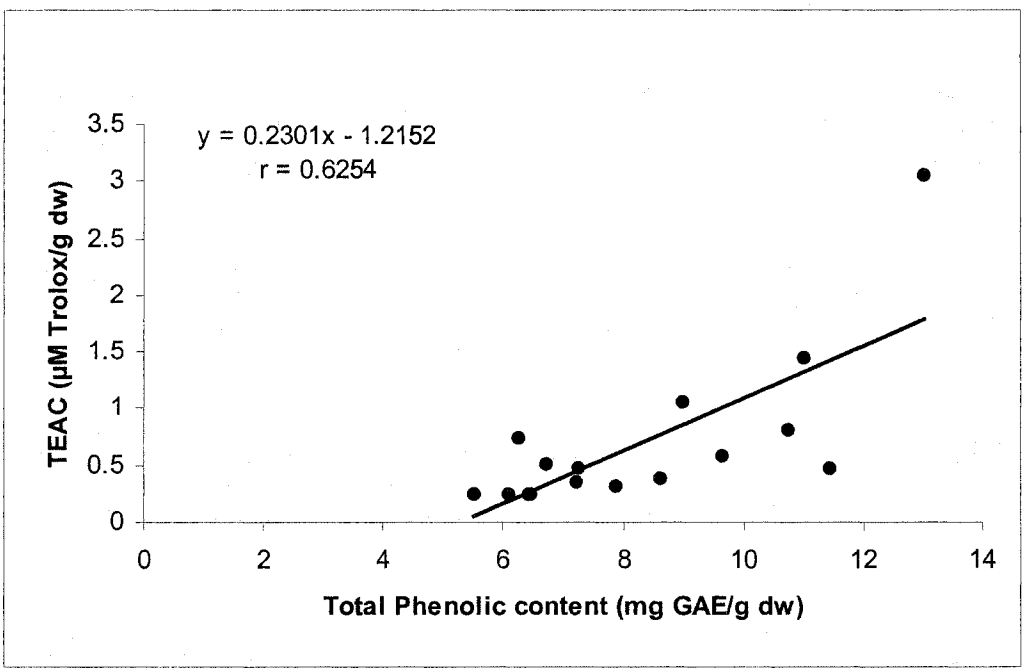

Fig 4.3 Linear correlation between TEAC and TP for boiled samples of 17 potato cultivars grown in San Luis valley, Colorado.

