THE EFFECTS OF COOKING, STORAGE, AND IONIZING IRRADIATION ON CAROTENOIDS, ANTIOXIDANT ACTIVITY, AND PHENOLICS IN POTATO (*Solanum tuberosum* L.)

A Thesis

by

TYANN BLESSINGTON

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

MASTER OF SCIENCE

August 2005

Major Subject: Horticulture

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Approved by:

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ABSTRACT

The Effects of Cooking, Storage, and Ionizing Irradiation on Carotenoids, Antioxidant Activity, and Phenolics in Potato (*Solanum tuberosum* L.). (August 2005)

Tyann Blessington, B.S., Texas A&M University

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

Past research conducted by our lab demonstrated that potatoes contain significant levels of phytochemicals important to human health. However, since potatoes are not consumed raw, it is important to determine the effects of processing on these levels. Therefore, the changes in carotenoid content, antioxidant activity, and phenolic content were investigated using combinations of cultivars, cooking methods, storage treatments, and low-dose ionizing irradiation. Carotenoid content was measured via absorbance at 445 nm, 450 nm, and HPLC identification. Antioxidant activity was measured initially and at stabilization via the DPPH method and phenolic content was measured via the Folin method and HPLC identification.

Microwaved, baked, fried, and raw potato samples contained more carotenoids than boiled samples. The samples microwaved, baked, and fried contained higher antioxidant activity and phenolics than the boiled or raw samples. However, the compound quercetin dihydrate appeared to decrease with cooking.

Carotenoids, antioxidant activity, and phenolics appeared to decrease with storage; however, high storage temperatures and long storage times were believed to cause a dehydration and concentration of compounds, which caused levels to be equal to or greater than before storage. However, this decreasing trend was not linear and there were multiple significant interactions. The compound chlorogenic acid appeared to be quite sensitive to high temperature storage.

Irradiation dose appeared to have only a minor, if any, effect on carotenoid levels. The interaction between storage time and irradiation dose was very influential on antioxidant activity. In early stages of storage, higher doses of irradiation had greater

antioxidant activity, while, with continued storage, low doses had higher antioxidant activity. Exposure to irradiation appeared to cause an increase in phenolic content, determined by the Folin method.

There may be a stimulation, induction, or release of some compounds due to processing; however, its magnitude is not believed to be as great as genetic control. The effects of processing can not be denied and should continue to be investigated. Future studies investigating the health properties of fruits and vegetables, particularly potatoes should include processing effects.

DEDICATION

This study is dedicated to my family, Thomas and Terry Blessington, Trisha, Christopher, and Emma Kate Haley, and Rita Zimmer, and to my splendid friends who have supported and taught me throughout the years.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

TABLE Page

TABLE Page

TABLE Page

TABLE Page

TABLE Page

TABLE Page

TABLE Page

TABLE Page

TABLE Page

TABLE Page

CHAPTER I
INTRODUCTION

The correlation between diet and health is becoming recognized. Numerous epidemiological studies have reported that societies consuming more fruits and vegetables have a lower incidence of chronic disease. It is believed that one of the main reasons for this is the antioxidant and other phytochemical content of fruits and vegetables. Although many studies have reported that disease prevention is related to increased consumption of fruits and vegetables, the average consumption of fruits and vegetables has decreased. According to the CDC 5-a-day program, people reporting never eating or consuming less than 1 fruit or vegetable a day was 3.6% in 1996, 4.7% in 2002, and 6.0% in Texas in 2002; those reporting eating 1 or 2 fruits and vegetables in 1996 were 32.3%, 35.9% in 2002, and 37.7% in Texas in 2002; those reporting eating 3 or 4 fruits and vegetables in 1996 was 40.4%, 36.1% in 2002, and 32.5% in Texas in 2002; and those eating 5 or more fruits and vegetables in 1996 was 23.6%, 22.6% in 2002, and 23.9% in Texas in 2002.

While not fully appreciated, the potato (*Solanum tuberosum* L.) contains high levels of nutritious and health promoting compounds, including antioxidants. Past experiments have shown that there is significant variability among potato genotypes. Hale (2003) reported that the antioxidant activity of 191 potato genotypes from Field Day Trials in 2000 and 2001 averaged from 104 to 590 µg trolox eq/gfw; the carotenoid content for the same 191 genotypes ranged from 97 to 536 µg eq/100gfw. The highest genotypes had significant levels of specific phenolics such as chlorogenic acid (26 to 329 µg /gfw), caffeic acid (33 to 41 µg /gfw), and rutin hydrate (7 to 306 µg /gfw). The highest genotypes also had significant levels of specific carotenoids such as lutein (14.25 to 48.75 µg /100gfw), violaxanthin (11.95 to 39.55 µg /100gfw), and antheraxanthin (found in one genotype, 18.40 µg /100gfw) (Hale, 2003).

This thesis follows the format and style of the Journal of the American Society for Horticultural Science.

The antioxidant activity trait is heritable and can be used in breeding programs to select new cultivars for the potato industry, or for use as parental material in developing improved genotypes of potato (Al-Saikhan, 1994, 2000; Hale, 2003).

There are significant levels of antioxidants, phenolics and carotenoids within cultivars and advanced breeding lines in the Texas Potato Variety Development Program. However, it is unknown how these levels are affected by postharvest processing. Historically, the potato has been a food of convenience, as well as a model system for new food processing technologies. Currently, numerous food products are derived from various cooked forms of potato. Potatoes are a food that can, and often is, stored over long time periods prior to consumption. Lengthy storage can cause some undesirable changes to the food product, such as sprouting and dehydration. Low-dose ionizing irradiation has been proposed to prevent sprouting in potatoes. This method is not used on a large scale currently, but it may be used in the future. Any postharvest processing technique might cause changes in the levels of important antioxidant phytochemicals. Therefore, the present investigation was designed to analyze the effects of the postharvest processing techniques of cooking, storage, and low-dose irradiation on popular processing cultivars and selected advanced selections from the Texas Potato Variety Development Program.

CHAPTER II
LITERATURE REVIEW

Background

HISTORY OF THE POTATO. It is believed that over 13,000 years ago wild potatoes grew on the Chilean coast. Later, some wild species are believed to have migrated to the altiplano in the central Andean highlands. The altiplano's environment consists of windy valleys and plateaus with poor soils that are at least 12,000 feet above sea level. The potato was able to survive the stressful conditions of poor soil, high altitude, and drought due to its ability to produce tubers that supply it with energy through hard times. Andean altiplano people between Lake Titicaca and Poopó began farming potatoes about 7,000 years ago (Burton, 1989).

The Spanish Conquistadores encountered the Inca empire around 1537 and found potato cultivation well established. By 1600, the potato had been introduced into Spain, Italy, Austria, Belgium, Holland, France, Switzerland, England, Germany, Portugal and Ireland. At this time it was solely a garden crop, which is unfortunate due to the large population in Europe that was undernourished. Many viewed the potato as evil and never touched it. To them the plant looked like a savage; the tuber was believed to be magical, and therefore evil because it could re-grow. Many viewed the potato as a "Frankenstein plant" because the flower looked like the eggplant, the stems like the tomato, and the berries like the mandrake or the nightshade. Many feared any plant from the nightshade (*Solanum* sps.) family, which was derived from the Latin word *solamen* meaning "quieting". The mandrake had a reputation of hastening menstruation, tobacco had been known to deter insects, and the potato was believed to produce leprosy and skin diseases due to its rough skin. Also at that time, any food that came from the ground represented death. Many misconceptions about the potato still live today. One can commonly hear the insults of *couch potato* or *potato head*, while someone with two left feet "dances like a sack of potatoes". Also the French use the statement "potato blood" if one is sluggish. Numerous other jokes are related to the fact that potato is a food that was enjoyed by both slaves and masters (Zuckerman, 1998).

The English feared the potato because it was a vegetable. Vegetables occupied a lower rank because the proper English diet mainly consisted of meat and bread. The poor also ate many vegetables such as onions, leeks, and parsnips. The view was that root crops were unhealthy because they grew in the ground. Not only did potatoes grow in the ground, but they grew more deeply than onions and leeks and were not attached to the foliage. Those that ate potatoes were viewed as desperate and inferior people. The French ate more fruits and vegetables than the English, but they did not eat the potato because the Bible never mentioned it. In Prussia and Russia, peasants feared that the potato would bring cholera, scrofula, rickets, and tuberculosis (Zuckerman, 1998).

Legend states that Sir Walter Raleigh brought the potato to Ireland in the 1590s. Oats were a staple food for most Irish, but many went hungry during the winter. Bread was also often eaten but was difficult for many poor people to obtain. The Irish began growing the potato as a field crop in the seventeenth century and it became a staple in the eighteenth century. For many poor Irish, the potato provided the balance between sustenance and starvation. The diet for most poor Irish was milk and potatoes which supplied all essential nutrients needed for good health. It is believed that each member of a peasant family consumed on average 5.5 pounds of potato daily, and one acre could feed six people for a year (Zuckerman, 1998). The Irish farmed, but sold the grain they grew and ate potatoes that they grew in small plots. Depending on a single crop was extremely risky, high rents and a miserable wage made the potato the only food choice for many. The Irish were not only depended on a single crop, they were also depended on a single cultivar to sustain themselves. At one time, the cultivar Irish Apple was used, but due to growing hunger, a higher yielding potato, the cultivar Lumper was almost exclusively cultivated. The combination of no genetic diversity and complete dependence on one food crop created a disastrous epidemic. Many were reluctant to help the poor because they were viewed as immoral, and it was believed that charity caused idleness and corruption. The Irish Potato Famine of 1845 to 1847 caused one million people to die and millions to emigrate to other parts of Europe and the United

States. Before the famine in Ireland, the population was roughly 8.2 million people, but by 1911, the population fell to 4.4 million. The potato blight, caused by a fungus-like organism, *Phytophthora infestans*, was one of the worst blight disasters in human history.

Most of the English-born colonists forgot their potato prejudice once they arrived in America. George Washington had the tuber planted on his estate in 1767, and Thomas Jefferson listed the potato in his farm journal in 1772. The United States, along with the rest of the world, was beginning to understand that consumption of the potato could be a labor saving device. The consumption of potatoes at this time was in urban, not rural areas. The Industrial Revolution was beginning, and many poor laborers lived in cities. It became necessary to ship food to centrally located cities, and potatotes became attractive because they needed no milling and spoiled less than grains during postharvest storage. Canals used to ship supplies into the city began potato commerce. In many working-class families all family members, including wives and children, worked so as to earn the most income. Time for preparation of big meals was scarce. Bread became too costly for many workers in 1800, while potato consumption increased in prominence. Laborers were looking for a source of food that would allow them to feel full. Bread did not satisfy, but potato, with it its bulky starch, was much more satisfying. Around 1820, street vendors began selling food for hungry employees of the industrial revolution, and potato became one of the more prominent items. The potato finally become part of the Western culture due to the ideals of the Industrial Revolution, such as stretching every resource, self-sufficiency as a type of nationalism, and thrift as an economic weapon (Zuckerman, 1998).

BREEDING. Gardeners and botanists began breeding the potato as a hobby in the 1600s. By the 1800's, however, potato consumption increased, and in America it ranked fourth among foods after wheat, corn, and oats, and breeding was conducted for yield and consumption. In 1847, Reverend Chauncey E. Goodrich proposed reinvigorating potato hardiness, especially to late blight, with new varieties from South America. He obtained the Chilean cultivar Rough Purple Chile and bred the potato Garnet Chile. In

1871, Luther Burbank developed the cultivar Burbank, and later the cultivar Russet Burbank, currently one of the six most widely planted potato cultivars in North America. Russian scientist and plant collector Nikolai I. Vavilov gathered potato samples from the five continents during the 1920s and 1930s. His pursuits resulted in creation of the largest seed collection and crop research institute in the world.

Goals of the modern breeding programs include processing objectives (dry matter content, reducing sugar content, discoloration of raw flesh, and resistance to damage), industrial objectives (production of starch), disease objectives (pathogen resistance such as late blight), and health objectives (such as vitamin content, carbohydrate content, and antioxidant content).

VARIATION WITHIN GENUS. The genus *Solanum* is very large, containing over 2,000 species, with less than one tenth tuber-bearing. There are two subgenera, *Pachystemonum* and *Leptostemonum.* The second is sub-divided into five sections, one of which is *Tuberarium* (now *Petota*). *Tuberarium* is sub-divided into *Basarthrum* and *Hyperbasarthrum* (now *Potatoe*). The former sub-division is the only one which is tuberous (Burton, 1989). Genetic control determines morphological features, resistance or susceptibility, yield, percentage of dry matter, time needed for tuber initiation, rate of bulking, length of growing season, response to environmental factors and cooking quality; therefore, there is wide variability among cultivars.

Tuber shapes found most commonly in commercial cultivars include: compressed, round, ovate, obviate, elliptic, oblong, long-oblong, oval, long, flattened, clavate, reniform, and fusiform. Falcate, coiled, digitate, concertina-shaped, and tuberosed tubers are often found in primitive Andean cultivars. The skin of the tuber may be smooth, rough, partially netted, totally netted or very heavily netted. The sole, or predominant and secondary colors of the skin may be white-cream, yellow, orange, brownish, pink, red, purplish red, purple, or dark purple-black. In parti-colored tubers, the colors may be confined to the eyes only, eyebrows only, splashed, spectacled, scattered, or stippled. The flesh color of the tuber may be uniform or involve secondary coloration. The flesh colors include: white, cream, pale yellow, yellow, deep yellow,

red, violet or purple. Secondary color may be present as scattered spots, scattered areas, in the vascular ring, in the medulla, or in all the flesh except the medulla. White-fleshed potatoes are preferred in the United States, while most European countries prefer yellow-fleshed potatoes.

The coloring of potatoes is an indication of the content of carotenoids and/or anthocyanins. There is a direct correlation between flesh color and total carotenoid content (yellowness of the flesh), while total anthocyanin content is correlated with the redness or blueness of the flesh.

GLOBAL PRODUCTION. The potato is the fourth most important food crop in the world, with an annual production of some 300 million tons. In 2003, the world's top potato producing countries were as follows: China, the Russian Federation, India, United States, Ukraine, Poland, Germany, Belarus, United Kingdom, and France (Economic Research Service, The United Nations Food and Agriculture Organization, 2004). The potato has high potential for production in many developing countries and is well suited for intensive small farming. It is one of the most efficient crops in converting land, water, labor and capital into a highly nutritious food (Horton, 1980). The tuber seed can be the single, most costly input for farmers in many developing nations. Interest in potato in developing nations seems to be increasing, especially in Afghanistan, Burundi, Columbia, India, Indonesia, Iraq, Rwanda, Tanzania, Sri Lanka and Viet Nam. One reason may be that potatoes rank first in energy production per hectare per day and are significantly above cassava, the cereals, and pulses (Horton, 1980).

PRODUCTION IN AMERICA. Potato is the most important vegetable crop in the United States on a cash-crop basis (Miller, 1992). The United States potato production in 2003 was 463,214,000 cwt., with a value of $3,151,178,000 (National Potato Council, 2004). Potato is grown in 34 states on at least 500 acres, and is grown in four distinct seasons. Potatoes are harvested across a large area during the fall, which constitutes about 88 % of the crop, followed by spring (6 %), summer (5 %), and winter (1 %) (National Potato Council, 2004).

PRODUCTION IN TEXAS. In 2003, there were 22 thousand acres of potatoes planted in Texas. Although this is only 1.7% of the US area planted, Texas production should not be overlooked. The state produces around 6.5 million cwt of potatoes, and receives one of the highest prices per cwt, $10.40. Texas 2003 production accounted for over $68 million. Texas can grow potatoes 11 months out of the year. The summer crop is by for the largest, and this is advantageous for Texas because this is a lower producing time for the rest of the US. The summer crop is planted in the Rolling Plains in February and harvested in June; while the High Plains is planted in April through May and harvested in July through late September. The Spring crop is planted in the Rio Grande Valley in December and harvested in April, while the Winter Garden is planted in January and harvested in May.

THE POTATO AND HUMAN NUTRITION. Even though the potato has many health benefits, numerous studies show that some people, especially followers of the Atkin's diet, believe that starches and potatoes are fattening. Many dieters avoid potatoes when they are on a self-prescribed weight loss diet. Potato is actually a low fat food, and it supplies ample amounts of vitamins and minerals to the diet. This has contributed to the use of potato as a staple source of food. Potato is well known as an important source of vitamin C, iron and B vitamins, along with a multitude of other vitamins and minerals needed to maintain health (Table 2.1).

Table 2.1 Potato nutrition facts.

Serving size	1 medium potato, 1/3 lb
Calories	100
Protein	6% US RDA
Fat	0 grams
Dietary Fiber	3 grams
Sodium	10 mg
Vitamin C	50% US RDA
Thiamin	8% US RDA
Niacin	2% US RDA
Vitamin B_6	15% US RDA
Calcium	10% US RDA
Iron	8% US RDA
Folic Acid	8% US RDA
Phosphorus	8% US RDA
Magnesium	8% US RDA
Zinc	2 % US RDA
Copper	8% of US RDA
Potassium	750 mg
Pantothenic Acid	4% of US RDA
Iodine	15% of US RDA

(Kolasa, 1993).

The potato supplies all vital nutrients except vitamins A and D. One medium potato provides 50% of the current US RDA for vitamin C. In the past, the tuber was used to prevent scurvy, benefiting populations that had little or no access to fruit. By the twentieth century, Americans obtained more vitamin C from fresh potatoes than any other single source. Potatoes are also the second most- important contributor of vitamin B_6 for the elderly and the third largest source for adults (Kolasa, 1993).

The potato is also a well-known source of fiber in the diet. Insoluble fiber contributes to laxation and some cancer prevention, while soluble fibers contribute to improved glucose and cholesterol control. The recommended consumption of dietary fiber is 25-30 g per day, and the potato is an important source of dietary fiber for many groups, such as low income US women who obtain 11.1% of their dietary fiber from potato (Thompson et al., 1992).

Although often unnoted, the potato can serve as a significant source of protein in the diet. The ratio of protein to carbohydrates in potato is much higher than many

cereals and other tuber and root crops (Table 2.2). The vitamins and minerals in cooked products, although often decreased, still contain significant levels (Table 2.3).

Table 2.2 Biological value[z] of foods.

Food crop	Biological Value
Egg	96
Potato	73
Soybean	72
Maize	54
Wheat Flour	53
Peas	48
Beans	46

[z] the index of the proportion of absorbed nitrogen retained by the body for growth and maintenance, or both
(Horton, 1980).

Table 2.3 Vitamins and minerals in cooked potatoes.

Vitamin (mg per 100 g)	Raw	Boiled	Baked (with skins)	Roasted	Fries	Chips
thiamine	0.11	0.08	0.10	0.10	0.10	0.19
riboflavin	0.04	0.03	0.04	0.04	0.04	0.07
Nicotinic acid	1.7	1.1	1.8	1.9	2.1	6.1
pyridoxine	0.25	0.18	0.18	0.18	0.18	0.89
Pantothenic acid	0.30	0.20	0.20	0.20	0.20	0.20
Folic acid (µg/ 100g)	14	10	10	7	10	20
Vitamin C	8-20	4-14	5-16	5-16	5-16	17
Vitamin E	-	-	-	-	-	6.1

(Mervyn, 1984).

CONSUMPTION OF THE POTATO. The US per capita consumption of potatoes in 2004 was 136 lbs (46 lbs fresh and 80 lbs processed) (National Potato Council, 2004), where more than half of potatoes are processed. The demand for processed potatoes has risen, while fresh potato consumption has fallen, and total potato consumption has risen

(USDA / Economic Research Service, 2004). Although the intake of fruits and vegetables is low in individuals living in the United States, the vegetable intake of adults is heavily influenced by white potato (1.0 serving a day) (Krebs-Smith et al., 1995).