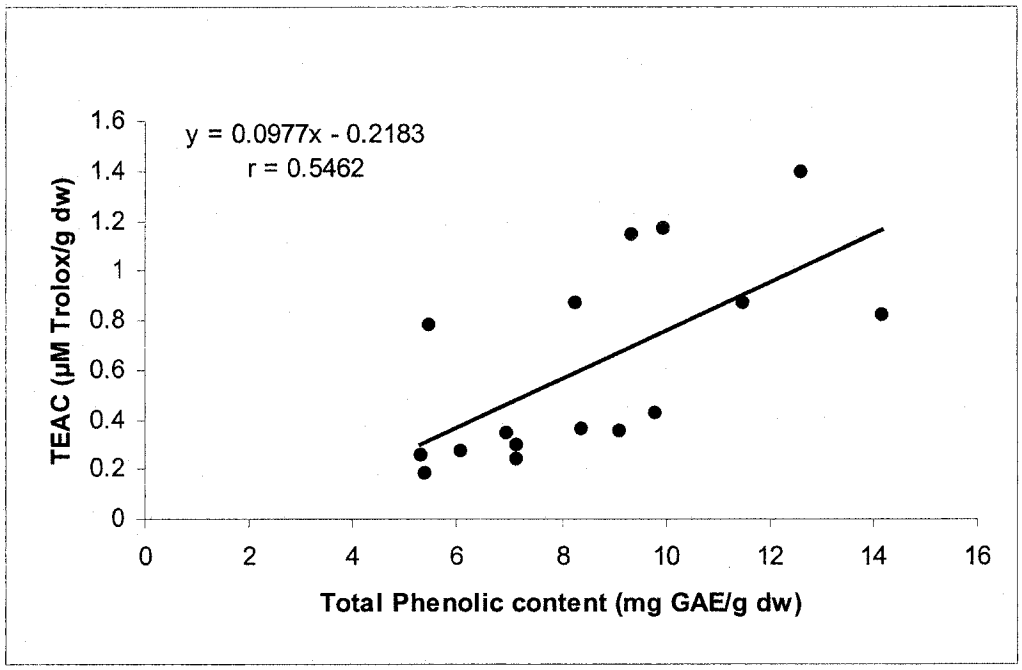

Fig 4.4 Linear correlation between TEAC and TP for baked samples in 17 potato cultivars gown in San Luis valley, Colorado.

References

Alsaikhan MS, Howard LR, Miller JC (1995). Antioxidant activity and total phenolics in different genotypes of potato (Solanum tuberosum L). *J Food Sci* 60 (2): 341-348

Amarine MA, Ough CS (1980). Methods for analysis of musts and wines. *Wiley, New York* 181-184

AOAC (1975). Official methods of analysis of the AOAC, 12th Ed, AOAC, Washington, DC pp 830-831

Arrigoni O, DeTullio MC (2000). The role of ascorbic acid in cell metabolism: between gene-directed functions and unpredictable chemical reactions. *J Plant Physiol* 157: 481-488

Augustin J (1975). Variation in the nutritional composition of fresh potatoes. *J Food Sci* 40: 1295-1299

Augustin J, Johnson C, Teitzel R, Toma R, Shaw R, True J, Hogan, Deutsch R (1978). Vitamin composition of freshly harvested and stored potatoes. *J Food Sci* (43): 1566-1574

Azuma K, Nakayama M, Koshioka M, Ippoushi K, Yamaguchi Y, Kohata K, Yamauchi Y, Ito H, Higashio H (1999). Phenolic antioxidants from the leaves of *Corchorus olitorius* L. *J Agric Food Chem* 47: 3963-3966

Benzie IF, Strain JJ (1999). Ferric reducing (antioxidant) power as a measure of antioxidant capacity: the FRAP assay. *Meth Enzy* 299: 15–27

Berg R. Guido R. Haenen M, Berg H, Vijgh W, Bast A (2000). The predictive value of the antioxidant capacity of structurally related flavonoids using the Trolox equivalent antioxidant capacity (TEAC) assay. *J Food Chem* 70 (3): 391-395

Brandl, W, Herrmann K (1984). On the occurrence of chlorogenic acids in potatoes. *Z* Lebensm Unters.-Forsch 178: 192-194

Brierley ER, Bonner PLR, Cobb AH (1997). Aspects of amino acid metabolism in stored potato tubers (cv. Pentland Dell). *Pla Sci* 127 (1): 17-24

Brown CR, Wrolstad R, Durst R, Yang CP, Clevidence B (2003). Breeding studies in potatoes containing high concentrations of anthocyanins. *Amer J Potato Research* 80 (4): 241-249

Bruin, S, Luyben K (1980). Food process engineering. London: *Applied Science Publishers*

Bu-Contreras R, Rao MA (2001). Influence of heating conditions and starch on the storage modulus of Russet Burbank and Yukon Gold potatoes. *J Sci & Agri* 81 (15): 1504-1511

Buratti S, Pellegrini N, Oreste V, Brenna, Mannino S (2001). Rapid Electrochemical Method for the Evaluation of the Antioxidant Power of Some Lipophilic Food Extracts. *J Agric Food Chem* 49 (11): 5136 – 5141

Burbank, L (1921). How Plants are Trained to Work for Man: Grafting and Budding, *Collier, New York* 2: 352

Burg P, Fraile P(1995). Vitamin C destruction during the cooking of a potato dish. *Lebensm Wiss Technol* 28, 506-514

Burton, WG (1969). Potato. In: *Encyclopedia Britannica* 18: 95-134

Busling BS, Wilson CW, Shaw PE (1982). High performance liquid chromatographic separation of carboxylic acids with anion-exchange and reverse-phase columns. *J Agr Food Chem* 30 (2): 342-345

Cano A, Hernandez-Ruiz J, Garcia-Canovas F, Acosta M, Arnao MB (1998). An end-point method for estimation of the total antioxidant activity in plant material. *Phyto Anal* 9 (4): 196-202

Che Man y, Jaswir I (2000). Effect of rosemary and sage extracts on frying performance of refined, bleached and deodorized (PBD) palm olein during deep-fat frying. *Food Chem* 69: 301-307

Chipault JR (1962). Antioxidants for food use. *Wiley* 477-542

Chu YF, Sun J, Wu XZ, Liu RH (2002). Antioxidant and anti proliferative activities of common vegetables. *J Agric Food Chem* 50 (23): 6910-6916

Coffin R, Moorehead S, Dawson D (1997). Potato research and extension from private industry: Potato production, genetically engineered varieties, pest control and fertility. *Can J Agri Econo-Revue* 45 (4): 461-469

Davey MW, Van Monatgu M, Sanmatin M, Kanellis A, Smirnoff N, Benzie IJJ, Strain JJ, Favell D, Fletcher J (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *J Sci Food Agric* 80: 825-860

Dennison DB, Brawley TG, Hunter GL (1981). Rapid high-performance liquid chromatography determination of ascorbic acid and combined ascorbic acid – dehydroascorbic acid in beverages. *Food Technol* 32 (6): 927-929

Dodds, KS (1962). Classification of cultivated potatoes. In. The Potato and its Wild Relatives: *Section Tuberarium of the Genus Solanum* 517-539

Douches, D (1996). Assessment of potato breeding progress in the United States over the last century. *Crop Sci* (in press)

Everett, T. H (1981). The New York Botanical Garden Encyclopedia of Horticulture. Garland, New York and London 3596

Feustel, I. C (1987). Miscellaneous products from potatoes. In: Potato Processing 4: 727-746

Finlay M, Dale B, Griffiths D, Drummond T (2003). Effects of genotype, environment, and postharvest storage on the total ascorbate content of potato (*Solanum tuberosum*) tubers. *J Agric Food Chem* 51 (1): 244 -248

Food and Agriculture Organization (FAO). 2001 Statistical databases, www.fao.org, as of 10.12.2004

Fox BA. Cameron AG (1995). Food science, nutrition and health. Vitamins. J. W. Arrowsmith Ltd., Bristol, UK 13: 236-242

Frankel EN, Meyer AS (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *J Sci Food Agri* 80 (13): 1925-1941

Friedman M (1997). Chemistry, biochemistry, and dietary role of potato polyphenols. J *Agri Food Chem* 45: 235-277.

Geigert J, Hirano DS Neidleman SL (1981). High-performance liquid chromatography method for the determination of L-ascorbic acid and D-ascorbic acid. *J Chrom* 206: 396-399

George J. Soleas, George Tomlinson, Eleftherios P. Diamandis, and David M. Goldberg (1997). Relative contributions of polyphenolic constituents to the antioxidant

status of wines: Development of a predictive model. *J Agric Food Chem* 45 (10): 3995-4003

Gil M, Francisco A. Barberan T, Hess-Pierce B, Holcroft D, Kader A (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J Agric Food Chem* 48 (10): 4581- 4589

Graham WD, Stevenson MH (1997). Effect of irradiation on vitamin C content of strawberries and potatoes in combination with storage and with further cooking in potatoes. *Sci Food Agri* 75 (3): 371-377

Granda C, Moreira RG, Tichy SE (2004). Reduction of Acrylamide Formation in Potato Chips by Low-temperature Vacuum Frying. *J Food Sci* 69: (8) 405-11

Haines M, Berger P, Fellma J, Shiel P (2003). Abnormalities in growth, development and physiological responses to biotic and abiotic stress in potato (Solanum tuberosum) transformed with Arabidopsis ETR1. *J Agri Sci* 141: (3) 333-347

Halliwell B, Gutteridge JM (1989). Free radicals in biology and medicine. Clarendon, oxford, UK 22-85

Hamama A, Nawar W (1991). Thermal decomposition of some phenolic antioxidants. *J Agri Food Chem* 39: 1063-1069