CONSUMPTION OF FRUITS AND VEGETABLES. Fruits and vegetables play a very significant role in human nutrition, providing the largest amounts of the vitamins A and C, and significant levels of vitamin B_6, magnesium, iron, thiamin, and niacin. Fruits and vegetables are also very low in calories, supplying only 9 % of the total (Goddard et al., 1979). Even with the numerous choices and health benefits, many people, especially in the United States, do not eat the recommended amounts of fruits and vegetables. On any given day, only about 49 % of the population consumes at least the minimum number of servings of vegetables recommended (3 servings per day). About 10 % of the population consumes less than one serving of vegetables per day. About 29 % of the population consumes at least the minimum number of servings of fruit recommended (2 servings per day), while about 48 % consume less than one serving of fruit a day. (USDA 1994-1996 Continuing Survey of Food Intakes by Individuals).

CONSUMPTION AND CHRONIC DISEASE. Today, nutrition is less focused on preventing deficiency diseases and more on enhancing immunity and preventing chronic disease. This has caused an increased interest in functional foods, those selected for the diet in order to improve human health (Brown, 2000).

Numerous epidemiological studies have been conducted correlating diets rich in fruits and vegetables with low levels of certain diseases, as opposed to diets poor in fruits and vegetables correlating with higher levels of certain diseases (Ames et al., 1993) (Table 2.4).

Table 2.4 Inverse correlation of chronic disease and antioxidant consumption from fruits and vegetables.

Disease	Antioxidant Source	Investigator
Melanoma and cancers of the lung and bladder	β-carotene intake in fruit and vegetables	Comstock et al., 1991
Oxidative cellular damage	Fruits/ vegetables	Thompson et al., 1999
Prostate cancer	Tomato products	Giovannucci et al., 1995
Esophageal cancer	Tea	Dreosti et al., 1997
Stroke (both hemorrhagic and ischemic)	Fruit and vegetable	Gillman et al., 1995
Age-related macular degeneration	Vegetables	Seddon et al., 1994
Heart disease	Flavonoid intake in fruit and vegetables	Hertog et al., 1993
Blood glucose response (glycemic index)	Potatoes, legumes and cereals	Thompson et al., 1983
Oxidation of lipoproteins (LDL), atherosclerosis	Ach berry (high phenolics)	Miranda-Rottmann et al., 2002

CARCINOGENESIS. Most recent estimates indicate that diet is responsible for 20 % to 33 % of all cancers that occur in economically developed countries (Willett and Trichopoulsos, 1996). Many studies have been conducted on cancer prevention with fruit and vegetable consumption. Although fruits and vegetables provide a rich source of dietary fiber, cancer prevention goes well beyond this notion. It has been proposed that the preventive actions start at the cancinogenic process, which begins with a long delay between the first exposure to a carcinogen and the occurrence of cancer. There are three stages to the carcinogenic process: initiation, promotion, and progression (Tanaka, 1994). Free radicals and the auto-oxidation process are involved in each of these stages: (1) several cancer initiators appear to either produce or cause production of free radicals, (2) free radicals can cause base damage, single strand and double-strand breaks in DNA, cross-linking between two DNAs, and chromosomal aberrations, (3) free-radical-generating carcinogens can induce the formation of thymine glycol, a major product of base damage in DNA, and (4) free radicals may activate the pro-carcinogen to its active

carcinogenic form (Al-Saikhan, 2000). Numerous studies have reported that compounds within fruits and vegetables can prevent and/or control the growth of cancers (Dillard and German, 2000).

Reduced glutathione, GSH, might protect cells from cancer through a number of mechanisms: (1) by functioning as an antioxidant, (2) by binding with mutagenic chemical compounds, (3) by directly or indirectly acting to maintain functional levels of other antioxidants such as vitamin C, E and β-carotene, (4) through its involvement in DNA synthesis and repair, and (5) by enhancing the immune response. It should be noted that potatoes, French fries, fried potatoes and potato chips are good sources of glutathione (Jones et al., 1992).

As a related theory, cancer initiation and or inhibition may be related to Phase I and Phase II enzymes. It is believed that Phase I enzymes activate and Phase II enzymes detoxify carcinogens. An example of a Phase I enzyme is cytochrome P-450, while an example of a Phase II enzyme is glutathione transferase. It is believed that many chemicals found in fruits and vegetables either promote or are themselves Phase II enzymes. These Phase II enzymes inactivate reactive carcinogens by destroying their reactive centers or by conjugating them with endogenous ligands, thereby facilitating their elimination from the body (Fahey, 1999). This process is not fully understood. It is believed that the compounds found in fruits and vegetables should be most protective at the initiation stage of cancer; however in recent animal studies, these compounds were found to be most protective during the later promotional phases of cancer development (Krinsky, 1991; Moon, 1989).

FREE RADICAL PRODUCTION. As noted previously, free radical production is highly linked to cancer initiation. This link between free radical production is also associated with other chronic diseases (Table 2.5). Damage caused by free radicals reacting with polyunsaturated fatty acids in cellular membranes, nucleotides in DNA, and sulfhydryl bonds in proteins contributes to many chronic health problems such as arthritis, atherosclerosis, emphysema, cardiovascular and inflammatory diseases, stroke, retrolental fibroplasias, cirrhosis, adult respiratory distress syndrome, cataracts, macular

degeneration and cancers (Machlin and Bendich, 1987; Byers and Perry, 1992; Pryor, 1986; and Thomas, 1995).

Table 2.5 Diseases that involve radical-mediated reactions

Disease	Strength of the evidence for some radical involvement
Emphysema	+++
Cancer	+++
Arthritis	++
Atherosclerosis	++
Cirrhosis	+
Stroke	+
Retrolental fibroplasias	+++
Cataract	++
Adult respiratory distress syndrome	++
Aging	+

(Pryor, 1986).

Cellular sources of free radicals include: 1) phagocytes, 2) mitochondrial electron transport system, 3) microsomal electron transport systems, 4) soluble oxidase enzymes, 5) autoxidation of endogenous or exogenous substrates, and 6) transition metals (Kehrer, 1993). Free radicals can be produced by reactive oxygen species (ROS) which include the superoxide radical, hydrogen peroxide, hydroxyl radical, peroxyl radicals, phagocyte-derived reactive oxygen species, heme proteins, peroxides, peroxynitrites, and singlet oxygen which has no unpaired electrons; therefore, it is not classified as a radical but is another important and powerful oxidizing agent.

ANTIOXIDANTS. In living systems, cells create free radicals which are by-products of reactions and often cause oxidation. Free radicals seek to be oxidized due to their unpaired electrons. The oxidation process causes damage in cells, including cell wall damage, cell structure damage and genetic damage within a cell. Antioxidants can be defined as any substance, when present at low concentrations compared to those of an oxidizable substrate, which significantly delays or inhibits oxidation (oxidation is the process of losing electrons) of that substrate (Halliwell and Gutteridge, 1990).

Antioxidants have the ability to act as defensive or protective agents against

oxidation of a substrate. A compound might exert antioxidant actions by inhibiting generation of radical oxygen species by directly scavenging free radicals, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors, or synergists (metal chelating agent or reducing agent) (Namiki, 1990). They can protect against oxidation by: (1) decreasing localized O_2 concentrations, (2) preventing initiation of oxidation by scavenging species capable of abstracting hydrogen atoms, (3) quenching or scavenging singlet O_2 which reacts directly with membrane lipids to produce peroxides, (4) binding metal ions (metal chelating agent or reducing agent) in forms that will not generate reactive species and / or will not decompose lipid peroxides to peroxyl and alkoxyl radicals, (5) removing peroxides by converting them into nonradical products such as alcohols, (6) chain breaking to prevent continued hydrogen abstraction from fatty acid side chains (Halliwell and Gutteridge, 1990).

PHYTOCHEMICALS. Phytochemicals or phytonutrients are secondary metabolites of plants thought to promote health. Unlike traditional nutrients, phytochemicals are not essential in the diet. The major classes of phytochemicals include: 1) carotenoids; 2) flavonoids, phenols and cyclic compounds; 3) inositol phosphates (phytates); 4) lignans (phytoestrogens); 5) isothiocyanates and indoles; 6) saponins; 7) sulfides and thiols; and 8) terpenes (Clevidence et al., 2003). Harborne (1999), offering an alternative in classification, identified three major classes of phytochemicals including: terpenoids, phenolic metabolites, and alkaloids along with other nitrogen-containing plant constituents. He placed carotenoids in the terpenoid classification. Phytochemicals have been reported to 1) serve as antioxidants, 2) enhance immune response, 3) enhance cell-to-cell communication, 3) alter estrogen metabolism, 4) convert to vitamin A, 5) cause cancer cells to die (apoptosis), 6) repair DNA damage caused by smoking and other toxic exposures, 7) detoxify carcinogens through activation of the cytochrome P-450 and Phase II enzyme systems (USDA Beltsville Agricultural Research Center, 2004). Potatoes have significant levels of both phenolics and carotenoids (Al-Saikhan, 1994, 2000; Hale, 2003).

Phenolics. The main function of the polyphenols in plants seems to be as a protective agent against pathogens. It is believed that polyphenols can accumulate during stressful conditions, and an accumulation of polyphenols has been found adjacent to injured or stressed tissues (Friedman, 1997). Phenolic compounds are distributed mostly between the cortex and skin of the potato. About 50% of the phenolic compounds are located in the peel and adjoining tissues, while the remainder decreases in concentration from the outside toward the center of the potato tuber (Friedman, 1997). Flavonoids, a major group of plant phenols, include compounds that are potent antioxidants (Table 2.6). Flavonoids are polyphenolic compounds that have 15 carbons and 2 benzene rings. The structure can have a third chromate ring or five-member ring. This ring is labeled ring C, and the various subgroups of flavonoids are classified according to patterns of subgroup ring C. The flavonoids and related compounds include: anthocyanidins, anthochlors, benzofurans, chromones, coumarins, minor flavonoids, flavonones, flavonols, flavones, isoflavonoids, lignans, phenols, phenolic acids, phenolic ketones, phenyl-propanoids, quinonoids, stilbenoids, tannins and xanthones (Dillard and German, 2000).

Table 2.6 Common flavonoids and their classification.

Classes	Examples
Flavonols	kampferol, quercetin, myricetin, rutin, luteolin, chryin, apigenin, naringenin, epicatechin, catechin, epigallocatechin, epicatechin, gallate, epigallocatechin gallate
Falvonone	naringin, taxifolin
Flavone	chrysin, apigenin, luteolin
Anthocyanidins	anthocyanins such as malvidin, cyanidin, apigenidin, pelargonidin, delphinidin, petunidin, peonidin
Phenyl-propanoids	ferulic acid, caffeic acid, p-coumaric acid, chlorogenic acid

(Rice-Evans 1996).

The majority of flavonoids are intermediates and derivatives of the shikimate and phenylpropanoid pathways (Cheng and Breen, 1991), and flavonoids are well known as antioxidant compounds. Miranda-Rottmann et al. (2002) reported that the correlation

between phenol content and total antioxidants present is r = 0.94; therefore, phenolics have a high amount of antioxidant activity. Flavonoids can prevent lipid peroxidation by the following means: (1) scavenging lipid peroxidation-initiating radicals such as HO^* and O_2* (2) binding metal ions, (3) scavenging lipid peroxyl radicals, and (4) inhibiting enzymatic systems responsible for free radical production (Brivba and Sies, 1994).

The flavonoids most common in potato include: flavonols, cinnamic acid, p-coumaric acid, caffeic acid, ferulic acid, quinic acid, and chlorogenic acid. Chlorogenic acid constitutes up to 90% of the total phenolic content of potato tubers. The coloring of the darker fleshed and darker skinned potatoes may be related to anthocyanin content, which is the second most important group of plant pigments (ranging from yellow, red to blue) following chlorophyll. Coloring of the skin or flesh may also be due to carotenoids, which are another type of phytochemical.

Carotenoids. Of all the phytochemicals, carotenoids have been studied the most. Carotenoids are responsible for the yellowness/ orange ness of many fruits and vegetables (Burton, 1989). There are two main types of carotenoids, the hydrocarbon carotenes and the xanthophylls or oxycarotenoids. The hydrocarbon carotenes, such as alpha and beta-carotene, contain only carbon and hydrogen. These compounds are extremely lipophylic and some have provitamin A activity. Others, such as lycopene, do not have pro-vitamin A activity. The xanthophylls or oxycarotenoids contain at least one oxygen, and some of these compounds include lutein, zeaxanthin, and beta-cryptoxanthin. Beta-cryptoxanthin also has pro-vitamin activity. The most prominent carotenoids found in plants include: lycopene, lutein, carotene, auoxanthin, violaxanthin, isolutein, aflavoxanthin, cryptoxanthin, antheraxanthin, neoxanthin, astaxanthin, canthaxanthin, bixin, zeaxanthin, crocin, alpha-tocopherol, lipoic acid, glutathione and derivatives of the former (Burton, 1989).

Carotenoids quench singlet oxygen, and the excess energy of singlet oxygen is transferred to the carotenoid's structure, where it neutralizes free radicals by adding them to its structure (long double chain); the structure also has a polar end which attacks radicals near fat/water surfaces. The main antioxidant mechanism of carotenoids in

biological systems is quenching singlet oxygen and scavenging free radicals (Klein and Kurilich, 2000). The physical structure of the carotenoid remains unchanged; therefore, it is then able to protect against further radical damage (DiMascio et al., 1989, 1990, 1991).

β-carotene was first isolated from carrots in 1831. In the 1940s it was determined that both yellow and white potatoes contained carotenoids. Pendlington (1965) identified β-carotene, β-carotene-5,6-di-epoxide, lutein, *cis*-violaxanthin, *cis*-antheraxanthin-5,6-mono-epoxide and *cis*-neoxanthin in potato. Tevini et al. (1984) determined that carotenoid content is not distributed equally among the potato tuber skin, cortex, and pith. The skin has the greatest amount of lutein, while the cortex has the greatest amounts of neoxanthin, violaxanthin, lutein epoxide, carotenoid diester, and total carotenoids. Lepage (1968) reported that 48.5 % of the carotenoids were lutein; 14.2 % were lutein 5,6-epoxide; 6.4 % were α-carotene; 16.3 % were β-carotene; and 14.2 % were an unidentified pigment in potato. Gross (1991) determined that varieties with carotene content of about 300 μg (100 g fresh wt)$^{-1}$ had an intense yellow color, whereas varieties with only 30 to 70 μg (100 g fresh wt)$^{-1}$ had a white color. Total carotenoids of ten yellow flesh potato varieties grown in Texas ranged from 39 to 128 μg (100 g fresh wt)$^{-1}$ and white flesh potato ranged from 29 to 76 μg (100 g fresh wt)$^{-1}$ (Al-Saikhan, 1994).

Boileau et al. (1999) determined that carotenoids often interact with fat and possibly fiber. The food matrix, fat content and fiber content affect intestinal absorption; therefore, humans will often not absorb carotenoids if the fat content and/or fiber content of the food are low.

FACTORS AFFECTING ANTIOXIDANT LEVELS. The important phytochemicals, phenolics and carotenoids, are both genetically controlled and are heritable traits (Al-Saikhan, 1994, 2000; Hale, 2003**)**. However, this is not the only important factor involved in determining phytochemical content in a product. Numerous reports have concluded that cultural and environmental conditions along with postharvest processing methods affect the content and quantity of phytochemicals in a

product (Burton, 1989; Connor et al. 2002; Howard et al., 2000; K'osambo et al., 1998; and Pendlington et al., 1965).

CULTURAL AND ENVIRONMENTAL FACTORS. Numerous studies have been conducted on the level of antioxidants and the degree of maturity in fruits and vegetables. Pendlington et al. (1965) studied the carotenoid distribution in potato cultivars over different maturity stages. Level of pigmentation was maturity dependent. K'osambo et al. (1998) determined that, in sweet potato, there was a significant interaction with cultivar, root age and carotenoid content. Younger roots contained less carotenoids than older roots. Howard et al. (2000) determined that the concentration of L-ascorbic acid, carotenoids, phenolic acids, capxanthin, and zeaxanthin in *Capsicum* species generally increased during maturation, whereas the level of lutein declined.

Climatic conditions also affect antioxidant levels. Pendlington et al. (1965) determined that total carotenoid content correlated with those climatic conditions that favored rapid growth. As the potatoes matured, the value approached unity. K'osambo et al. (1998) determined that total carotenoid content also was dependent on both farming site and cultivar. Burton (1989) determined that the highest intensity of skin and flesh color occurred in sandy soils. Weather differences from year to year can also have a significant effect on antioxidant content. Connor et al. (2002) reported that there was a significant genotype by year interaction for antioxidant activity among blueberry cultivars.

POSTHARVEST PROCESSING FACTORS. Processing techniques are known to affect the quality of produce. Concentrations of vitamins (such as vitamin C and thiamin) in fruits and vegetables tend to decline during postharvest handling, storage, and processing. Buescher et al. (1999) refer to the loss of nutrient content of fruits and vegetables after processing as a "hidden loss", because there may not be any other detectable changes, such as in color, flavor or texture.

PEELING. Peeling the skin of potato is a common practice in home preparation. Tevini et al. (1984) stated that the carotenoid content of potato is not distributed equally among the potato tuber skin, cortex, and pith. Peeling the skin of the potato caused a

loss of 20% to 30% of the total carotenoids. When dicing, a further loss of about 10% was due to the enzymatic activity of peroxidase and lipoxygenase. The most significant losses of quercetin found in food processing dealt with the peeling process and the removal of the outer layers of plant tissue. Peeling and blanching of onions reduced flavonoid content to approximately half of the starting level. When different processing steps for onions were compared, the only significant losses of flavonoids took place during the peeling and trimming processes (39%). Further processing by cooking, frying, and warm-holding of blanched onion, beans, and peas had small effects on flavonoid content (Ewald, 1999).

COOKING. Most potato products are served after cooking or some sort of heat treatment. Heat treatment can cause changes in the nutritients and composition of a food product. The Institute of Food Technologists (1986) stated that heat processing destroys antidigestive factors such as trypsin and amylase inhibitors, thus providing higher bioavailability. Heat also destroys some enzymes that could promote spoilage and/or reduce nutritive value. Heat processing increases the digestibility of starch and protein (gelatinization and denaturation) and also increases the bioavailability of niacin. Excessive heat treatment can result in lower protein and carbohydrate bioavailability because of interactions. Fat reactions with heat include lipolysis (degradation of fat to free fatty acids and glycerol), oxidation of PUFAs in the presence of air, and conversion of cis-PUFAs to trans-PUFAs.

Minerals can combine with other food components and become non-available. Also, minerals are often susceptible to leaching. Water soluble vitamins (thiamin, riboflavin, vitamin C) are more susceptible to losses due to leaching during washing or blanching. Fat soluble vitamins (A, D, E) are more sensitive to oxidation during processing or storage. Minerals, on the other hand, are often more stable to oxygen and heat. Vitamin C and thiamin are the vitamins that are most heat sensitive (Table 2.7).

Table 2.7 Stability (S) or instability (U) of vitamins and minerals when exposed to oxygen, light, and heat.

	Air or Oxygen	Light	Heat
Vitamin A	U	U	U
Vitamin C	U	U	U
Biotin	S	S	U
Beta- Carotene	U	U	U
Chlorine	U	S	S
Cobalamin (B_{12})	U	U	S
Vitamin D	U	U	U
Folic Acid	S	U	S
Inositol	S	S	U
Vitamin K	S	U	S
Niacin	S	S	S
Pantothenic acid	S	S	U
Pyridoxine (B_6)	S	S	S
Riboflavin	S	U	U
Thiamin	U	S	U
Vitamin E	U	U	U
Mineral Salts	S	S	S

(Institute of Food Technologists, 1986).

Kala et al. (2001) reported that the vitamin and mineral nutrition aspects of cooked food remained similar to the raw product. Three different methods of cooking; boiling, pressure cooking, and microwave radiation had similar effects on contents of moisture, total ash, protein, carbohydrate, dietary fiber, ascorbic acid, calcium, phosphorus, and iron.

Phytochemicals do not necessarily react to cooking processes as do other vitamins and minerals. Macheix et al. (1990) stated that high temperatures will destroy anthocyanins. Several enzymes are involved in anthocyanin degradation: B-glycosidase, peroxidases and polyphenol oxidases (PPO). B-glycosidase's mechanism of degradation involves enzymatic hydrolysis of anthocyanin to aglycone and glycoside, followed by the degradation of the aglycone. Peroxide is found to enhance the phenolic compound to form o-quinone, which will in turn oxidize the anthocyanin, thus contributing to the degradation of the molecule. In order to prevent degradation, Short time/high temperatures should be used for color retention. Carotenoids of yellow pepper (*Capsicum annuum*, L.) are also heat sensitive, and the vitamin A values are

smaller (a reduction of 21-30 %) after 10 minutes of cooking (Bianchini and Penteado, 1998).

Cooking methods affect food components differently. The conjugated quercetin content of tomatoes and onions declined with microwave cooking, and boiling produced an even larger reduction. Boiling reduced quercetin, but further warm-holding had no effect. Losses due to frying were less severe (Crozier et al., 1997; Ewald, 1999). The reduced quercetin found in boiling, baking or microwaving compared to frying may be due to flavonoid breakdown during cooking and/or conjugated quercetin being extracted from tissues by hot water more efficiently than by hot oil (on tomatoes and onions). Leaching occurs in water used for cooking, and frying causes a thermal degradation (Miean, 2001). Much of this loss is due to the leaching that occurs with procedures that involve water or steam. Cooking extracted less flavonoid glycosides and acylated derivates from spinach than glucuronide derivatives, which were the predominant compounds in the cooking water. In addition, the glucuronide derivatives were more highly degraded due to the cooking process, since a 30% loss was detected when compared to the original tissue. Compounds in the tissue were found to be more stable than those in water, where they were more degraded (Gil et al., 1999). Using UV spectroscopy, it was determined that oven baked potatoes contained no chlorogenic acid, boiled potatoes 35%, and microwaved potatoes 55% of the original amount. French fried potatoes, mashed potato flakes, and potato skins contained no chlorogenic acid (Friedman, 1997). Fresh potato has the highest amount of chlorogenic acid, followed by microwaved and boiled, while baked potato has been reported to have no chlorogenic acid (Table 2.8).

Table 2.8 Heat stability of chlorogenic acid in cooked potatoes determined by UV spectrophotometry.

Potato	Chlorogenic acid (mg/ g of freeze-dried weight)
Fresh	0.800 ± 0.05
Baked	0.000
Boiled	0.319 ± 0.01
Microwaved	0.434 ± 0.02

(Dao and Friedman, 1992).

There is believed to be a leveling-off point in the degradation of carotenoids. Boiling of sweet potato roots for 30 minutes caused a reduction in total carotenoids, which varied by cultivar; however, further boiling for up to 60 minutes did not exacerbate the reduction in total carotenoids (K'osambo et al., 1998). Blanching in water or steaming caused a further loss of 10% to 20%, with no differences between the two methods, and the total carotenoid retention was 40% (Gross, 1991). Cooking and further processing can also cause an isomerization from *trans* to *cis* isomers of carotenoids (Klein and Kurilich, 2000).

Although individual compounds can be degraded, the total antioxidant activity might also increase. Dewanto et al. (2002) stated that boiling or steaming, (115 ^{o}C for 10-15 minutes) of corn before canning increased total antioxidant activity by approximately 21.9% and 52.6%, respectively. Increased levels of lycopene were found in cooked corn as compared to raw. One explanation postulated was that processing breaks down the cell matrix and releases some of the bound phenolics. Thermal treatment at 115 ^{o}C decreased total antioxidant activity of the bound phenolics in sweet corn extract, while it increased the antioxidant activity of free phenolics (Dewanto et al., 2002). Granado et al. (1992) analyzed the quantity of alpha and beta carotene, lutein, lycopene, and zeaxanthin. They reported that cooked samples contained more carotenoids than raw. Raw potato contained 12 μg/ 100 g lutein, 4 μg/ 100 g zeaxanthin and 1 μg/ 100 g β-carotene, while cooked potato contained 44 μg/ 100 g lutein , 21 μg/ 100 g zeaxanthin, and 1.5 μg/ 100 g β-carotene. Granado et al. (1992) also saw a similar trend with other vegetables. Dietz et al. (1988) explained that the process of heating tomato juice or steaming spinach, for example, increased the amount of carotenoids extracted. One explanation is that processing breaks down the cell matrix and releases some of the bound phenolics. Carotenes can be bound either in carotenoproteins or the plant matrix (Boileau et al., 1999). Following heating, the carotenes are released, resulting in apparent increases in carotene content after cooking or thermal processing (Klein and Kurilich, 2000). Bioavailability may also be increased with cooking due to both the destruction of antidigestive factors and enzymes that

promote spoilage and the increased digestibility of starch and protein through the processes of gelatinization and denaturation.

There is a large discrepancy between losses and gains in phytochemicals using various cooking methods. This may be due to the different means of sample preparation. Many investigators cooked the food first, and then weighed out the allotment for analysis (Dewanto et al., 2002; Hunter, 2002; Scita, 1992; Sistrunk, 1977; Shahidi, 1997; Sharma, 2000; Shirsat and Thomas, 1998; Toma, 1978). Using this method, the allotment of fresh weight could change depending on cooking method due to the degree of dehydration. Some investigators pre-peeled the food for some cooking methods (Muneta and Kalbfleisch, 1987; Oruna-Concha, 2002; Thomas and Joshi, 1977). Other investigators freeze-dried their samples after cooking then weighed an allotment for analysis (Dao and Friedman, 1992; Finglas, 1984). Friedman and Dao (1990) used a milling and flour mixture, while Gazzani (1998) and Spanos (1990) used a juicing procedure before cooking. Also, other variability may be due to different species, cultivars used for experiments, and interactions involving other factors.

MAILLARD REACTION. The Maillard reaction, a non-enzymatic browning reaction, was first reported in 1946. The Maillard reaction occurs when aldehydes, ketones and reducing sugars condense by heat with free amino acids, peptides or proteins, leading to the formation of a wide variety of brown melanoidins (Hodge, 1953; Nicoli et al., 1997). The Maillard reaction is desirable in many food products, e.g., baking, cooking, roasting, and frying. Examples of where one can see the melanoidins are on toasted bread, grilled chicken or coffee. It is not clear whether the Maillard reaction products (MRPs) exhibit mutagenic or antimutagenic activity (Cuzzoni et al., 1988; 1989; Yen et al., 1993; Yen and Tsai, 1993). Antimutagenic activity has been recently attributed to the fact that certain MRPs can act like antioxidants, such as chain breakers, oxygen scavengers, and metal chelating agents (Lingnert and Waller, 1983). The antioxidant activity of coffee beverages was greatly enhanced as the roasting time was increased (Nicoli et al., 1997). There may be a loss of some original antioxidant compounds in cooked products as compared to the raw products, but there is a possibility of increased load of antioxidant

compounds in cooked products due to the formation of MRPs (Areana et al., 2001; Lee, 1992; Nicoli et al., 1997; Polydera et al., 2004).

STORAGE. Storage is another procedure which can cause changes in the quality and nutrition of food products. The amount of change is dependent on the storage temperature and duration. Both phenolics and carotenoids have been reported to be affected by storage. Storage at -18 ^{o}C or lower resulted in an excellent retention of vitamins for up to 6 months (Klein and Kurilich, 2000). Piironen et al. (1986) determined that tocopherol content appeared to be stable during freezing and storage of vegetables. However, most home-refrigerator freezer units are maintained at temperatures above -18 ^{o}C. Major causes of losses during storage are oxygen and light permeability (Institute of Food Technologists, 1986).

Mondy et al. (1966) found that higher respiration rates occurred during storage. This increased respiration might have other physiological effects on a food product. Reducing sugar contents are higher at lower temperatures, and high reducing sugar content in potatoes can cause darkening of cooked products (probably forming MRPs).

Potatoes stored at 10 ^{o}C had higher cytochrome and polyphenol oxidase (PPO) activities than those stored at 4 ^{o}C. An increase in PPO activity causes a greater transformation of monomeric polyphenols to polymeric polyphenols; therefore, cold-stored tubers may have higher total phenol content (Mondy et al., 1966). Storage period significantly affected the magnitude of light-induced chlorogenic acid response. Tuber chlorogenic acid concentrations declined during prolonged cold storage at 5 ^{o}C. Rates of accumulation in response to light were cultivar dependent (Percival et al., 2000). Friedman (1997) reported a large increase in chlorogenic acid and glycoalkaloid levels of potatoes stored in well-lit areas, and a smaller increase of chlorogenic acid and glycoalkaloids in potatoes stored in the dark.

Each food product and compound responds to storage differently, as some compounds increase, others decrease, and others might not change at all. Awald (2000) stated that quercetin flavonoids that are present in apple are stable during storage (2 to 6 months) and shelf life (1 to 2 weeks). It is believed that there are few gross changes in

overall level or composition of quercetin glucosides during normal commercial storage (Miean, 2001).

IRRADIATION. When potatoes sprout they decrease in weight, quality, and market value. Sprouts are high in glycoalkaloids which could pose a health threat. Low temperature storage (4 ^{o}C -10 ^{o}C), chemical sprout inhibitors (chlorpropham, CIPC and maleic hydrazide, MH) or low-dose ionizing irradiation (75-200 Gy) can delay sprouting. Ionizing radiation is a type of radiation that has sufficient energy to eject electrons from electrically neutral atoms, leaving charged atoms or ions.

There are four basic types of ionizing radiation: alpha particles (helium nuclei), beta particles (electrons), neutrons, and gamma rays (high frequency electromagnetic waves; x-rays are generally identical to gamma rays except for their place of origin.) Neutrons are not themselves ionizing, but their collisions with nuclei leads to the ejection of other charged particles that do cause ionizing reactions. The term radiation should be used for the energy and the source it is produced from, while the term irradiation should be used for an absorbed dose. Since food receives an absorbed dose and does not produce radiation, the food product has received irradiation. The term Gray (1Gy = 1 J/kg) should be used for the absorbed dose of ionizing radiation. The term Rad (1 rad = 10^{-2} Gy = 10^{-2} J/kg) should be used to define the amount of radiation (Panel on gamma and electron irradiation, 2002).

In 1964, the FDA approved the use of low-dose irradiation treatment for potatoes to inhibit sprouting. Irradiation doses up to 100 krad or 1 kGy have been approved to inhibit the growth and maturation of fruits and vegetables, as well as kill insects which are present after harvest. A dose of 75-150 Gy has been recommended for potato sprout control, depending on cultivar, time of irradiation, post-irradiation storage conditions, and storage duration. The mechanism of dormancy is controlled by endogenous hormones, and the effect of irradiation is believed to inhibit the metabolism of these hormones (Thomas, 1984).

A combination of cold storage at 10-15 ^{o}C and low-dose irradiation is a viable alternative to conventional storage at 2-4 ^{o}C. Irradiation is a relatively cheap (other than

initial costs) means to reduce the application of chemicals to potatoes and reduce the costs of low-temperature storage (Bhushan and Thomas, 1990; Mondy and Gosselin, 1989; Morehouse, 2002; Saour and Makee, 2002). The technology of sprout inhibition with gamma- irradiation is feasible but is not being used commercially, except at one industrial potato irradiation facility operating in Hokkaido, Japan since 1973.

While produce is in storage, there seems to be an immediate increase in phenolics and carotenoids after irradiation, followed by a steady decrease until a steady state is reached. Patil et al. (1999) stated that gamma- irradiation can be used to increase quercetin (a phenolic) content in specific onion cultivars. Aglycone content increased due to partial hydrolysis and/or autolysis. Penner and Fromm (1972) studied the chlorogenic acid content in irradiated potatoes at a dose of 8-15 krad. Chlorogenic acid content rose immediately after irradiation, then returned to normal values. This increase may be due to the induction of the PAL (phenylalanine ammonia lyase) enzyme. Pendharker and Nair (1975, 1987) reported activation of PAL after irradiation.

Electron radiation, or an e-beam system for electronic pasteurization, is a less studied means of irradiation. This method is believed to be more precise and causes less change to a food product. Electron radiation penetrates only shallowly into food products, and the depth depends on the energy of the electrons. In practice, potatoes that are electron irradiated must be fed under the radiation source in a single layer. The tubers must also be turned as they pass below the source to ensure that all sides of the tuber are exposed to the radiation (Rastovski, 1987). Buitelaar (1987) determined that 200 Gy of electron radiation supplied by a source of maximum electron energy of 1.7 MeV (MeV = mega electron volt) is sufficient for most cultivars to remain spout free under storage.

IRRADIATION AND STORAGE. Storage of potatoes at 10-15 ^{o}C has been found to decrease the concentration of carotenoids present in raw potatoes, and irradiation further enhances the disappearance of carotenoids during storage at these temperatures (Bhushan and Thomas, 1990; Janave and Thomas, 1979; Thomas and Joshi, 1977). While tubers are in storage, there seems to be an immediate increase in phenolics and

carotenoids following irradiation followed by a steady decrease until a steady state is reached.

Penner and Fromm (1972) studied the chlorogenic acid content in irradiated potatoes at a dose of 8-15 krad. Chlorogenic acid content rose immediately after irradiation, then returned to normal values after several weeks of storage. Bhushan and Thomas (1990) stated that carotenoid content of irradiated (100 Gy) tubers increased in storage similar to non-irradiated potatoes, but the increase was not as high as in non-irradiated tubers. Storage of 4 ^{o}C and 25-30 ^{o}C resulted in a distinctive increase in carotenoids over time, while storage at 15 ^{o}C and 20 ^{o}C did not cause an increase.

Other biochemical interactions with irradiation and storage exist. Leszcznski et al. (1992) stated that irradiated (150 Gy) tubers were lower in starch, but higher in sugar content, especially sucrose. Pendharkar and Nair (1975) reported two activations of PAL, one immediately following irradiation which did not require protein synthesis, and the other during subsequent storage after irradiation which required a de novo synthesis of the enzyme protein.

STRESS INDUCTION OF PAL. The changes in phytochemical content in fruit and vegetables due to processing may be due to the induction of stress on the food product. Phenolic compounds have been reported to be synthesized in a plant as a protection against adverse conditions such as mechanical bruising, light, and injury by predators (Friedman, 1997; Ghanekar et al., 1984).

The activity of PAL and the phenylpropanoid pathway increases under stressful conditions, and this is associated with the accumulation and synthesis of phenolic compounds (Blankenship and Unrath, 1988; Kang and Saltveit, 2002). Patil et al. (1999) stated that the increase in total quercetin content after irradiation treatment may be due to simulation of PAL (phenylalanine ammonia lyase) and flavonoid biosynthesis. According to Pendharkar and Nair (1987) the PAL enzyme is resistant to low doses of irradiation (10 krad).

ENZYMATIC DISCOLORATION. Enzymatic browning may also play a role in the production of phenolics and could therefore affect antioxidant activity levels. The most

prominent enzymes, polyphenol oxidase (PPO) and peroxidase (POD), catalyze oxidative reactions that give rise to melanins (Tudela et al., 2002; Friedman, 1997). Melanin production and enzymatic discoloration are highly correlated with phenolic levels ($r = 0.89$) (Dean et al. 1992).

PPO concentrations remained relatively constant during cold storage (Coseteng and Lee, 1987). Less PPO activity was observed at the lower temperature (Friedman, 1997). Sawyer and Dallyn (1955) noticed that irradiation also increases the incidence of enzymatic darkening such as "blackspot" in potato, while Mondy and Gosselin (1989) determined that the higher the irradiation (10 and 100 krad) the greater the discoloration. This increase of enzyme content may also be related to the combination of storage and irradiation. An increase in the formation of potato phenolics was observed during storage of tubers following irradiation (Ramarmurthy et al., 1992). The extent of browning also depends on the storage period from harvest to irradiation. Ogawa and Uritani (1970) stated that, in order to minimize browning, potatoes should be stored at ambient temperatures for about 1 month before irradiation.

Patil et al. (1999) suggested that the increase in phenolic content in irradiated food may also be related to ethylene biosynthesis, and that this ethylene synthesis could be related to stress or wounding. The effect of wounding or stress is also a factor in enzymatic coloring. Kang and Saltveit (2002) determined that wounding increased the phenolic content in iceberg lettuce by 330%, whereas it increased the antioxidant capacity by 140%. Also, wounding increased the phenolic content of romaine lettuce by 305% and the antioxidant capacity by 255%. The products that were wounded contained numerous phenolics, including chlorogenic acid, isochlorogenic, caffeoyltartaric, and dicaffeoyltartaric acids.

COMBINATIONS. There are individual effects of processing resulting from cooking, storage, and irradiation, and the combination of these three may act independently or in concert on certain compounds. Shirsat and Thomas (1998) studied the effects of cooking, storage, and irradiation on potatoes and their effect on ascorbic acid. Both storage at 15 ^{o}C and cooking method reduced total ascorbic acid levels (22- 45 %

losses); irradiation resulted in additional losses (5- 13 %). Cooking tubers in boiling water resulted in maximum loss, while pressure and microwave cooking produced smaller losses. The gains/losses in phytochemical levels may or may not be similar to those of ascorbic acid. The research involved with combinations of postharvest treatments and phytochemicals is limited. There are also many possibly significant interactions that might occur with these processing techniques. Multiple factors and multiple interactions may also cause discrepancies between past research and future research due to a) differences in radiation doses and exposure times used by different investigators, b) storage history of the potatoes before and after exposure to irradiation, c) differences in genotype, d) year of harvest, e) location of harvest, f) fertilizer application, g) other processing techniques, h) preparation techniques, and i) different methods of analysis (Friedman, 1997; Goddard et al. 1979).

The objectives of this study were to determine the effects of cooking, storage, and ionizing irradiation on carotenoid content, antioxidant activity, and phenolic content.

CHAPTER III
THE EFFECTS OF CULTIVAR AND COOKING METHOD ON CAROTENOID CONTENT, ANTIOXIDANT ACTIVITY, AND PHENOLIC CONTENT IN POTATO

Synopsis

In spite of the low consumption of fruits and vegetables in many industrialized nations, potato consumption has remained high. Past research has shown that there are significant levels of antioxidants, and phenolic and carotenoid contents within cultivars and advanced breeding lines from the Texas Potato Variety Development Program. However, it is unknown how these phytochemical levels are affected by cooking. The objective of this experiment was to study the effects of cooking methods (no cooking, microwaving, boiling, baking, and frying) on total carotenoid content, individual carotenoid content, total phenolic content, individual phenolic content, and total antioxidant activity in a number of named cultivars and advanced selections harvested at two different locations. Seventeen cultivars and advanced selections were chosen for this study from a harvest near McCook, Texas; fourteen cultivars and advanced selections were chosen from a harvest near Springlake, Texas. Five-gram samples of potato were subjected to one of five cooking methods and frozen until extraction and quantification of phytochemicals. Carotenoid content, both carotene and xanthophyll, was determined via absorbance at 450 nm and 445 nm, respectively. Individual carotenoid compounds were quantified via HPLC identification, based on retention time, spectra, and the combination of retention time and spectra corresponding to standards. Antioxidant activity was determined by DPPH and the kinetic reaction was quantified twice, initially and at stabilization. Phenolic content was determined by the Folin-Ciocalteau method and individual phenolic compounds were also quantified via HPLC. Results indicated there is wide variability for carotenoid content, antioxidant activity, and phenolic content within the cultivars and advanced selections studied. The cultivars Russet Norkotah, Krantz, and Innovator ranked high in all tests. The cooking methods of frying and, microwaving and the raw samples had the highest levels of both

xanthophylls and carotenes, while raw samples had the highest amount of individual carotenoid compounds. Microwaving, frying and baking resulted in higher antioxidant activity and phenolic content, as compared to boiled and raw samples. These results were also supported by the individual phenolic compound quantification via HPLC. The interaction between cultivar and cooking method was not significant.