Hamouz K, Lachman J, Vokal B, Pivec V (1999). Influence of environmental conditions and way of cultivation on the polyphenol and ascorbic acid content in potato tubers. *Ros Vyr* 45 (7): 293-298

Han JS, Kozukue N, Young KS, Lee KR, Friedman M (2004). Distribution of ascorbic acid in potato tubers and in home-processed and commercial potato foods. *J Agric Food Chem* 52: 6516-6521

Hasse NU, Plate J (1996). Properties of potato starch in relation to varieties and environmental factors. *Starch/Stake* 48: 167-171

Hizukuri S (1985). Relationship between the distribution of the chain length of amylopectin and the crystalline structure of starch granules. *Carbohydr Res* 141: 295-306

Huaman Z, Schmiediche P (1999). The potato genetic resources held in trust by the International Potato Center (CIP) in Peru. *Pot Res* 42 (3-4): 413-426

Ibarz, A, Pagan J, Garza, S (1999). Kinetic models for color changes in pear puree during heating at relatively high temperatures. *J Food Eng* 39: 415–422

Jadhar S, Steele L, Hadziyer D (1975). Vitamin C losses during production of dehydrate mashed potatoes. *Lebensm Wiss U. Tech* 8: *225-228*

James A, Okeyo, Mosbah M. (1995). Composition of four potato cultivars in relation to cold storage and reconditioning. *HorTech* 5: 250-252

James AO, Mosbah MK (1995). Composition of four potato cultivars in relation to cold storage and reconditioning. *HortTech* 5: 250-253

Jaswir I, Kitts DD, Che Man YB (2000). Use of natural antioxidants in refined palm olein during repeated deep-fat frying. *Food Res Inter* 33: 501-508

Jesus F, Alcolea, Cano A, Acosta A, Marino B. Arnao (2002). Hydrophilic and lipophilic antioxidant activities of grapes. Nahr Food 46 (5): 353 – 356

Jewell, L. D. 1988. Agricultural Statistics 1987. U. S. Department of Agriculture, Washington, D.C 541

Joe A, Yong H, Xuehui S, Ligia Z (1998). Phenol antioxidant quantity and quality in foods: vegetables. *J Agric Food Chem* 46: 3630-3634

Joshi MR, Srirangarajan AN, Thomas P (1990). Effects of gamma irradiation and temperature on sugar and vitamin C changes in five Indian potato cultivars during storage. *Food Chem* 35 (3): 209-216

Justenes U, Knuthsen P, Leth T (1997). Determination of plant polyphenols in Danish foodstuff by HPLC-UV and LC-MS detection. *Cancer Lett* 114: 165-167.

Katsube T, Tabata H, Ohta Y, Yamasaki Y, Anuurad E, Shiwaku K, Yamane Y (2004). Screening for Antioxidant Activity in Edible Plant Products: Comparison of Low-Density Lipoprotein Oxidation Assay, DPPH Radical Scavenging Assay, and Folin-Ciocalteu Assay. *J Agric Food Chem* 52 (8): 2391-2396

Keijbets M, Ebbenhorst G (1990). Loss of vitamin C during long-term cold storage of Dutch table potatoes. *Potato Res* 33: 125-130

Kelly GJ, Latezeko E (1981). Prospect of a specific of enzymatic assay for ascorbic acid (vitamin C). *J Agri Food Chem* 28: 1320-1321

Khraisheh MA, McMinn WA, Magee TR (2004). Quality and structural changes in starchy foods during microwave and convective drying. *Food Res Inter* 37 (5): 497-503

Kim Y, Lee K, lee H (2004). Total antioxidant capacity of arginine-conjugated-linoleic acid (CLA) complex. *J Agric Food Chem* 52 (3): 439-444

Koutroubakis IE, Malliaraki N, Dimoulios PD, Karmiris K, Castanas E, Kouroumalis EA (2004). Decreased total and corrected antioxidant capacity in patients with inflammatory bowel disease. *Dig Dis & Sci* 49 (9): 1433-1437