Introduction

Fruit and vegetable consumption has decreased, and one of the main reasons is that more processed meals are being consumed. In the U.S. the number of hours worked has increased over the past two decades. Consequently, snacks are replacing meals and meal preparation time has decreased. At the turn of the century, typical homemakers spent 44 h a week preparing meals and clean up (Bowers, 2000). Food preparation time in 1980 averaged 60 min, while it averages 20 min today. Potato has been a food of convenience since the early 1900s, which may be one reason why the consumption of potatoes is quite high, even though the consumption of fruits and vegetables has decreased. The Indiana government reported that the percent of people eating potatoes (not including French fries, fried potatoes, or potato chips) once daily is 6.2%; 3-6 times per week, 30.3%; 1-2 times a week, 46.5%; 1-3 times a month, 12.0%; and less than once per month, 4.4% (Indiana State Department of Health, 2000). These are high consumption rates even with the exclusion of fried potato products.

Significant levels of antioxidants, primarily phenolics and carotenoids, have been identified within cultivars and advanced breeding lines in the Texas Potato Variety Development Program; however, it is unknown how these photochemical levels are affected by various cooking methods. Buescher et al. (1999) refer to the loss of nutrient contents of fruits and vegetables after processing as a “hidden loss”, because there may not be any other detectable changes such as changes in color, flavor or texture. There have been a number of studies that have investigated the effects of cooking on antioxidant compounds, although they differ in results. Crozier et al. (1997), Friedman (1997), Ewald et al. (1999), Gil et al. (1999) and Tudela et al. (2002) reported losses in phenolic content with cooking, while Dewanto et al. (2002), Zafrilla et al. (2001), and

Amakura et al. (2000) reported increases in antioxidant activity and phenolic content. Bianchini and Penteado (1998) have reported losses in carotenoids with heat, while Boileau et al. (1999), Dietz et al. (1988), Granado et al. (1992), Klein and Kurilich (2000), and Van den Berg et al. (2000) reported increases in extractability and bioavailability of carotenoids with cooking.

In the present study, the most prominent methods of cooking potato were studied. Four methods are the most prominent in American cuisine, including microwaving, boiling, baking, and frying. Over 90% of American households currently have a microwave (Bowers, 2000) and this is probably due to the convenience that the microwave provides. Boiling is the most common method primarily used for mashed potatoes and potato salad. Baked potatoes alone are a very common meal. Frying is the most common method of cooking potatoes for French fries (chips in UK) and chips (crisps in UK).

The objective of this experiment was to determine the effects of cooking methods (no cooking, microwaving, boiling, baking, and frying) on total carotenoid content, individual carotenoid content, total phenolic content, individual phenolic content, and total antioxidant activity in a number of named cultivars and advanced selections harvested from two different locations. A broader goal of this study was to provide the Texas Potato Variety Development Program and the potato industry with information about cooking effects on a number of named cultivars and advanced selections. This study will also provide information to nutritionists, chefs, and consumers as to which method of cooking will result in the healthiest potato product.

Materials and Methods

HARVEST LOCATION. Two planting locations were used in this study, McCook and Springlake, Texas. McCook is located near the Mexican border in the Lower Rio Grande Valley, 30 miles northwest of McAllen in west central Hidalgo County. Springlake is located in north central Lamb Country in the High Plains of Texas, 59 miles northwest of Lubbock.

PLANT MATERIAL. Named processing cultivars and advanced selections were harvested in April 2003 near McCook, evaluated, and seventeen were selected for this study (Table 3.1). Named processing cultivars and advanced selections were also grown and evaluated in July of 2003 near Springlake, and fourteen were selected for this study (Table 3.1).

Table 3.1 Processing cultivars and advanced selections selected in McCook and Springlake, 2003.

McCook	Springlake
A84420-5	A84420-5
Atlantic	Atlantic
ATX84706-2Ru	ATX84706-2Ru
ATX85404-8W	ATX85404-8W
F88042	F88042
Innovator	Innovator
Krantz	Krantz
NDTX4930-5W	NDTX4930-5W
Russet Burbank	Russet Burbank
Russet Norkotah	
Santana	Santana
Shasta	Shasta
Shepody	Shepody
Superior	Superior
TX1523-1W/Y (Sierra Gold ^{TM})	
Umatilla	Umatilla
Yukon Gold	

The selected cultivars represent variability among popular processing potatoes. The carbohydrate (starch and sugar) composition and water content of tubers determines the use of the potato cultivar. Processing cultivars must have high starch (dry matter) and low reducing sugar (glucose/fructose) levels. Reducing sugars create undesirable dark chips. Potatoes with high dry matter and low reducing sugar levels tend to be more desirable for frying. Potato cultivars used for French fries should have a long, cylindrical shape, while potato cultivars with a round shape are often used for chipping. Table 3.2 describes some characteristics of the cultivars used in this study.

Table 3.2 Characteristics of potato cultivars and advanced selections used in cooking studies.

Cultivar	Shape	Skin Color / Flesh Color	Utilization	Maturity
A84420-5	Oval	White / White	Fry Processing	Early
Atlantic	Oval to round	White, buff / White	Chipping, Boiling, and Baking	Early to Medium
ATX84706-2Ru	Oblong	Russet Light / White	Boiling, Baking, and French fries	Early
ATX85404-8W	Oval	White / White	Chipping	Medium to Late
F88042	Long	White / White	Fry Processing	Early to Medium
Innovator	Oblong to long	Russet / Yellow	Baking and French fries	Early to Medium
Krantz	Oblong	Russet / White	Boiling, Baking and French fries	Medium
NDTX4930-5W	Oval to long	Light, buff / White	Chipping and French fries	Early to Medium
Russet Burbank	Long	Russet / White	Baking and Fry Processing	Late
Russet Norkotah	Long to slightly oblong	Russet / White	Boiling and Baking	Early to Medium
Santana	Oval to long	White / Yellow	Fry Processing	Early to Medium
Shasta	Oval to long	White to Yellow / Cream	Fry Processing	Early to Medium
Shepody	Long	Buff / White	Boiling, Baking, and French fries	Medium
Superior	Oval to oblong	Buff / White	Boiling, Baking, and Fry Processing	Early to Medium
TX1523-1W/Y	Oval	Russet / Yellow	Boiling, Baking and French fries	Early
Umatilla	Long to oblong	Russet / White	Boiling, Baking, and Fry Processing	Medium to Late
Yukon Gold	Oval	White / Yellow	Boiling, Baking, and Fry Processing	Medium

SAMPLE PREPARATION. Three tubers from each field replication were diced with a manual vegetable dicer (The Redco Insta Cut 3.5, Lincoln Foodservice, Fort Wayne, IN). Size of the diced samples was roughly 0.64 cm cubes. The diced tubers were mixed, so a randomized sample was obtained. The 5 g samples were placed in extraction tubes and were frozen at -18 ^{o}C (0 ^{o}F) until cooking.

COOKING METHODS. Four cooking methods and a no cooking or raw control were used. Processing times and temperatures were based on the optimum times and required temperatures to cook the average sample. This was determined based on the texture and feel of the sample. The uncooked sample had a starchy texture that was firm and sticky, while cooked samples had an interior that was mealy and/or powdery. The raw samples remained frozen at -18 ^{o}C (0 ^{o}F) until extraction.

Microwave. Six samples were cooked in their respective tubes for 2.5 min on high with a microwave (model MW8985W, Emerson, St. Louis, MO). After 1 min, the cooking process was stopped to mix the contents. After cooking for the second minute, the cooking process was stopped to rotate the sample, then cooking continued for another thirty seconds. Cooked samples were then frozen at -18 ^{o}C (0 ^{o}F) until extraction.

Boil. Water was brought to a boil using a stove range (Montgomery Ward, Cedar Rapids, IA). Ten mL of nanopure, autoclaved water was added to each sample in the plastic extraction tubes. Samples were cooked for 25 min in the boiling water. After cooking, the leachate was removed and discarded. The cooked potato sample was patted dry and frozen at -18 ^{o}C (0 ^{o}F) until extraction.

Bake. A gas oven (Montgomery Ward, Cedar Rapids, IA) was brought to 204 ^{o}C (400 ^{o}F). One-hundred-sixty samples, in glass tubes were cooked for 15 min. Foil was used to cover the tubes to prevent water loss. After cooking, the samples were removed from the glass test tubes and placed back into the plastic tubes for extraction. Cooked samples were then frozen at -18 ^{o}C (0 ^{o}F) until extraction.

Fry. Canola oil was brought to 191 ^{o}C (375 ^{o}F) in a mini fryer (Rival CF250 Cool Touch Deep Fryer, El Paso, TX). Potato samples were placed in tea balls. The cooking time, which began once the tea ball entered the oil, was 1 min. After cooking, the

sample was removed from the tea ball and placed on paper towels to cool, and the sample was placed back into the plastic extraction tubes. Cooked samples were then frozen at -18 ^{o}C (0 ^{o}F) until extraction.

EXTRACTION OF CAROTENOIDS. Carotenoid extraction combined the use of two solvents for the extraction of two different classes of compounds, carotenes and xanthophylls. The carotenes were extracted with hexane, and the xanthophylls were extracted with methanol (plus 1 g/ L of BHT for stabilization). This double extraction procedure was used to quantify total carotenoid content based on the content of xanthophylls and carotenes, and the individual carotenoid content via HPLC. Twenty-five mL of methanol plus BHT was added to a 5-g sample of diced potato. This mixture was then homogenized with an ultra turrax tissumizer from Tekmar (Cincinnati, OH). Ten mL of hexane was added to the sample. Samples and solvent were stored at -20 ^{o}C (-4 ^{o}C) for at least 12 h to ensure that the solvent extracted all carotenoids. Samples were then placed in a J-17 rotor at 17,000 rpm for 20 min in a refrigerated centrifuge manufactured by Beckman (model J2-21, Fullerton, CA). Eight mL of the methanol and 8 mL of hexane were extracted. A second extraction procedure was then conducted to ensure all carotenoids were extracted from the cells. The left-over solvents were discarded and the pellet of tissue was used for the following extraction. Five mL of methanol (plus BHT 1g/L) and 10 mL of hexane were added to the pellet. The sample was shaken and placed back into the centrifuge at 17,000 rpm for 20 min. Four mL of the methanol and 4 mL of the hexane were extracted and added to the previous extraction. Two mL of the 12 mL from both extractions was saved for the analysis of total carotenoids and two mL of each replication (total 6 mL) were used for HPLC analysis on selected cultivars (Fig. 3.1). The extracted samples were stored at -29 ^{o}C (-20 ^{o}F).

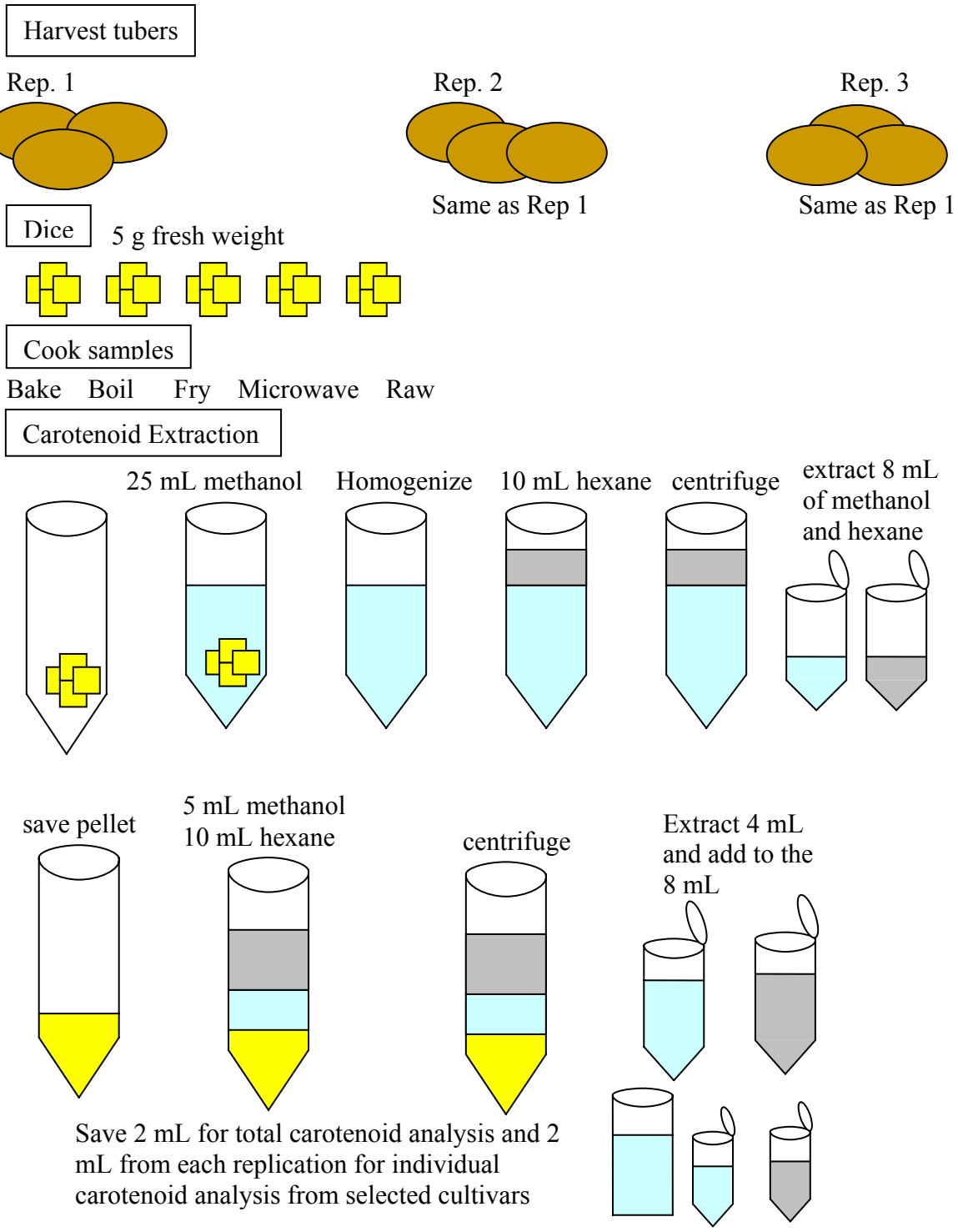

Fig 3.1 Carotenoid extraction procedure for the factors cultivar and cooking method.

HPLC ANALYSIS FOR INDIVIDUAL CAROTENOID COMPOUNDS. Selected cultivars (Innovator, Russet Burbank, and Santana) were analyzed for individual carotenoid compounds and were chosen based on high quantifications on all tests. The extracted samples were concentrated under nitrogen gas and filtered through a 0.45 μm syringe filter (Hale, 2003). The samples were resuspended in 0.5 mL ethanol and 0.5 mL nanopure water. A PC-operated Waters high performance liquid chromatograph was used to analyze individual carotenoid compounds through spectra and retention time. The samples were analyzed using Waters Millennium 3.2 software, Waters 515 binary pump system (Waters 515), an autoinjector (Waters 717 plus), and photodiode detector (Waters 996), along with a column heater (SpectraPhysics SP8792) maintained at 35 ^{o}C. A 4.6 x 250 mm, 5 μm, YMC Carotenoid Column (C-30 reverse phase) (Waters, Milford, MA) was used to separate the carotenoid compounds. The compounds analyzed and used to create a library included: 1) violaxanthin (CaroteNature, Lupsingen, Switzerland), 2) neoxanthin (CaroteNature, Lupsingen, Switzerland), 3) antheraxanthin (CaroteNature, Lupsingen, Switzerland), 4) β-cryptoxanthin (Hoffman La Roche, Basel, Switzerland), 5) canthaxanthin (Hoffman La Roche, Basel, Switzerland), 6) zeaxanthin (Hoffman La Roche, Basel, Switzerland), and 7) lutein (Hoffman La Roche, Basel, Switzerland). Two filtered and de-gassed solution solvents were used for carotenoid extraction. "Solvent A" consisted of methanol, water, and triethylamine (90:10:0.1), while "solvent B" consisted of methanol, MTBE, and triethylamine (6:90:0.1). The following was the gradient for the analysis: (min / %A) 0/99, 8/99, 8/99, 45/0, 50/0, and 53/99 (Breithaupt and Barmedi, 2002; Hale, 2003).

EXTRACTION OF PHENOLICS AND TOTAL ANTIOXIDANT ACTIVITY. The same extraction procedure was conducted for total phenolic content, individual phenolic content, and total antioxidant activity. Fifteen mL of methanol was added to a 5-g sample of diced potato. This mixture was then homogenized with an ultra turrax tissumizer from Tekmar. After homogenization, samples were placed in a J-17 rotor at 17,000 rpm for 20 min in a refrigerated centrifuge (Beckman model J2-21). Two mL of the methanol extract were saved in snap-cap tubes for analysis of total antioxidant

activity and total phenolic content. The selected cultivars (Innovator, Russet Burbank, and Santana) were chosen for individual phenolic analysis, and 6 mL of the methanol extracts were saved in glass vials (Fig. 3.2). The extracted samples were stored at -29 ^{o}C (-20 ^{o}F).

DPPH ASSAY FOR TOTAL ANTIOXIDANT ACTIVITY. Total antioxidant activity was measured using DPPH (1,1 Diphenyl-2 picrylhydrazyl), a colorimetric assay first described by Brand-Williams et al. (1995). DPPH is a stable radical which causes oxidation and can be reduced by natural antioxidants, which reduce the oxidizing power of DPPH. Non-reduced DPPH is dark purple, while reduction shifts the color from dark purple to lighter purple to light yellow. This decrease in color and reduction power can be measured at 515 nm. The reduction was correlated to absorbance. The lower the absorbance, the greater the antioxidant activity in the sample.

The DPPH solution was diluted by dissolving 24 mg DPPH in 100 mL methanol to create a 607 μM DPPH stock solution. The solution was then diluted to ~10:55 with methanol until the spectrophotometer read 1.1 at 515 nm. The extracted methanol sample of 150 μL was combined with 2.85 mL of diluted DPPH in a scintillation vial, along with a blank which contained 150 μL of pure methanol (instead of methanol extract) with the diluted 2.85 mL DPPH. The samples reacted for 15 min. After this time, the level of reduction was determined by absorption at 515 nm in a plastic UV-spectrophotometeric cuvette. This reading was based on the activity of the sample after 15 min (initial antioxidant activity, AOAI), but the total reaction is kinetic and continues for about 24 h until stabilization (stabilized antioxidant activity, AOAS). Each antioxidant compound reacts with the oxidizing substance at a given time; therefore, two readings were recorded. The second reading was taken after 24 h, when the samples and the DPPH had stabilized. The first reading (after 15 min) represents an initial response, whereas the second represents a final response. It is currently unknown how long consumed antioxidants are functional; therefore, these readings may represent two responses. Absorptions were subtracted from the blank. A standard curve using a known antioxidant, trolox (6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid),

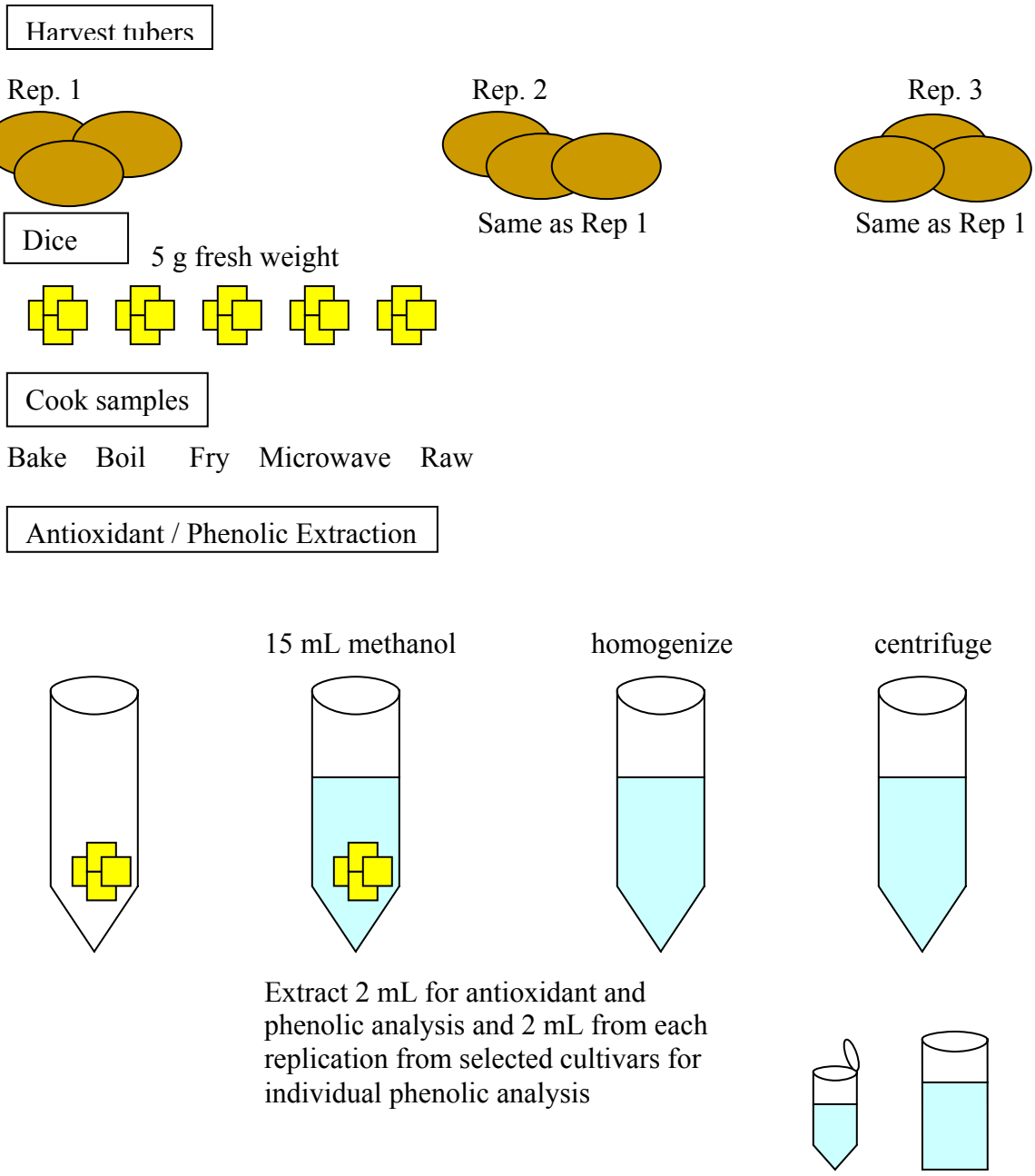

Fig. 3.2 Antioxidant/ phenolic extraction procedure for the factors cultivar and cooking method.

was prepared, and a regression curve was calculated to convert the antioxidant activity into trolox equivalents.

TOTAL PHENOLIC CONTENT. The Folin-Ciocalteau phenol method to determine phenolic content was first described by Swain and Hillis, (1959) and modified by Singleton and Rossi (1965). This method, like the total antioxidant activity method, is a colorimetric reaction that is determined by absorbance. A 0.25 N Folin - Ciocalteau phenol reagent solution with nanopure water and a 1.0 N Na_2Co_3 solution with nanopure water were prepared. The extracted methanol sample of 150 μL was combined with 2.4 mL of nanopure water in a scintillation vial, along with a blank which contained 150 μL of pure methanol (instead of methanol extract) with 2.4 mL of nanopure water. The samples and blank reacted with 150 μl of the 0.25 N Folin - Ciocalteau phenol reagent solution for 3 min. Then, 300 μL of the 1.0 N Na_2Co_3 solution was added to both the samples and blank. The reaction was also kinetic, and stabilization occurred after 1 h and 55 min. Data was recorded at stabilization. Absorption was determined at 725 nm in plastic UV-spectrophotometeric cuvettes. The blank was read first, and the sample absorption was based on the cleared response of the blank. The phenolic content was determined by a prepared regression curve to chlorogenic acid equivalents.

HPLC ANALYSIS FOR INDIVIUAL PHENOLIC COMPOUNDS. Selected cultivars (Innovator, Russet Burbank, and Santana) analyzed for individual phenolic compounds were chosen based on high quantifications on all tests. The extracted samples were concentrated under nitrogen gas or by using a heated speed vacuum centrifuge and filtered through a 0.45 μm syringe filter (Hale, 2003). A PC-operated Waters high performance liquid chromatograph was used to analyze individual phenolic compounds through spectra and retention time. The samples were analyzed using Waters Millennium 3.2 software, Waters 515 binary pump system (Waters 515), an autoinjector (Waters 717 plus), and photodiode detector (Waters 996), along with a column heater (SpectraPhysics SP8792) maintained at 40 ^{o}C (104 ^{o}F). A 4.6 x 150 mm, 5 μm, Atlantis C-18 reverse-phase column (Milford, MA) was used to separate phenolic compounds. The compounds analyzed included: 1) 5,7-trihydroxyflavanone, 2) sinapic

acid, 3) kampherol, 4) (-) epicatechin, 5) catechin, 6) quercetin dehydrate, 7) rutin hydrate, 8) protocatechuic acid, 9) salicylic acid, 10) myricetin, 11) syringic acid, 12) gallic acid, 13) vanillic acid, 14) t-cinnamic acid, 15) p-coumaric acid, 16) ferulic acid, 17) caffeic acid, and 18) chlorogenic acid; the standard compounds were obtained from Agros Organics (Pittsburgh, PA). Two filtered and de-gassed solution solvents were used for the phenolic extraction. "Solvent A" consisted of acetonitrile, and "solvent B" consisted of nanopure water, and HCL adjusted to pH 2.3. The following gradient was used, (min/%A) 0:85, 5:85, 30:0, 35:0 (Hale, 2003).

STATISTICAL ANALYSIS. The field plot was a completely randomized design with tuber sample replications collected from 3-4 different blocks. A separate multiple analysis of variance (MANOVA) general linear model was performed based on location and year. The statistical model of the experiment was a full factorial design. The dependent variables included total carotenoid content, total phenolic content, total antioxidant activity initially (AOAI) (measurements recorded after 15 min), and total antioxidant activity at stabilization (AOAS) (measurements recorded after 24 h). The fixed factors included cultivar and cooking method. Factor comparison was conducted using the post hoc multiple comparison methods of S-N-K tests. Also, a test to measure the estimate of magnitude of effect or strength of association was conducted. This test determines how strongly two or more variables are related, or the magnitude of difference between groups. The effect size is reported as eta squared values and is defined as the sums of squares of the effect of interest divided by the total sums of squares (Levine and Hullett, 2002). The analysis was conducted using the SPSS statistical package version 11.5.

Results

STANDARD CURVE FOR LUTEIN. The linear regression equation to equate the spectrophotometric absorbance readings of the methanol extract at 445 nm to lutein equivalents was as follows: $3028.6x + 8.1063$, where x was the absorbance at 445 nm and y was the µg lutein equivalents per hundred g fresh weight. The R^2 value of this equation was 0.9991.

STANDARD CURVE FOR β-CAROTENE. The linear regression equation to equate the spectrophotometric absorbance readings of the hexane extract at 450 nm to β-carotene equivalents was as follows: 373.59x + 2.0463, where x was the absorbance at 450 nm and y was the µg β- carotene equivalents per hundred grams fresh weight. The R^2 value of this equation was 0.9993.

McCook 2003. The average amount of xanthophylls or lutein equivalents for all cultivars and cooking methods was 163 µg/100gfw, the average amount of carotenes or β-carotene equivalents was 24 µg/100gfw, and the total carotenoid average was 187 µg/100gfw. Analysis of variance for xanthophylls indicated that there were significant differences for cultivar ($p < 0.00$), and cooking method ($p < 0.00$), but not for the interaction of cultivar and cooking method. Similar results were observed with carotenes, where there were significant differences for cultivar ($p < 0.00$) and cooking method ($p <0.00$), but not for the interaction. The addition of both xanthophylls and carotenes (total carotenoids) showed a similar trend (Table 3.3).

There were multiple factors that could affect carotenoid content of samples, although some factors had a greater influence than others. The eta squared is an estimate of the magnitude of the effect, which attempts to explain how strongly two or more variables are related (Levine and Hullett, 2002). All the eta squared values, including error, when added together equal 100 %. The eta squared value for cultivar for xanthophylls (eq. of lutein) was 42 %, while that for carotenes (eq. of β-carotene) was 35 %. This suggests that cultivar accounts for 42 % of the total variability in xanthophyll content and 35 % of the variability in carotene content. The eta squared value for cooking method for both the xanthophylls and the carotenes was 2 %, while the eta squared value for the interaction of cultivar and cooking method for both xanthophylls and carotenes was 6 %. Unknown causes or error that is not caused by variability in cultivar and cooking was 50 % for the xanthophylls and 57 % for the carotenes.

Table 3.3 Analysis of variance results for carotenoid content for the factors cultivar and cooking method, McCook 2003.

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	xanthophyll	1851769.292 [z]	84	22044.873	6.686	.000
	carotene	50313.678 [y]	84	598.972	4.782	.000
	total carotenoids	2099777.415 [x]	84	24997.350	7.408	.000
Intercept	xanthophyll	16202768.367	1	16202768.367	4914.104	.000
	carotene	361806.532	1	361806.532	2888.416	.000
	total carotenoids	21406998.854	1	21406998.854	6344.393	.000
Cultivar	xanthophyll	1530174.373	16	95635.898	29.005	.000
	carotene	40490.390	16	2530.649	20.203	.000
	total carotenoids	1738748.892	16	108671.806	32.207	.000
Cook	xanthophyll	87299.485	4	21824.871	6.619	.000
	carotene	2654.699	4	663.675	5.298	.000
	total carotenoids	108407.745	4	27101.936	8.032	.000
Cultivar * Cook	xanthophyll	235759.023	64	3683.735	1.117	.258
	carotene	7405.281	64	115.708	.924	.644
	total carotenoids	255499.274	64	3992.176	1.183	.167
Error	xanthophyll	1764000.358	535	3297.197		
	carotene	67014.767	535	125.261		
	total carotenoids	1805175.613	535	3374.160		
Total	xanthophyll	20176610.002	620			
	carotene	473360.376	620			
	total carotenoids	25678238.788	620			
Corrected Total	xanthophyll	3615769.650	619			
	carotene	117328.445	619			
	total carotenoids	3904953.028	619			

[z] $R^2 = .512$ (Adjusted $R^2 = .436$)
[y] $R^2 = .429$ (Adjusted $R^2 = .339$)
[x] $R^2 = .538$ (Adjusted $R^2 = .465$)

The main effect of cultivar was a significant factor for all dependent values (xanthophylls, carotenes, and total carotenoids), and a wide range of values was seen among cultivars (Table 3.4). 'Shepody' had the lowest amount of xanthophylls at 96µg/gfw, while 'Innovator' had the highest at 276 µg/100gfw, an almost three-fold difference. The carotene content within cultivars was much smaller than the xanthophyll content; however, the range for carotenes was greater than for the xanthophylls, with almost a four-fold difference. The selection F88042 was the lowest at 14 µg/100gfw,

while 'Yukon Gold' was the highest at 53 µg/100gfw. The total carotenoid content ranged from 116 µg/100gfw for 'Shepody' to 304 µg/100gfw in 'Innovator'. Although several cultivars (eg. Yukon Gold and Innovator) that had high levels of xanthophyll also had high levels of carotene, many of the rankings were not similar. 'Russet Norkotah' had a high ranking in xanthophyll level but ranked in the middle for carotene.

Table 3.4 Cultivar ranking for xanthophyll, carotene, and total carotenoid content, McCook 2003.

Cultivar	Xanthophylls Eq. of lutein (µg/100gfw)		Cultivar	Carotenes Eq. of β-carotene (µg/100gfw)		Cultivar	Total carotenoids (µg/100gfw)	
Innovator	276	a[z]	Yukon Gold	53	a	Innovator	304	a
Russet Norkotah	275	a	A84420-5	35	b	Russet Norkotah	297	a
Santana	215	b	Atlantic	33	b	Yukon Gold	250	b
Krantz	213	b	TX1523-1W/Y	33	b	Santana	244	b
Yukon Gold	198	bc	Santana	29	bc	Krantz	236	b
Russet Burbank	184	bcd	Innovator	28	bc	A84420-5	208	c
A84420-5	173	cd	Krantz	24	cd	Russet Burbank	203	cd
F88042	156	de	Umatilla	23	cde	TX1523-1W/Y	186	cd
TX1523-1W/Y	153	de	Russet Norkotah	22	cde	F88042	171	de
ATX85404-8W	150	de	Shepody	20	de	ATX85404-8W	170	def
Umatilla	134	ef	NDTX4930-5W	20	de	Umatilla	157	def
ATX84706-2Ru	133	ef	ATX85404-8W	20	de	Atlantic	155	efg
NDTX4930-5W	125	ef	Russet Burbank	20	de	ATX84706-2Ru	151	efg
Shasta	124	ef	ATX84706-2Ru	18	de	NDTX4930-5W	145	efg
Atlantic	122	ef	Superior	18	de	Shasta	140	efg
Superior	117	ef	Shasta	15	de	Superior	138	fg
Shepody	96	f	F88042	14	e	Shepody	116	fg

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

For cooking method, as with cultivar, the xanthophyll content was greater than the carotene content, although the range was smaller among all dependent values (Table 3.5). The xanthophyll content ranged from 141µg/100gfw for the boiling method to 173µg/100gfw for the microwave method. The carotenes ranged from 21 µg/100gfw for the baking method to 27 µg/100gfw for the fry method. The total carotenoid content ranged from 163 µg/100gfw for the boiling method to 199 µg/gfw for the fry method. Based on these results, the fried, microwaved, and raw samples were all in the first level

of significance. The baking and boiling methods were lower. Therefore, it appears that most cooking methods do not have a large effect on the carotenoid content of potatoes, except for boiling which generally resulted in slightly reduced levels.

Table 3.5 Cooking method ranking for carotenoid content, McCook 2003.

Cooking method	Xanthophylls Eq. of lutein (µg/100gfw)		Cooking method	Carotenes Eq. of β-carotene (µg/100gfw)		Cooking method	Total carotenoids (µg/100gfw)	
Micro	173	a[z]	Fry	27	a	Fry	199	a
Fry	172	a	Raw	25	ab	Micro	197	a
Raw	168	a	Micro	24	abc	Raw	193	a
Bake	163	a	Boil	22	bc	Bake	185	a
Boil	141	b	Bake	21	c	Boil	163	b

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Effects of cultivar and cooking method are presented in Table 3.6. The top five average values for the xanthophylls (expressed as equivalents of lutein µg/100gfw) were as follows: 'Russet Norkotah', raw (372); 'Innovator', microwave (314); 'Russet Norkotah', microwave (308); 'Innovator', raw (301); and 'Innovator', fry (293). The lowest five average values for the xanthophylls were as follows: 'Shepody', boil (84); 'Shepody', bake (87); 'Shepody', raw (91); 'Shepody', fry (100); and 'NDTX4930-5W', boil (107). Microwave, fry and raw ranked highest. Irregardless of cooking method, the cultivar Shepody was lowest.

The top five average values for the carotenes (expressed as equivalents of β-carotene µg/100gfw) were as follows: 'Yukon Gold', microwave (65); 'Yukon Gold', raw (59); 'Yukon Gold', fry (57); 'A84420-5', fry (56); and 'TX1523-1W/Y', microwave (43). The lowest five average values for the carotenes were as follows: 'F88042', bake (12); 'F88042', microwave (12); 'Shasta', bake (13); 'Shasta', boil (15); and 'F88042', raw (15). 'Yukon Gold' again was the highest in carotenes. The best cooking methods were microwave and fry.

The top five average values for total carotenoids were ‘Russet Norkotah’, raw (395); ‘Innovator’, microwave (340); ‘Russet Norkotah’, microwave (333); ‘Innovator’, raw (332); and ‘Innovator’, fry (323). The lowest five average values for total carotenoids were ‘Shepody’, boil (104); ‘Shepody’, bake (106); Shepody’, raw (113); ‘Shepody’, fry (119); and ‘Shasta’, boil (123). ‘Shepody’ again had the lowest total carotenoid ranking and ‘Russet Norkotah’ and ‘Innovator’ were the highest.

Table 3.6 The influence of cultivar and cooking method on contents of zanthophyll, carotene, and total carotenoids, McCook 2003.

Cultivar and cooking method	Eq. of lutein (µg/100gfw)	Eq. of β-carotene (µg/100gfw)	Total carotenoids (µg/100gfw)
A84420-5			
Bake	186	30	216
Boil	143	27	171
Fry	206	56	262
Micro	165	28	193
Raw	165	34	199
Atlantic			
Bake	124	31	155
Boil	109	30	140
Fry	145	42	187
Micro	112	29	141
Raw	121	33	155
ATX84706-2Ru			
Bake	139	17	156
Boil	110	16	126
Fry	159	17	177
Micro	128	21	148
Raw	128	20	148
ATX85404-8W			
Bake	135	18	153
Boil	149	21	170
Fry	151	21	172
Micro	176	20	197
Raw	140	19	159
F88042			
Bake	137	12	148
Boil	127	16	143
Fry	168	16	184
Micro	171	12	183
Raw	179	15	194
Innovator			
Bake	261	27	288
Boil	208	28	236
Fry	293	30	323

Table 3.6 (continued).

Cultivar and cooking method	Eq. of lutein (µg/100gfw)	Eq. of β-carotene (µg/100gfw)	Total carotenoids (µg/100gfw)
Micro	314	26	340
Raw	301	31	332
Krantz			
Bake	212	21	234
Boil	193	24	217
Fry	212	27	240
Micro	210	21	231
Raw	236	24	260
NDTX4930-5W			
Bake	130	18	148
Boil	107	20	127
Fry	145	19	164
Micro	129	22	150
Raw	115	21	136
Russet Burbank			
Bake	188	18	206
Boil	150	18	169
Fry	195	20	215
Micro	189	23	212
Raw	195	20	215
Russet Norkotah			
Bake	249	18	268
Boil	220	24	244
Fry	226	21	247
Micro	308	25	333
Raw	372	23	395
Santana			
Bake	209	27	235
Boil	179	28	207
Fry	222	31	253
Micro	216	29	245
Raw	248	32	280
Shasta			
Bake	156	13	170
Boil	108	15	123
Fry	124	17	140
Micro	110	16	126
Raw	123	17	140
Shepody			
Bake	87	19	106
Boil	84	20	104
Fry	100	19	119
Micro	115	20	135
Raw	91	22	113
Superior			
Bake	112	17	128
Boil	110	17	127
Fry	108	18	126
Micro	134	16	150
Raw	135	22	157

Table 3.6 (continued).