Kumari N, Mukherjee D (1990). Influence of low temperature on ascorbic acid changes in potatoes during storage. *Indian J Horti* 268-270

Laanest L, Tohver A, Palm E (1995). Soluble phenolics and alicyclic acids in aging potato tuber slices. *Eesti Tead Akad. Toim Biol* 44: 1-10

Labuza,T, McNally, L, Gallagher, D, & Hawkes, J (1977). Degradation of ascorbic acid in dehydrated food system. *J of Food Sci* 42 (50): 1274-1279

Lee SH, Labuza TP. (1975). Destruction of ascorbic acid as a function of water activity. *J Food Sci* 40: 370-373

Lewis CE, Walker JRL, Lancaster JE, Sutton KH (1998). Determination of anthocyanins, flavonoids and phenolic acids in potatoes. I: Colored cultivars of Solanum tuberosum L. *J of the Sci of Food & Agri* 1: 45-57

Linnemann AR, Andre VS, Hartmans KJ (1984). Changes in the content of L-ascorbic acid, glucose, fructose, sucrose and total glycoalkaloids in potatoes (cv. Bintje) stored at 7, 16, and 28°C. *Pot Res* 28: 271-278

Lister C (1999). Natural antioxidants in fruits and vegetables. *Leath Food RA Food Ind J* 2: 251-253

Lister C (2003). Antioxidants and health revolution. *The Vital vegetable project.* 52

Lister E, Wilson P (2001). Measurement of total phenolics and ABTS assay for antioxidant activity (personal communication). Crop Res Inst., Lincolon, New Zealand

Love SL, Salaiz T, Shafii B, Price WJ, Mosley AR, Thornton RE (2004). Stability of expression and concentration of ascorbic acid in North American potato germplasm. *Hort Sci* 39 (1): 156-160

Lozano JE, Ibarz, A (1997). Color changes in concentrated fruit pulp during heating at high temperatures. *J Food Eng* 31: 365–373

Mai-Houng, Bui-Nguyen (1980). Application of high performance liquid chromatography to the separation of ascorbic acid from isoascorbic acid. *J Chrom* 196: 163-165

Maltseva AP, Derid TF, Shalinova RT (1967). Effect of ionizing radiation on the change in the content of ascorbic acid in potatoes. *Mater Nauch Prakt Kont Ispotz ioniz Izuluch Nat Khoz* 137-140

Mareschi, JP, Belliot JP, Fourlon C, Gey KF (1994). Changes in vitamin C content of Bintje potatoes during storage and usual culinary preparation (in French). *Int. J Vit. Nutr Res* 53: 402-411

Marilyn G, Steven R, Norman P (1994). Chilling sensitivity of the frost-tolerant potato *Solanum commersonii. Phys Planta* 90: 319-326

Marino B, Arnao, Antonio C, Acosta M (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. *J Food Chem* 73 (2): 239-244

Marja P, Anu I, Heikki J, Jussi-Pekka R, Kalevi P, Tytti S, Marina H (1999). Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem* 47: 3954-3962

Marja P, Hopia A, Vuorela H, Rauha J, Pihlaja K, Kujala T, Heinonen M (1999). Antioxidant activity of plant extracts containing phenlic compounds. *J Agric Food Chem* 47: 3954-3962

Mark W Davey, Marc Van Montagu, Dirk Inze, Maite Sanmartin, Angelos Kanellis, Nicholas Smirnoff, Iris JJ Benzie, John J Strain, Derek Favell, John Fletcher (2000). Review Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *J Sci Food Agric* 80: 825-860

Marsili RT, Ostapenko H, Simmons RE, Green DE (1981). High performance liquid chromatographic determination of organic acids in dairy products. *J Food Sci* 46: 52-57

Mask an, M. (2001). Kinetics of color change of kiwifruits during hot air and microwave drying. *J Food Eng* 48: 169–175

Matsuura, EC, Kobayashi A, Noda N, Takeaway S, Yamauchi H, Mori M (2004). Changes in sugar content and activity of vacuolar acid inverts during low-temperature storage of potato tubers from six Japanese cultivars. *J Plant Res* 117: 131 - 137