Cultivar and cooking method	Eq. of lutein (µg/100gfw)	Eq. of β-carotene (µg/100gfw)	Total carotenoids (µg/100gfw)
TX1523-1W/Y			
Bake	162	25	188
Boil	128	32	160
Fry	147	33	180
Micro	207	43	250
Raw	121	32	153
Umatilla			
Bake	144	19	163
Boil	135	19	154
Fry	141	25	167
Micro	128	25	153
Raw	121	25	147
Yukon Gold			
Bake	213	42	255
Boil	191	39	230
Fry	233	57	290
Micro	245	65	311
Raw	108	59	166

DPPH ASSAY FOR TOTAL ANTIOXIDANT ACTIVITY- STANDARD CURVE FOR TROLOX. The linear regression equation to equate the spectrophotometric absorbance readings of the methanol extract and reduced DPPH at 515 nm into trolox equivalents was as follows: $y = 891.69x$, where x was the delta absorption calculated from the subtraction of the sample from the blank of methanol and DPPH at 515 nm and y was the µg trolox equivalents per g fresh weight. The R^2 value of this equation was 0.997.

McCook 2003. The average initial antioxidant activity, AOAI, (quantified after 15 minutes) was 120 trolox equivalents µg/gfw; while, the average of stabilized antioxidant activity, AOAS, (quantified after 24 hours) was 348 trolox equivalents µg/gfw. Analysis of variance revealed that the main effects of cultivar and cooking method were both significantly different for the dependent values AOAI ($p < 0.000$; $p < 0.000$, respectively) and AOAS ($p < 0.000$; $p < 0.000$, respectively). The cultivar by cooking method interaction was not significant either AOAI ($p = 0.052$) or AOAS ($p = 0.377$) (Table 3.7). The eta squared values for cultivar were 42% for both AOAI and AOAS,

the values for cooking method were 14% for AOAI and 11% for AOAS, and the interaction values were 12% and 11%, respectively. Error accounted for 32 % and 36% in AOAI and AOAS, respectively. Again, cultivar was the most influential factor on antioxidant activity. The eta squared values for cooking and the interaction between cooking and cultivar were much higher than those for carotenoid values. This indicates that antioxidant content can be manipulated by cooking.

Table 3.7 Analysis of variance results for antioxidant activity for the factors cultivar and cooking method, McCook 2003.

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	AOAI	1478302.994 [z]	87	16991.988	5.486	.000
	AOAS	6295305.818 [y]	87	72359.837	4.801	.000
Intercept	AOAI	4040484.510	1	4040484.510	1304.463	.000
	AOAS	33846606.012	1	33846606.012	2245.737	.000
Cultivar	AOAI	919919.653	16	57494.978	18.562	.000
	AOAS	4026874.426	16	251679.652	16.699	.000
Cook	AOAI	306086.233	4	76521.558	24.705	.000
	AOAS	1089462.971	4	272365.743	18.072	.000
Cultivar * Cook	AOAI	270500.637	64	4226.572	1.365	.052
	AOAS	1019642.496	64	15931.914	1.057	.377
Error	AOAI	687629.825	222	3097.432		
	AOAS	3345870.693	222	15071.490		
Total	AOAI	6200222.348	310			
	AOAS	45725326.029	310			
Corrected Total	AOAI	2165932.819	309			
	AOAS	9641176.510	309			

[z] R^2 = .683 (Adjusted R^2 = .558)
[y] R^2 = .653 (Adjusted R^2 = .517)

There was a high amount of variability among cultivars (Table 3.8). The AOAI range was 317 to 44 µg trolox eq./ gfw, with 'Russet Norkotah' the highest and 'Shepody' the lowest; the AOAS range was 727 to 206 µg trolox eq./ gfw, with 'Russet Norkotah' again the highest and 'Shepody' the lowest. The antioxidant content increased from the initial reading at 15 min to the stabilization reading at 24 h. This

increase, however, was not uniform across cultivars. Some cultivars increased slightly over two-fold, eg. Russet Norkotah (from 317 to 727 µg trolox eq./ gfw), while others, such as ATX84706-2Ru and F88042, increased to a greater extent (139 to 442 µg trolox eq./ gfw) and (71 µg trolox eq./ gfw to 391 µg trolox eq./ gfw), respectively. This may be due to variability in kinetic behavior of the compounds within samples. Generally, the cultivar rankings from AOAI to AOAS remained about the same.

Table 3.8 Cultivar ranking for antioxidant activity, McCook 2003.

Cultivar	AOAI[z] (µg/gfw)		Cultivar	AOAS[y] (µg/gfw)	
Russet Norkotah	317	a[x]	Russet Norkotah	727	a
Russet Burbank	181	b	Russet Burbank	500	b
Innovator	175	b	ATX84706-2Ru	442	bc
Yukon Gold	160	bc	Krantz	427	bc
ATX84706-2Ru	139	bcd	Innovator	414	bcd
Superior	134	bcde	F88042	391	bcde
Krantz	125	bcdef	ATX85404-8W	385	bcde
Umatilla	124	bcdef	Umatilla	346	cdef
ATX85404-8W	112	cdefg	NDTX4930-5W	321	cdefg
A84420-5	102	defgh	Yukon Gold	294	defg
Atlantic	92	defgh	Shasta	287	efg
TX1523-1W/Y	79	efgh	Santana	257	fg
Shasta	78	efgh	A84420-5	256	fg
F88042	71	fgh	TX1523-1W/Y	231	fg
Santana	61	gh	Superior	220	fg
NDTX4930-5W	51	h	Atlantic	210	g
Shepody	44	h	Shepody	206	g

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[x] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The main effect of cooking for AOAI ranged from 63 µg trolox eq./ gfw for the raw samples to 163 µg trolox eq./ gfw for the microwaved samples (Table 3.9). The main effect for AOAS ranged from 247 µg trolox eq./ gfw for the raw samples to 428 µg trolox eq./ gfw for microwaved samples. The fact that the raw samples had lower total antioxidant activity than the cooked samples, is an interesting phenomenon because many believe that cooked potatoes have less nutritious compounds than raw potatoes. Either a synthesis of compounds during or after cooking or a release of compounds due

to changes in texture of the starches such as gelatinization, might explain the increases seen during cooking. Consistent with earlier results, the microwaved samples were highest, with boiled samples significantly lower.

Table 3.9 Cooking method ranking for antioxidant activity, McCook 2003.

Cooking method	AOAI[z] (µg/gfw)		Cooking method	AOAS[y] (µg/gfw)	
Micro	163	a[x]	Micro	428	a
Fry	132	b	Fry	388	b
Bake	127	b	Bake	348	bc
Boil	116	b	Boil	328	c
Raw	63	c	Raw	247	d

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[x] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The effects of cultivar and cooking method were also analyzed (Table 3.10). The top five averages for AOAI were 'Russet Norkotah', microwave (450), 'Russet Norkotah', bake (435), 'Russet Norkotah', fry (360); 'Russet Norkotah', boil (278); and 'Yukon Gold', fry (258). The lowest five averages for AOAI were 'TX1523-1W/Y' raw (23); 'NDTX 4930-5W', raw (24); 'F88042', raw (26); 'Shepody', boil (33); and 'Santana', raw (35). The top five average values for AOAS were 'Russet Norkotah', boil (848); 'Russet Norkotah', fry (811); 'Russet Norkotah', bake (717); 'Russet Norkotah', microwave (680); and 'Russet Burbank', fry (630). The lowest five average values for AOAS were 'Atlantic', raw (70); 'A84420-5', raw (110); 'TX1523-1W/Y', raw (118); 'Atlantic', boil (126); and 'Shepody', boil (133). Over all, cooked samples had the highest levels of antioxidant activity, while raw or boiled samples were at the lower end of antioxidant activity. Furthermore, this data supports earlier results that cultivar is the most significant factor in determining antioxidant activity in processed potatoes.

Table 3.10 The influence of cultivar and cooking method on antioxidant activity, McCook 2003.

Cultivar and cooking method	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
A84420-5		
Bake	108	384
Boil	108	384
Fry	114	313
Micro	128	294
Raw	58	110
Atlantic		
Bake	87	283
Boil	63	126
Fry	135	250
Micro	131	318
Raw	42	70
ATX84706-2Ru		
Bake	154	451
Boil	93	388
Fry	162	519
Micro	169	479
Raw	114	373
ATX85404-8W		
Bake	93	342
Boil	128	395
Fry	126	435
Micro	149	469
Raw	66	284
F88042		
Bake	53	328
Boil	117	419
Fry	38	326
Micro	124	580
Raw	26	302
Innovator		
Bake	137	348
Boil	212	403
Fry	194	523
Micro	236	534
Raw	98	261
Krantz		
Bake	125	410
Boil	162	429
Fry	114	442
Micro	158	509
Raw	66	345
NDTX4930-5W		
Bake	54	292
Boil	47	447
Fry	46	278
Micro	86	349
Raw	24	242

Table 3.10 (continued).

Cultivar and cooking method	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Russet Burbank		
Bake	193	510
Boil	181	454
Fry	199	630
Micro	234	617
Raw	97	287
Russet Norkotah		
Bake	435	717
Boil	278	848
Fry	360	811
Micro	450	680
Raw	62	579
Santana		
Bake	93	326
Boil	36	162
Fry	56	255
Micro	86	334
Raw	35	207
Shasta		
Bake	89	309
Boil	80	275
Fry	51	236
Micro	112	366
Raw	59	248
Shepody		
Bake	38	216
Boil	33	133
Fry	37	173
Micro	77	287
Raw	38	222
Superior		
Bake	141	219
Boil	128	153
Fry	132	208
Micro	165	335
Raw	103	185
TX1523-1W/Y		
Bake	92	216
Boil	85	264
Fry	104	261
Micro	90	296
Raw	23	118
Umatilla		
Bake	137	357
Boil	121	327
Fry	118	416
Micro	163	441
Raw	83	190
Yukon Gold		
Bake	140	202
Boil	103	176

Table 3.10 (continued).

Cultivar and cooking method	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Fry	258	526
Micro	216	394
Raw	84	172

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox

Springlake, 2003. Analysis of variance for Springlake samples from 2003 shows that the main effects of cultivar and cooking method were significantly different ($p < 0.000$) for both AOAI and AOAS, while the interactions of cultivar and cooking method were not, with AOAI ($p = 0.323$) and AOAS ($p = 0.377$) (Table 3.11). The eta squared values for cultivar were 28 % for AOAI and 24 % for AOAS; the values for cooking method were 18 % for AOAI and 29 % for AOAS; the values for the interaction were 15 % for AOAI and 13 % AOAS; and the error values were 37% for AOAI and 33% AOAS. The eta squared values for cooking method and the interaction in this harvest were about twice as large as the values from the McCook harvest; therefore, cooking appeared to have a greater effect on the Springlake samples.

Table 3.11 Analysis of variance results for antioxidant activity for the factors cultivar and cooking method, Springlake 2003.

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	AOAI	1347881.902 [z]	71	18984.252	3.271	.000
	AOAS	3389156.288 [y]	71	47734.596	3.964	.000
Intercept	AOAI	7201690.243	1	7201690.243	1240.825	.000
	AOAS	22601896.321	1	22601896.321	1876.912	.000
Cultivar	AOAI	605708.199	13	46592.938	8.028	.000
	AOAS	1211282.864	13	93175.605	7.738	.000
Cook	AOAI	380877.149	4	95219.287	16.406	.000
	AOAS	1489292.886	4	372323.222	30.919	.000
Cultivar * Cook	AOAI	332674.187	52	6397.581	1.102	.323
	AOAS	667675.146	52	12839.907	1.066	.377
Error	AOAI	800945.512	138	5803.953		
	AOAS	1661804.766	138	12042.064		

Table 3.11 (continued).

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Total	AOAI	9350517.657	210			
	AOAS	27652857.375	210			
Corrected Total	AOAI	2148827.414	209			
	AOAS	5050961.054	209			

[z] R^2 = .627 (Adjusted R^2 = .435)
[y] R^2 = .671 (Adjusted R^2 = .502)

The cultivar main effect of varied widely in this harvest as in McCook. The range for AOAI was from 93 µg trolox eq./ gfw for 'Atlantic' to 259 µg trolox eq./ gfw for 'Santana'. The range for AOAS was from 206 µg trolox eq./ gfw with 'F88042' to 458 µg trolox eq./ gfw with 'Santana' (Table 3.12). 'Atlantic' was the lowest cultivar in AOAI and 'F88042' was the lowest cultivar in AOAS. This change in ranking of cultivars may be due to the kinetic variability of the compounds analyzed. As compared to the McCook harvest, this harvest had a smaller range of values for both AOAI and AOAS. 'Shepody' also was one of the lower cultivars in McCook, but ranked much higher at Springlake. The environmental and cultural conditions might enhance the antioxidant levels in 'Shepody', indicating that these factors can play a major role in the expression of antioxidant activity. Furthermore, some cultivars such as Krantz and Russet Burbank, appear to be stable in expressing AOA across environments, while Shepody and Santana are much less stable.

Table 3.12 Cultivar ranking for antioxidant activity, Springlake 2003.

Cultivar	AOAI[z] (µg/gfw)		Cultivar	AOAS[y] (µg/gfw)	
Santana	259	a[x]	Santana	458	a
Shepody	253	a	Krantz	439	ab
Krantz	235	a	Shepody	402	abc
Superior	233	a	Superior	381	abcd
Russet Burbank	226	ab	Russet Burbank	376	abcd
NDTX4930-5W	204	ab	NDTX4930-5W	344	abcde
Innovator	203	ab	Umatilla	336	bcde
Umatilla	192	ab	Shasta	332	bcde
Shasta	182	abc	ATX84706-2Ru	315	cde
ATX85404-8W	148	bc	ATX85404-8W	270	def
ATX84706-2Ru	146	bcd	Innovator	270	def
F88042	109	cd	A84420-5	248	ed
A84420-5	108	cd	Atlantic	217	f
Atlantic	93	d	F88042	206	f

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[x] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The main effects of cooking method were similar to the results for McCook (Table 3.13). The microwave method remained high, and boiling and the raw control remained low. The AOAI ranged from 113 for raw samples to 235 µg trolox eq./ gfw for baked samples, while AOAS ranged from 220 for raw samples to 418 µg trolox eq./ gfw for microwaved samples.

Table 3.13 Cooking method ranking for antioxidant activity, Springlake 2003.

Cooking method	AOAI[z] (µg/gfw)		Cooking method	AOAS[y] (µg/gfw)	
Bake	235	a[x]	Micro	418	a
Micro	218	ab	Bake	397	a
Fry	190	bc	Fry	373	a
Boil	169	c	Boil	233	b
Raw	113	d	Raw	220	b

[z] AOAI = Initial antioxidant activity eq. trolox.
[y] AOAS = Stabilized antioxidant activity eq. trolox.
[x] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The effect of cultivar and cooking method was also analyzed (Table 3.14). The top five average values for AOAI were 'Santana', bake (344); 'Shasta', bake (341); 'Santana', boil (329); 'Shepody', bake (328); 'Innovator', microwave (320). The lowest five average values for AOAI were 'F88042', raw (52); 'Innovator', raw (69); 'A8440-5', raw (70); 'ATX85404-8W', raw (72); and 'Atlantic', boil (73). The top five average values for AOAS were 'Shasta', bake (567); 'Santana', bake (559); 'Krantz', microwave (559); 'Santana', microwave (548); 'Krantz', bake (537). The lowest five average values for the AOAS were 'Innovator', raw (108); 'Atlantic', boil (127); 'F88042', raw (134); 'Shasta', boil (142); and 'ATX85404-8W', boil (150). Similar trends were observed in the McCook 2003 samples.

Table 3.14 The influence of cultivar and cooking method on antioxidant activity, Springlake 2003.

Cultivar and cooking method	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
A84420-5		
Bake	124	294
Boil	97	198
Fry	115	249
Micro	132	331
Raw	70	165
Atlantic		
Bake	93	229
Boil	73	127
Fry	102	249
Micro	122	286
Raw	74	192
ATX84706-2Ru		
Bake	169	364
Boil	107	164
Fry	136	284
Micro	131	368
Raw	189	392
ATX85404-8W		
Bake	187	336
Boil	114	150
Fry	166	313
Micro	203	385
Raw	72	166
F88042		
Bake	128	180
Boil	106	164
Fry	152	293
Micro	108	259

Table 3.14 (continued).

Cultivar and cooking method	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Raw	52	134
Innovator		
Bake	168	283
Boil	184	262
Fry	275	342
Micro	320	355
Raw	69	108
Krantz		
Bake	278	537
Boil	218	371
Fry	253	461
Micro	307	559
Raw	120	267
NDTX4930-5W		
Bake	242	423
Boil	175	191
Fry	237	398
Micro	260	479
Raw	108	226
Russet Burbank		
Bake	285	472
Boil	242	291
Fry	229	463
Micro	247	460
Raw	129	193
Santana		
Bake	344	559
Boil	329	444
Fry	224	495
Micro	274	548
Raw	123	244
Shasta		
Bake	341	567
Boil	95	142
Fry	192	378
Micro	194	397
Raw	90	178
Shepody		
Bake	328	474
Boil	272	358
Fry	220	421
Micro	282	464
Raw	161	293
Superior		
Bake	305	518
Boil	245	228
Fry	192	381
Micro	243	442
Raw	181	336

Table 3.14 (continued).

Cultivar and cooking method	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Umatilla		
Bake	304	327
Boil	107	173
Fry	164	490
Micro	236	508
Raw	149	184

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox

A separate analysis compared the antioxidant activity at the two harvest locations. Location was a significant factor for AOAI ($p < 0.000$) but not for AOAS ($p = 0.648$). The average value of AOAI for the McCook samples was 120 µg/gfw, while for Springlake it was 185 µg/gfw. The average value of AOAS at McCook was 348 µg/gfw, while for Springlake it was 328 µg/gfw. The interaction between cultivar and location was significant for both AOAI ($p < 0.000$) and AOAS ($p < 0.000$). Also, the interaction of cooking method and location was a significant factor for both AOAI ($p < 0.000$) and AOAS ($p = 0.004$). The interaction of cultivar, cooking method, and location was not significant for either AOAI ($p = 0.657$) or AOAS ($p = 0.516$).

TOTAL PHENOLIC CONTENT. The linear regression equation to equate the spectrophotometric absorbance readings of the Folin test at 727 nm to chlorogenic acid equivalents was the following: $y = 0.5775x - 0.0279$, where x was the absorbance at 727 nm after zeroing the spectrophotometer, with a blank lacking antioxidant extract but containing all other solutions, and y was the µg chlorogenic acid equivalents per gram fresh weight. The R^2 value of this equation was 0.970.

McCook 2003. The over all average phenolic content was 352 µg chlorogenic acid eq./ gfw. Analysis of variance revealed that the main effects of cultivar and cooking method were significant ($p < 0.000$), while the two-factor interaction was not significant ($p = 0.972$) (Table 3.15). The eta squared values were as follows: cultivar 49 %, cooking method 6 %, the interaction of cultivar and cooking method 7 %, and error 38 %. Cultivar was an influential factor for phenolic content in this harvest.