Mendez C, Delgado M, Rodriguez E, Romero (2004). Content of free phenolic compounds in cultivars of potatoes harvested in Tenerife (Canary Islands). *J Agric Food Chem* 52 (50): 1323-1327

Miller NJ, Rice-Evans CA (1996). Spectrophotometric determination of antioxidant activity. *Redox Rep* 2: 251-264

Moledina KH, Flink JM (1982). Determination of ascorbic acid in plant food products by high performance liquid chromatography. *Lebensm Wiss u-Technol* 15: 351-358

Mondy NI, Gosslin B (1988). Effect of peeling on total phenols, total glycoalkaloids, discoloration and flavor of cooked potatoes. *J Food Sci* 53: 756-759

Nenadis N, Wang L, Tsimidou M, Zhang H (2004). Estimation of Scavenging Activity of Phenolic Compounds Using the $ABTS^{\bullet+}$ Assay. *J Agric Food Chem* 52 (15): 4669-4674

Nirmala K, Mukherjee D (1984). Influence of low temperature on ascorbic acid changes in potatoes during storage. *Ind J Hort* 268-270

Nourian F, Ramaswamy H, Kushalappa A (2003). Kinetic changes in cooking quality of potato stored at different temperatures. *J Food Engi* 60: 257-266

Orsak M, Lachman J, Vejdova M, Pivec V, Hamouz K (2001). Changes of selected secondary metabolites in potatoes and buckwheat caused by UV, gamma- and microwave irradiation. *Rost V* 47 (11): 493-500

Ortiz R., Golmirzaie A (2004). Genotype by environment interaction and selection in true potato seed breeding. *Exp Agri* (40): 99-107

Pachla LA, Kissinger PT (1976). Determination of ascorbic acid in food stuff, pharmaceuticals, and body fluid by liquid chromatography with electrochemical detection. *Annal Chem* 48 (2): 364-367

Paganga G, Miller N, Rice-Evans C (1998). The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute. *Free Rad Res* 30: 153-162

Page, O. T. 1982. Utilization of the Genetic Resources of the Potato III. International Potato Center, Lima, Peru. Page 235

Page, O. T. 1985. Innovative Methods for Propagating Potatoes. International Potato Center, Lima, Peru. Page 342

Park, W. J (1989). Park Seed: Flowers and Vegetables, Seed Catalogue. Park Seed Company, Greenwood, South Carolina. Page 132

Pellegrini N, Visioli F, Buratti S, Brighenti F (2001) *J Agric Food Chem* 49 (5): 2532-2538

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Rad Bio Med* 26: 1231-1237

Reyes LF, Cisneros-Zevallos L (2003). Wounding stress increases the phenolic content and antioxidant capacity of purple-flesh potatoes (Solanum tuberosum L). *J Agri and Food Chem* 51 (18): 5296-5300

Reyes LF, Miller JC, Cisneros-Zevallos L (2004). Environmental conditions influence the content and yield of anthocyanins and total phenolics in purple- and red-flesh potatoes during tuber development. *Ameri J Pot Res* 81 (3): 187-193

Rhodes M (1985). The physiological significance of plant phenolic compounds. The biochemistry of plant phenolics. *Annu Proce Phytoche Scei Eur* 25: 99-117

Rice-Evans C, Miller N, Bolwell P, Bramley p, pridham J (1995). The relative antioxidant activities of plant derived polyphenolic flavonoids. *Free Rad Res* 22: 375-383

Rivero R, Rodriguez E, Romero C (2003). Effects of current storage conditions on nutrient retention in several varieties of potatoes from Tenerife. *Food Chem* 80 (4): 445-450

Rizzolo A, Forni E, Polesello A. (1984). HPLC assay of ascorbic acid in fresh and processed fruit and vegetables. *Food Chem* 14:189-199

Rodriguez De Sotillo D, Holm ET, Hadley, M (1994). Potato peel waste: stability and antioxidant activity of a freeze-dried extract. *J Food Sci* 59 (5): 1031-1033

Rogan JR, Bookout, JT, Duncan DR, Fuchs RL, Lavrik PB, Love SL, Mueth M, Olson T, Owens E, Raymont P, Zalewski J (2000). Compositional analysis of tubers from insect and virus resistant potato plants. *J Agric Food Chem* 48: 5936–5945

Rogan JR, Marks BP, Gonzalez AR, Fuchs RL, Lavrik PB, Love SL, Mueth M, Olson T, Owens E, Raymont P, Zalewiski J (2000). Compositional analysis of tubers from insect and virus resistant potato plants. *J Agric Food Chem* 48: 5936-5945

Ross H (1986). Potato Breeding: Problems and perspectives: Advances in plant breeding, Supplement 13 to Journal of Plant Breeding. Paul Parey, Berlin and Hamburg. Page 132

Salkova EG (1975). The influence of irradiation with radioactive cobalt 60 on vitamin C content in potatoes. *Dokl Akad Nauk* 114: 757-759

Schlack JE (1974). Quantitative determination of L-ascorbic acid by gas-liquid chromatography. *JAOAC* 57 (6): 1346-1348

Shahidi F (2004). Functional foods: Their role in health promotion and disease prevention. *J Food Sci* 69 (5): 146-149

Shaker V, Iritani W, Arteca R (1978). Changes in ascorbic acid content during growth and short-term storage of potato tubers. *Am Potato* J 55: 663-670

Shehidi F, Naczk M (1995). Phenolic compounds in fruits and vegetables. *Food Pheno* 75-107

Shepard, JF, Bidney, D, Shahin E (1980). Potato Protoplasts in Crop Improvement. *Science* 208: 17-24

Sinden S, Webb R, Sandford L (1978). Genetic potential for increasing ascorbic acid content in potatoes. *Am potato J* 55: 394-395

Singh N, Rajini PS (2004). Free radical scavenging activity of an aqueous extract of potato peel. *FOOD Chem* 85 (4): 611-616

Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Oxidants and antioxidants. *Pt A* 299: 152-178

Sook Han J,Kozukue N, Young K, Lee K, Friedman M (2004). Distribution of Ascorbic Acid in potato tubers and in home-processed and commercial potato foods. *J Agric Food Chem* 52: 6516-6521

Sponos GA, Worlstad RE (1990). Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. *J Agric Food Chem* 38: 1565-1571

Stephen L, Jeffrey C, Joseph F (2003). Potato production systems. Ch 1 p 1.

Storey R, Davies H (1992). Strawberries and Potatoes in Combination with Storage and with Further Cooking in Potatoes. *J Sci Food Agric* 75: 317-377

Stushnoff C, Cox S, Sampson D (2003). Relationship of fruit color and light exposure to lycopene content and antioxidant properties of tomato. *Can J Plant Sci* 913-919

Suttle JC (2004). Physiological regulation of potato tuber dormancy. *Amer J Potato Res* 81 (4): 253-262

Swain T, Hillis WE (1959). The phenolic constituents of Prunus domestica . The quantitative analysis of phenolic constituents. *J Sci Food Agri* 10: 63-68

Takatsuki S, Nemoto S, Sasaki K, Maitani T (2003). Production of acrylamide in agricultural products by cooking. *J Food Hyg Soc Jap* 45 (1): 44-48

Takuya K, Hiromasa T, Yukari O, Yukikazu Y, Erdembileg A, Kuninori S,Yosuke Y (2004). Screening for Antioxidant Activity in Edible Plant Products: Comparison of Low-Density Lipoprotein Oxidation Assay, DPPH Radical Scavenging Assay, and Folin-Ciocalteu Assay. *J Agric Food Chem* 52 (8): 2391-2396

Talburt, WF (1987). History of potato processing. In: Potato processing , 4th Ed., pp. 1-10. Talburt, W. F., Smith, O., Eds. Van Nostrand, New York. 796 pp

Thomas P (1984). Radiation preservation of foods of plant origin. Part 1. Potatoes and other tuber crops. *CRC Crit Rev Food Sci Nut* 19(4): 327-379

Treadway R (1975). Potato starch (Chapter 15) in: Talburt W.F. and Smith O. (Eds.): Potato Processing. 3rd ed. Avi Publ. Co. Westport. CT. USA. 546-562.