Table 3.15 Analysis of variance results for phenolic content for the factors cultivar and cooking method, McCook 2003.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	6464225.371[z]	84	76955.064	4.437	.000
Intercept	35799341.622	1	35799341.622	2063.932	.000
Cultivar	5066650.211	16	316665.638	18.257	.000
Cook	578254.519	4	144563.630	8.334	.000
Cultivar * Cook	739636.710	64	11556.824	.666	.972
Error	3902673.537	225	17345.216		
Total	46711457.020	310			
Corrected Total	10366898.908	309			

[z] $R^2 = .624$ (Adjusted $R^2 = .483$)

The phenolic content of the cultivars ranged from 177 for Atlantic to 672 µg chlorogenic acid eq./ gfw for Russet Norkotah. This 500 µg range was the largest cultivar range seen in this present study (Table 3.16). The rankings were similar to the antioxidant activity results, which may indicate that phenolic content is the major contributor to antioxidant activity.

Table 3.16 Cultivar ranking for phenolic content, McCook 2003.

Cultivar	Chlorogenic acid eq. (µg/gfw)	
Russet Norkotah	672	a[z]
Krantz	542	b
Innovator	538	b
Russet Burbank	509	b
F88042	397	c
Yukon Gold	378	cd
ATX84706-2Ru	360	cd
Shasta	327	cde
TX1523-1W/Y	302	cdef
Umatilla	290	cdef
Santana	290	cdef
ATX85404-8W	274	cdef
A84420-5	248	def
NDTX4930-5W	243	def
Superior	234	def
Shepody	201	ef
Atlantic	177	f

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The main effect of cooking method ranged from 280 µg for raw to 406 µg for microwaved samples (Table 3.17). This ranking is similar ranking to those for antioxidant activity in McCook and Springlake (Tables 3.9 and 3.13).

Table 3.17 Cooking method ranking for phenolic content, McCook 2003.

Cooking method	Chlorogenic acid eq. (µg/gfw)	
Micro	406	a[z]
Bake	382	a
Fry	368	ab
Boil	325	bc
Raw	280	c

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The effects of cultivars and cooking methods were also analyzed (Table 3.18). The top five average values for phenolic content (as expressed as equivalents of µg chlorogenic acid / gfw) were 'Russet Norkotah', fry (802); 'Russet Norkotah', bake (699); 'Russet Norkotah', boil (674); 'Russet Burbank', microwave (666); and 'Russet Norkotah', raw (645). The lowest five averages for phenolic content were 'Atlantic', raw (139); 'Atlantic', fry (147); 'Shepody', fry (153); 'A84420-5', raw (159); and 'Umatilla', raw (160). 'Russet Norkotah' had the highest phenolic content irregardless of cooking method, with microwaved and baked samples the highest.

Table 3.18 The influence of cultivar and cooking method on phenolic content, McCook 2003.

Cultivar and cooking method	Chlorogenic acid eq. (µg/gfw)
A84420-5	
Bake	303
Boil	244
Fry	256
Micro	278
Raw	159
Atlantic	
Bake	207
Boil	173
Fry	147
Micro	219

Table 3.18 (continued).

Cultivar and cooking method	Chlorogenic acid eq. (µg/gfw)
Raw	139
ATX84706-2Ru	
Bake	426
Boil	229
Fry	448
Micro	372
Raw	323
ATX85404-8W	
Bake	263
Boil	284
Fry	298
Micro	292
Raw	235
F88042	
Bake	433
Boil	445
Fry	316
Micro	464
Raw	328
Innovator	
Bake	519
Boil	515
Fry	619
Micro	624
Raw	414
Krantz	
Bake	574
Boil	575
Fry	580
Micro	568
Raw	414
NDTX4930-5W	
Bake	242
Boil	221
Fry	221
Micro	280
Raw	251
Russet Burbank	
Bake	590
Boil	421
Fry	550
Micro	666
Raw	319
Russet Norkotah	
Bake	699
Boil	674
Fry	802
Micro	538
Raw	645

Table 3.18 (continued).

Cultivar and cooking method	Chlorogenic acid eq. (µg/gfw)
Santana	
Bake	383
Boil	229
Fry	298
Micro	348
Raw	191
Shasta	
Bake	385
Boil	297
Fry	252
Micro	390
Raw	311
Shepody	
Bake	205
Boil	162
Fry	153
Micro	300
Raw	184
Superior	
Bake	265
Boil	173
Fry	191
Micro	368
Raw	175
TX1523-1W/Y	
Bake	271
Boil	307
Fry	351
Micro	346
Raw	236
Umatilla	
Bake	294
Boil	279
Fry	332
Micro	385
Raw	160
Yukon Gold	
Bake	427
Boil	289
Fry	450
Micro	457
Raw	268

Springlake 2003. The over all average phenolic content was 384 eq. µg chlorogenic acid / gfw from the Springlake harvest, which was slightly higher than the McCook average. Analysis of variance indicated that the main effects of cultivar and cooking

method were both significant ($p < 0.000$), while the two-way interaction of cultivar and cooking method was not a significant factor ($p = 0.720$) (Table 3.19). The eta squared value for cultivar was 48 %; the value for cooking method was 15 %; the interaction value was 9 % and the error value was 28 %. Again, cultivar was a very influential component in phenolic content.

Table 3.19 Analysis of variance results for phenolic content for the factors cultivar and cooking method, Springlake 2003.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	3256342.523 [z]	69	47193.370	5.290	.000
Intercept	31023534.437	1	31023534.437	3477.368	.000
Cultivar	2177637.352	13	167510.566	18.776	.000
Cook	676896.867	4	169224.217	18.968	.000
Cultivar * Cook	401808.305	52	7727.083	.866	.720
Error	1249017.842	140	8921.556		
Total	35528894.802	210			
Corrected Total	4505360.365	209			

[z] $R^2 = .723$ (Adjusted $R^2 = .586$)

The phenolic content of cultivar ranged from 196 µg for 'Atlantic' to 591 for 'Krantz'. As with the McCook results, the cultivars Krantz and Russet Burbank were at the top of the ranking. Atlantic was the lowest cultivar at both harvest locations (Table 3.20).

Table 3.20 Cultivar ranking for phenolic content, Springlake 2003.

Cultivar	Chlorogenic acid eq. (µg/gfw)	
Krantz	591	a[z]
Santana	490	b
Russet Burbank	467	bc
NDTX4930-5W	441	bc
Superior	438	bc
Umatilla	433	bc
Shepody	426	bc
Shasta	369	cd
Innovator	366	cd

Table 3.20 (continued).

Cultivar	Chlorogenic acid eq. (µg/gfw)	
ATX84706-2Ru	315	de
ATX85404-8W	309	de
F88042	292	de
A84420-5	246	ef
Atlantic	196	f

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Phenolic content as affected by cooking method ranged from 279 µg for boiling to 441 µg for baking (Table 3.21). As in McCook, boiled and raw samples were low in phenolic content, while the baked, microwaved, and fried samples were the highest.

Table 3.21 Cooking method ranking for phenolic content, Springlake 2003.

Cooking method	Chlorogenic acid eq. (µg/gfw)	
Bake	441	a[z]
Micro	418	ab
Fry	411	ab
Raw	373	b
Boil	279	c

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The influence of cultivar and cooking method on phenolic content is shown in Table 3.22. The top five average values for phenolic content were 'Krantz', micro (659); 'Krantz', bake (651); 'Krantz', fry (634); 'Krantz', raw (560); and 'Santana', bake (555). The lowest five average values for phenolic content were 'Atlantic', boil (94); 'Shasta', boil (164); 'A84420-5', boil (167); 'ATX84706-2Ru', boil (184); and 'ATX85404-8W', boil (195) (Table 3.22).

Table 3.22 The influence of cultivar and cooking method on phenolic content, Springlake 2003.

Cultivar and cooking method	Chlorogenic acid eq. (µg/gfw)
A84420-5	
Bake	268
Boil	167
Fry	237
Micro	289
Raw	268
Atlantic	
Bake	205
Boil	94
Fry	206
Micro	220
Raw	257
ATX84706-2Ru	
Bake	370
Boil	184
Fry	340
Micro	322
Raw	360
ATX85404-8W	
Bake	334
Boil	195
Fry	339
Micro	379
Raw	298
F88042	
Bake	313
Boil	206
Fry	376
Micro	269
Raw	297
Innovator	
Bake	362
Boil	318
Fry	468
Micro	371
Raw	313
Krantz	
Bake	651
Boil	454
Fry	634
Micro	659
Raw	560
NDTX4930-5W	
Bake	471
Boil	335
Fry	464
Micro	530
Raw	406
Russet Burbank	
Bake	552
Boil	385

Table 3.22 (continued).

Cultivar and cooking method	Chlorogenic acid eq. (µg/gfw)
Fry	532
Micro	489
Raw	376
Santana	
Bake	555
Boil	466
Fry	497
Micro	507
Raw	428
Shasta	
Bake	514
Boil	164
Fry	380
Micro	483
Raw	305
Shepody	
Bake	500
Boil	350
Fry	405
Micro	447
Raw	429
Superior	
Bake	538
Boil	345
Fry	448
Micro	359
Raw	499
Umatilla	
Bake	538
Boil	249
Fry	424
Micro	525
Raw	429

Location was a significant factor for phenolic content ($p < 0.000$). The average value for McCook was 352 µg/gfw, while for Springlake it was 384 µg/gfw. The interactions between cultivar and location ($p < 0.000$) and between cooking method and location ($p < 0.000$) were significant. The three-way interaction of cultivar, cooking method, and location was not significant ($p = 0.988$).

HPLC ANALYSIS FOR CAROTENOID COMPOUNDS. Although there were seven compounds analyzed via HPLC for carotenoid content in the individual experiments, only two compounds, antheraxanthin and canthaxanthin, were found

through retention time in this study (Table 3.23). The other compounds either degraded or were not contained in the cultivars. The cultivars chosen were Innovator, Russet Burbank and Santana. The analysis of variance indicated that there were no significant differences between cultivars for content of carotenoid compounds.

Table 3.23 Cultivar ranking for individual carotenoid compounds (µg/100gfw) based on retention time, McCook 2003.

Cultivar	Antheraxanthin	Canthaxanthin	Total
Innovator	2 a[z]	7 a	8 a
Russet Burbank	0 a	8 a	8 a
Santana	3 a	7 a	10 a

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

As with cultivar, there were no significant differences in carotenoids content among cooking methods (Table 3.24). However, the raw samples tended to have higher carotenoids when compared to the other cooking methods.

Table 3.24 Cooking method ranking for individual carotenoid compounds (µg/100gfw) based on retention time, McCook 2003.

Cooking method	Antheraxanthin	Canthaxanthin	Total
Bake	0 a[z]	0 a	0 a
Boil	0 a	8 a	8 a
Fry	0 a	7 a	7 a
Micro	0 a	11 a	11 a
Raw	8 a	11 a	18 a

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

HPLC ANAYSIS FOR PHENOLIC COMPOUNDS. Although there were 18 compounds in the library, only 13 were found based on retention time (Table 3.25). Unlike carotenoid content, analysis of variance results revealed significant differences in phenolic content among cultivars. 'Russet Burbank' contained high relative levels for all compounds, followed by 'Innovator', then 'Santana'. Total phenolic content was

determined by the addition of all 13 compounds. Analysis of variance was unable to detect significant differences among cultivars.

Table 3.25 Cultivar ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, McCook 2003.

Cultivar	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
Innovator	94	66	14	219	41	48	14	7	89	26	16	303	21	957
	a[y]	a	a	b	ab	a	a	a	a	a	a	a	b	a
Russet	83	67	12	278	50	78	16	2	110	27	16	304	30	1047
Burbank	ab	a	a	a	a	a	a	b	a	a	a	a	a	a
Santana	50	68	15	219	29	35	12	4	91	26	15	326	16	906
	b	b	a	a	b	a	a	b	a	a	a	a	b	a

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics
[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The results based on spectra and the combination of retention time and spectra are shown in Table 3.26. Only chlorogenic acid and caffeic acid matched the spectra. There were no significant differences among cultivars based on spectra or the combination of retention time and spectra.

Table 3.26 Cultivar ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both spectra and retention time, McCook 2003.

Cultivar	CH-SP	CA-SP	Total-SP	CH-RSP	CA-RSP	Total-RSP
Innovator	131 a[y]	389 a	520 a	29 a	53 a	82 a
Russet Burbank	126 a	320 a	446 a	20 a	34 a	54 a
Santana	93 a	245 a	338 a	11 a	38 a	49 a

[z]CH-SP : Chlorogenic acid based on spectra
CA-SP : Caffeic acid based on spectra
Total-SP : the addition of all measured phenolics based on spectra
CH-RSP : Chlorogenic acid based on retention time and spectra
CA-RSP : Caffeic acid based on retention time and spectra
Total-RSP : the addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Cooking method was also analyzed for phenolic content via HPLC. Analysis of variance revealed that there were significant differences between cooking methods based on retention time (Table 3.27). Baking was in the highest level of significance 12 out of 13 times (for the 13 compounds that were found in the samples), and had the highest values four times. Boiled samples were in the highest level of significance 12 times, and ranked the highest four times. Fried samples were in the highest level of significance 12 times and ranked the highest seven times. Microwaved samples were in the highest level of significance 12 times, and ranked the highest six times. Raw samples were in the highest level of significance 11 times and ranked the highest two times. Total phenolics content was calculated by addition of all values for individual phenolics. Baking had the highest value of 1029 µg/ gfw, but there were no significant differences between cooking methods for total value. Over all, all cooking methods seemed to result in higher phenolics than the raw control.

Table 3.27 Cooking method ranking for individual phenolic compounds[z] (μg/gfw) based on retention time, McCook 2003.

Cooking method	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
Bake	69	68	14	277	41	58	15	3	106	26	16	312	23	1029
	ab[y]	a	a	a	a	a	a	b	a	a	a	a	a	a
Boil	69	66	14	213	39	59	13	4	87	25	15	308	19	890
	ab	a	a	a	a	a	a	b	a	a	a	a	a	a
Fry	104	68	14	239	43	56	15	1	102	27	16	312	25	1013
	a	a	a	a	a	a	a	b	a	a	a	a	a	a
Micro	101	70	12	243	40	58	16	3	97	25	16	312	25	1018
	a	a	a	a	a	a	a	b	a	a	a	a	a	a
Raw	35	63	15	221	37	47	11	10	80	27	14	308	21	899
	b	a	a	a	a	a	a	a	a	a	a	a	a	a

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The results for the spectra and the combination of spectra and retention time are shown in Table 3.28. There were no significant differences among the cooking methods based on spectra and the combination of retention time and spectra; however the baked, microwaved, and fried samples ranked the highest.

Table 3.28 Cooking method ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both spectra and retention time, McCook 2003.

Cooking method	CH-SP	CA-SP	Total-SP	CH-RSP	CA-RSP	Total-RSP
Bake	95 a[y]	390 a	486 a	0 a	33 a	33 a
Boil	107 a	324 a	430 a	20 a	42 a	62 a
Fry	124 a	344 a	468 a	22 a	57 a a	80 a
Micro	149 a	332 a	481 a	58 a	46 a	104 a
Raw	108 a	200 a	308 a	0 a	30 a	30 a

[z]CH-SP : Chlorogenic acid based on spectra
CA-SP : Caffeic acid based on spectra
Total-SP : the addition of all measured phenolics based on spectra
CH-RSP : Chlorogenic acid based on retention time and spectra
CA-RSP : Caffeic acid based on retention time and spectra
Total-RSP : the addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Discussion and Conclusion

Based on the spectrophotometric results, there was wide variability in carotenoid content among the 17 cultivars. 'Shasta' had the lowest amount of xanthophylls, while 'Innovator' had the highest. 'F88042' had the lowest carotene content, while 'Yukon Gold' had the highest. The total carotenoid content ranged from 116 µg/gfw for 'Shepody' to 304 µg/gfw for 'Innovator'. Fried, microwaved and raw samples had the highest levels of both xanthophylls and carotenes, while baking and boiling appeared lower. Boileau et al. (1999), Dietz et al. (1998), and Van den Berg et al. (2000) reported that there was an increased extractability of carotenoids due to cell matrix breakage in cooked samples. The spectrophotometric results support this claim. Although HPLC analysis of the carotenoid compounds had limited quantification, the raw samples appeared to have slightly more carotenoid compounds than the cooked samples. Bianchini and Penteado (1998) reported that carotenoids of pepper were heat sensitive and therefore there were losses during cooking. Spectrophotometric results reported an increase, while HPLC identification reported a decrease in carotenoid content with cooked potato samples. This may be due to changes in the structure of the carotenoids due to cooking, which would cause a decrease in identification based on HPLC.

Based on the spectrophotometric results, there was wide variability of antioxidant activity among the 17 cultivars from McCook and the 14 cultivars from Springlake. Over all, there was a greater range in the McCook trial. 'Shepody' had one of the lower values at McCook and 'Russet Norkotah' the highest. Microwaved samples had the highest antioxidant activity, while raw samples had the lowest. The increase in antioxidant activity in cooked samples may be due to Maillard Reaction products. Nicoli et al. (1997) also reported an increase in antioxidant activity in coffee with an increase in roasting time. These increases may also be due to better extractability in cooked samples as reported by Amakura et al., 2000; and Dewanto et al., 2002

Based on the spectrophotometric results, there was wide variability for phenolic content among the 17 cultivars from McCook and the 14 cultivars from Springlake. Again, there was greater variability in the McCook trial, where the phenolic content in cultivars ranged from 177 for Atlantic to 672 μg chlorogenic acid eq./ gfw for Russet Norkotah. Over all, the phenolic content was greater at Springlake than at McCook. Microwaving, frying and baking seemed to result in higher amounts of phenolics as compared to boiling and no cooking. This was also supported by the results for individual compounds via HPLC. It is believed that the cooking methods may break open the potato cells and release bound phenolics (Dewanto et al., 2002), while boiling may result in a leaching effect (Gil et al., 1999).

In conclusion cooking, with the exception of boiling generally does not appear to have a detrimental effect on content of carotenoids or phenolics, or on antioxidant activity. In fact, based on this study, cooking resulted in increased levels of carotenoids, phenolics, and antioxidant activity. While location (growing conditions) had a significant effect, cultivar had the greatest influence on these levels, irrespective of cooking method.

CHAPTER IV
THE EFFECTS OF STORAGE, CULTIVAR, AND COOKING METHOD ON CAROTENOID CONTENT, ANTIOXIDANT ACTIVITY, AND PHENOLIC CONTENT IN POTATO

Synopsis

The consumption of processed potato products has increased in recent years. With this increase, there has been a demand for stored potato to ensure production of processed goods throughout the year. Past research has shown that there are significant levels of antioxidants, phenolics and carotenoids within cultivars and advanced selections in the Texas Potato Variety Development Program. However, it is unknown how these photochemical levels are affected by cooking and storage, and the interaction of storage and cooking. The objective of this experiment was to study the effects of storage treatments (no storage, 4 ^{o}C for 110 days, 4 ^{o}C for 110 days plus 10 days of reconditioning at 20 ^{o}C, and 20 ^{o}C for 110 days) and cooking methods (no cooking, microwaving, boiling, baking, and frying) on carotenoid content, antioxidant activity, and phenolic content in 8 named cultivars and advanced selections. Whole tubers were subjected to one of four storage treatments, then diced. A five-gram sample of the diced tubers was subjected to one of five cooking methods and frozen until extraction and quantification of phytochemicals. Carotenoid content (xanthophyll content) was determined via absorbance at 445 nm. Individual carotenoid compounds were quantified via HPLC identification based on retention time, spectra and the combination of retention time and spectra corresponding to standards. Antioxidant activity was determined by the DPPH method, and the kinetic reaction was quantified at two times, initially and at stabilization. Phenolic content was determined by the Folin-Ciocalteau method and individual phenolic compounds were quantified via HPLC identification as described for carotenoids. The cultivars Santana, Russet Burbank, and Krantz ranked high in carotenoid content, antioxidant activity and phenolic content. Boiling ranked significantly lower in carotenoid content measured spectrophotometrically. The cooking methods of fry, microwave, and baked ranked significantly higher than boiling and raw

for both antioxidant activity and phenolic content. The HPLC results for phenolics and carotenoids generally supported the spectrophotometric results. Storage treatments generally increased carotenoids levels; the storage treatment of 4 ^{o}C for 110 days produced significantly greater antioxidant activity; while the storage treatment of 4 ^{o}C for 110 days with reconditioning was significantly greater than other storage methods in phenolic content via the Folin method, while both no storage and 4 ^{o}C for 110 days with reconditioning ranked highest in total phenolic content via HPLC identification. The interaction of cultivar and storage treatment was significant for carotenoid content, antioxidant activity, and phenolic content, while the interactions of cultivar and cooking method, and the interaction of cooking method and storage treatment were significant in both carotenoid content and phenolic content.