Tudela J, Tomas A, Gil M, Cantos E, Espin J (2002). Induction of antioxidant flavonol biosynthesis in fresh-cut potatoes. Effect of domestic cooking. *J Agric Food Chem 50* (21): 5925-5931

Van Del Berg H. Faulks R. Granado F. Hischberge J. Olmedilla B. Sandmann G. Southon S. Stahl W (2000). The potential for the improvement of carotenoid levels in foods and the likely systemic effects. *J Sci Food Chem* 45: 590-595

Van Der Slice J, Higgs D, Hayes J, Block G (1990). Ascorbic acid and dehydroascorbic acid content of foods-as-eaten. *J Food Comp Anal* 13: 105-110

Velioglu Y, Mazza G, Gao L, Oomah B (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J Agric Food Chem* 46: 4113-4117

Villano D, Fernandez-Pachon MS, Troncoso AM, Garcia-Parrilla MC (2004). The antioxidant activity of wines determined by the ABTS(*+) method: influence of sample dilution and time. *Talanta* 64 (2): 501-509

Vokal B, Hamouz K, Cepl J (2003). The effect of different ecological conditions of growing on cooking quality of potato tubers. *Ros Vyr* 46 (11): 487

Wang H, Cao G, Prior R (1996). Total antioxidant capacity of fruits. *J Agric Food Chem* 44: 701-705

Wang QB, Zhang W (2004). China's potato industry and potential impacts on the global market. *Amer J Potato Res* 81 (2): 101-109

Wang XY, Kozempel MG, Hicks KB, Seib PA (1992). Vitamin C stability during preparation and storage of potato flakes and reconstituted mashed potatoes. *J Food Sci* 57: 1136-1139

Wang-Pruski G, Nowak J (2004). Potato after-cooking darkening. *Amer J of Potato Res* 81 (1): 7-16

Weber L, Putz B (1998). Vitamin C in Kartoffeln, Kartoffelbau 49: 278-281

Wheeler GL, Jones MA, Smirnoff N (1998). The biosynthetic pathway of vitamin C in higher plants. *Nature* 393: 365-369

Will R, Suthilucksanavanish K (1991). Seasonal variation in vitamin C and nutrient composition of processed potato product. *Food Aust* 43(1): 19-29

William A (1951). Potato crisps. Food Trade Press: London.

Wills BH. McGlasson W B, Graham D, Lee T H, hall E G (1989). Commodity treatments. In: postharvest- An introduction to the physiology and handling of fruit and vegetables, (3rd edn). BSP Professional Books, Oxford UK, pp 114-131

Wills R, Silalahi J (1990). Effect of storage temperature on loss of vitamin C in potato crisps. *J Sci Food Agric* (53): 275-278

Wills RB, Jansen S (1990). Effect of storage temperature on loss of vitamin C in potato crisps. *J Sci Food Agric* 53: 275-278

Wilson PE, Lister CE (2000). Can a single measurement reveal the true antioxidant potential of vegetables? *Anti & Nutri* 121

Winston G, Regoli F, Dugas A. Fong J, Blanchard K (1998). A rapid gas chromatography assay for determining oxyradical scavenging capacity of antioxidants and biological fluids. *Free Rad Biol Med* 24: 480-493

Wioletta B, Jadwiga S, Fornal JZ, Josef V, Bogdan F, Włodzimierz ZR (2004). Influence of cooking and microwave heating on microstructure and mechanical properties of transgenic potatoes. *Nahrung/Food* 48: 169 – 176

Yusuph M. Tester RF, Ansell R and Snap CE (2003). Composition and properties of starches extracted from tubers of different potato varieties grown under the same environmental conditions. *Food Chem* 82: 283-289

Zafrilla P. Ferreres F. Tomas-Barberan F (2001). Effects of processing and storage of the antioxidant ellagic acid derivatives and flavonoids of red raspberry jams. *J Sci Food Agri* 49: 3651-3655