Introduction

Fresh potato consumption has declined over the years, although that of processed vegetables has increased. The per capita consumption of frozen vegetables has increased 108 % from 28.5 to 59.3 pounds in the last 25 years (King et al., 2004). Currently, over 50% of potatoes grown in the U.S. are destined for processing, e.g., frozen products, rather than fresh potato consumption. The carbohydrate (starch and sugar) composition and water content of tubers determines the usage of the potato cultivar. Processing varieties must have high starch (dry matter) and low reducing sugar (glucose/fructose) levels. Reducing sugars create undesirably dark chips. Potatoes with high dry matter and low reducing sugar levels tend to be more desirable for frying.

With the increase in processed products, there is also an increase in stored potatoes to ensure production of processed goods throughout the year. Similarly, commercial storage of fresh potatoes also ensures a steady supply of potatoes during the off season. The most challenging aspect of storage is to ensure consistent quality of the tubers. Storage losses are caused by respiration, sprouting, changes in chemical composition of the tuber, spreading of disease, damage by extreme temperatures, and evaporation of water from the tubers (shrinkage). All factors are influenced by storage conditions (Rastovski et al., 1987). However, a good-quality stored potato is mainly

dependent on the quality of the harvested material. Storage rot organisms which have the greatest potential for losses during storage include soft rot (*Erwinia* spp.), Fusarium dry rot (*Fusarium* spp.), Pythium water rot (*Pythium* spp.), and early blight tuber blemish (*Alternia solani*). Stress on the growing plant can cause uneven tuber growth, often resulting in undesirable traits in stored potato. Some examples of stress expression include malformed shapes, accumulation of sugars during storage, development of sugar and jelly-end tubers, premature physiological aging, and sprouting of tubers during storage. Also, tubers should be harvested when mature because dry matter peaks, sugar formation is minimized, and the skin sets properly, all desirable traits for potatoes going into storage.

It is essential to control temperature and humidity during storage. The temperature required is dependent on the potato's future use. Temperature affects both the metabolic processes of the potatoes such as respiration, sprouting, sweetening, and the activity of microorganisms; therefore, temperature control is essential. Storage losses are often greater in higher temperatures because there is an increased metabolic rate and higher activity of most spoilage organisms. There is greater moisture loss, higher dry matter losses through sprouting, and increased spread of diseases (Rastovski et al., 1987). Most often, storage of potatoes is separated into two phases, curing or sweating and holding. The process of curing ensures that wounds are healed and skin is suberized. The curing process requires that potatoes are stored at 13- 15.5 ^{o}C (55- 60 ^{o}F) and kept at this temperature for 10-14 days, with a relative humidity of 92-97%. Holding or storage temperature depends on future use of the potatoes. Seed potatoes are stored at 2- 4 ^{o}C (35- 40 ^{o}F), and at this temperature, physiological aging and sprouting can be kept to a minimum. Table stock potatoes are stored at 4 to 5 ^{o}C (40- 41 ^{o}F). Processing stock storage depends on how long potatoes will remain in storage and also the anticipated means of processing. Chipping potatoes are often stored at 4-10 ^{o}C (40- 50 ^{o}F), while French frying potatoes are stored at 5-8 ^{o}C (41- 46 ^{o}F). Relative humidity should remain at 92-97% (Rastovski et al., 1987).

If potatoes are stored at low temperatures, sweetening occurs. The mechanism for this process of sugar accumulation is still unknown. This phenomenon of increasing sugars, and especially the reducing sugars, glucose and fructose, was first noted in 1882 by Müller –Thurgau (Rastovski et al., 1987). The sweetening is undesirable, especially in processing potatoes, because sugar accumulation results in a dark fry color and poor texture in cooked potatoes. Variation in sugar content during storage depends on cultivar, maturity, location grown, environmental factors during growth, age of the tuber, and storage conditions (Burton, 1989). This sweetening occurs at different temperatures and is dependant on many factors, but often occurs around 6 ^{o}C (43 ^{o}F).

The starch in potato cells consists of carbohydrate polymers. During storage, potato starch breaks down. This starch consists of 21 to 25% amylose and 75 to 79% amylopectin, both composed of glucose chains. Glucose has a free reducing group; therefore, the starch of potatoes is labeled a reducing sugar (Rastovski et al., 1987). During cold storage, the starch is broken down to glucose, which causes the increase in reducing sugars and sweetening. If potatoes become sweet, exposing them to ordinary room temperature for a few days tends to restore natural flavor, a process called reconditioning. During this process, much of the formed reducing sugar content is converted back to starch, and most of the remaining sugar is lost during respiration. Long-term storage facilities that need to store potatoes at lower temperatures often add a reconditioning step to their process. Sprouting has also been associated with changes in carbohydrates due to storage. Sucroses within tubers are broken down to glucose and fructose before sprouting, and the suppression of sprouting results in an increase in sucrose (Rastovski et al., 1987). Sugar accumulation and sucrose degradation may influence antioxidant levels. Flavonoids often occur as glycosides, i.e., they are bound to sugars, and sugar degradation may cause flavonoids to be affected in antioxidant analyses. Reducing sugar levels are directly proportional to Maillard reaction products, which also may influence antioxidant levels. Past research has indicated that antioxidants are influenced by storage. Craft and Wise (1993) reported a decrease in carotenoid content with storage and greater decrease as storage temperature increased,

while Bhushan and Thomas (1990) and Janave and Thomas (1979) reported an increase in carotenoid content with ambient storage. Korableva et al. (1973) found a loss of caffeic acid content during storage. Rumpf (1972) reported that the concentration of malic acid increased during storage, while the concentration of citric acid declined.

Significant levels of antioxidants, phenolics and carotenoids exist within cultivars and advanced selections in the Texas Potato Variety Development Program (Al-Saikhan, 1994, 2000; Hale, 2003). It is unknown how these photochemical levels are affected either by storage and cooking or the interactions of storage and cooking.

The objectives of this experiment were to investigate the effects of storage treatments and cooking methods on total carotenoid content, individual carotenoid content, total phenolic content, individual phenolic content, and total antioxidant activity in several named cultivars and advanced selections. The long term objective of this study was to provide the Texas breeding program and the potato industry with information about storage effects, cooking effects, and the interaction of storage and cooking on selected named cultivars and advanced selections.

Materials and Methods

HARVEST LOCATION. Planting and harvesting was conducted near Dalhart, Texas, which is located on the border of Dallam county, in the northwest corner of the Texas Panhandle.

PLANT MATERIAL. Named cultivars and advanced selections were harvested in October 2003 in Dalhart, graded, and eight were selected for this study. Their characteristics are described in Table 4.1.

Table 4.1 Characteristics of potato cultivars and advanced selections used in cultivar, storage and cooking studies.

Cultivar	Shape	Skin Color / Flesh Color	Utilization	Maturity
Atlantic	Oval to round	White, Buff / White	Chipping, Boiling, and Baking	Early to Medium
ATX85404-8W	Oval	White / White	Chipping	Medium to Late
Innovator	Oblong to long	Russet / Yellow	Baking and French fries	Early to Medium
Krantz	Oblong	Russet / White	Boiling, Baking and French fries	Medium
NDTX4930-5W	Oval to long	Light buff / White	Chipping and French fries	Early to Medium
Russet Burbank	Long	Russet / White	Baking and Fry Processing	Late
Santana	Oval to long	White / Yellow	Fry Processing	Early to Medium
Shepody	Long	Buff / White	Boiling, Baking, and French fries	Medium

STORAGE TREATMENTS. Four different storage treatments were conducted, no storage, 4 ^{o}C for 110 days, 4 ^{o}C for 110 days plus 10 days of reconditioning at 20 ^{o}C, and 20 ^{o}C for 110 days. The no storage treatment included analysis of fresh harvested samples. The other three treatments (4 ^{o}C, 4 ^{o}C with reconditioning, and 20 ^{o}C) involved controlled temperature storage for 110 days. The recondition process involved a secondary treatment after the 110 days of an additional 10 days at 20 ^{o}C.

SAMPLE PREPARATION. Three potatoes from each field replication were diced with a manual vegetable dicer (The Redco Insta Cut 3.5, Lincoln Foodservice, Fort Wayne, IN). The size of the diced samples was roughly 0.64 cm cubes. The diced potatoes were mixed, so a randomized sample was obtained. A 5 g sample was used. Once diced, samples were placed in extraction tubes and frozen at -18 ^{o}C (0 ^{o}F) until cooking.

COOKING METHODS. Four cooking methods and a control of no cooking or raw were used in this experiment. Processing times and temperatures were based on the optimum times and required temperatures to cook the average sample. This was determined based on the texture and feel of the potato sample. The uncooked sample had a starchy texture that was firm and sticky, while cooked samples had an interior that was mealy and/or powdery. The raw samples remained frozen at -18 ^{o}C (0 ^{o}F) until extraction.

Microwave. Six samples were cooked in their respective tubes for 2.5 min on high with a microwave (model MW8985W, Emerson, St. Louis, MO). After one min, the cooking process was stopped to mix the contents. After cooking for a second min, the cooking process was stopped to rotate the sample, then cooking continued for another thirty seconds. Cooked samples were then frozen at -18 ^{o}C (0 ^{o}F) until extraction.

Boil. Water was brought to a boil using a stove range (Montgomery Ward, Cedar Rapids, IA). Ten mL of nanopure, autoclaved water was added to each sample in the plastic extraction tubes. Samples were cooked for 25 min in boiling water. After cooking, the leachate was removed and discarded. The cooked potato sample was patted dry and frozen at -18 ^{o}C (0 ^{o}F) until extraction.

Bake. A gas oven (Montgomery Ward, Cedar Rapids, IA) was brought to 204 ^{o}C (400 ^{o}F). One-hundred-sixty samples in glass tubes were cooked for 15 min. Foil was used to cover the tubes to prevent water loss. After cooking, the samples were removed from the glass test tubes and placed back into the plastic tubes for extraction. Cooked samples were then frozen at -18 ^{o}C (0 ^{o}F) until extraction.

Fry. Canola oil was brought to 191 ^{o}C (375 ^{o}F) in a mini fryer (Rival CF250 Cool Touch Deep Fryer, El Paso, TX). Potato samples were placed in tea balls. The cooking time, which began once the tea ball entered the oil, was one min. After cooking, the sample was removed from the tea ball and placed on paper towels to cool, and the sample was placed back into the plastic extraction tubes. Cooked samples were then frozen at -18 ^{o}C (0 ^{o}F) until extraction.

EXTRACTION OF CAROTENOIDS. Due to the lack of carotenes found in the McCook 2003 trial (Chapter III) only the xanthophylls were analyzed in this experiment. The xanthophylls were extracted with methanol (plus 1 g/ L of BHT for stabilization). This extraction procedure was used to quantify the total carotenoid content based on the content of xanthophylls, and the individual carotenoid content via HPLC. Twenty-five mL of methanol plus BHT were added to a 5 g sample of diced potato. This mixture was then homogenized with an ultra turrax tissumizer from Tekmar (Cincinnati, OH). Samples and solvent were stored at -20 ^{o}C (-4 ^{o}F) for at least 12 h to ensure that the solvent extracted all carotenoids. Samples were then placed in a J-17 rotor at 17,000 rpm for 20 min in a refrigerated centrifuge manufactured by Beckman (model J2-21, Fullerton, CA). Two mL of the methanol were extracted and saved for the analysis of total carotenoids, and 2 mL of each replication (total 6 mL) were used for HPLC analysis of select cultivars (Fig. 4.1). The extracted samples were stored at -29 ^{o}C (-20 ^{o}F).

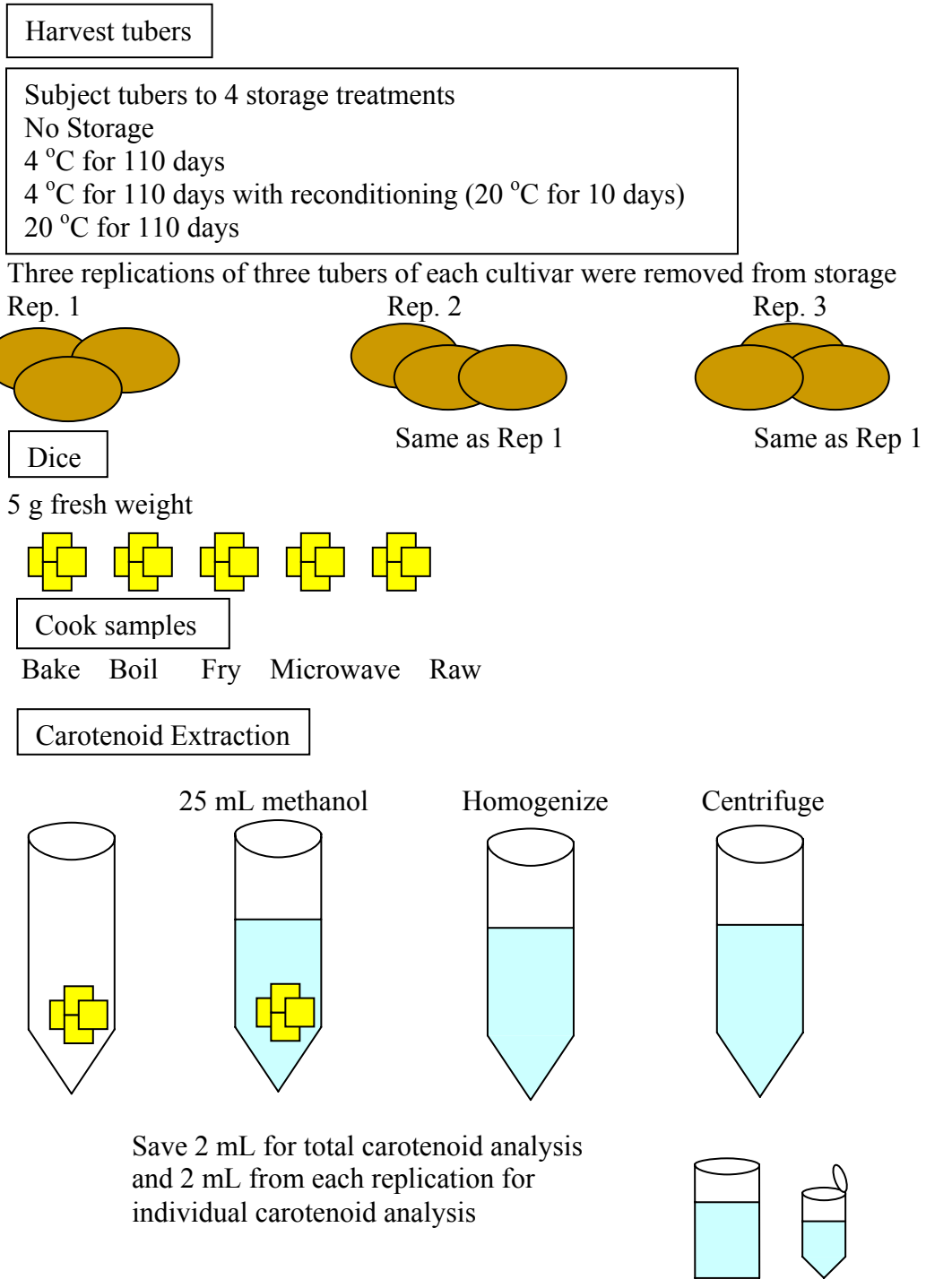

Fig. 4.1 Carotenoid extraction procedure for the factors storage, cultivar, and cooking method.

HPLC ANALYSIS FOR INDIVIDUAL CAROTENOID COMPOUNDS. Selected cultivars (Innovator, Russet Burbank, and Santana) were analyzed for individual carotenoid compounds and were chosen based on high quantifications on all tests. The extracted samples were concentrated under nitrogen gas and filtered through a 0.45 μm syringe filter (Hale, 2003). The samples were resuspended in 0.5 mL ethanol and 0.5 mL nanopure water. A PC-operated Waters high performance liquid chromatograph was used to analyze individual carotenoid compounds through spectra and retention time. The samples were analyzed using Waters Millennium 3.2 software, Waters 515 binary pump system (Waters 515), an autoinjector (Waters 717 plus), and photodiode detector (Waters 996), along with a column heater (SpectraPhysics SP8792) maintained at 35 ^{o}C (95 ^{o}F). A 4.6 x 250 mm, 5 μm, YMC Carotenoid Column (C-30 reverse phase) (Waters, Milford, MA) column was used to separate the carotenoid compounds. The compounds analyzed and used to create a library included: 1) violaxanthin (CaroteNature, Lupsingen, Switzerland), 2) neoxanthin (CaroteNature, Lupsingen, Switzerland), 3) antheraxanthin (CaroteNature, Lupsingen, Switzerland), 4) β-cryptoxanthin (Hoffman La Roche, Basel, Switzerland), 5) canthaxanthin (Hoffman La Roche, Basel, Switzerland), 6) zeaxanthin (Hoffman La Roche, Basel, Switzerland), and 7) lutein (Hoffman La Roche, Basel, Switzerland). Two filtered and de-gassed solution solvents were used for carotenoid extraction: "solvent A" consisted of methanol, water, and triethylamine (90:10:0.1), while "solvent B" consisted of methanol, MTBE, and triethylamine (6:90:0.1). The following was the gradient for the analysis: (min/ %A) 0/99, 8/99, 8/99, 45/0, 50/0, and 53/99 (Breithaupt and Barmedi, 2002; Hale, 2003).

EXTRACTION OF PHENOLICS AND TOTAL ANTIOXIDANT ACTIVITY. The same extraction procedure was conducted for total phenolic content, individual phenolic content, and total antioxidant activity. Fifteen mL of methanol was added to a 5 g sample of diced potato. This mixture was then homogenized with an ultra turrax tissumizer from Tekmar (Cincinnati, OH). After homogenization, samples were placed in a J-17 rotor at 17,000 rpm for 20 min in a refrigerated centrifuge (Beckman model J2-

21). Two mL of the methanol extract was saved in snap-cap tube for analysis of total antioxidant activity and total phenolic content. Selected cultivars (Innovator, Russet Burbank, and Santana) were chosen for individual carotenoid analysis, and 6 mL of the methanol extracts were saved in glass vials (Fig. 4.2). The extracted samples were stored at -29 ^{o}C (-20 ^{o}F).

DPPH ASSAY FOR TOTAL ANTIOXIDANT ACTIVITY. Total antioxidant activity was analyzed using DPPH (1,1 Diphenyl-2 picrylhydrazyl), which is a colorimetric assay first described by Brand-Williams et al. (1995). DPPH is a stable radical which causes oxidation and can be reduced by natural antioxidants, which reduce the oxidizing power of DPPH. Non-reduced DPPH is dark purple, while reduction shifts the color from dark purple to lighter purple to light yellow. This decrease in color and reduction power can be measured at 515 nm. The lower the absorbance, the greater the amount of antioxidant activity in the sample.

The DPPH solution was diluted by dissolving 24 mg DPPH in 100 mL methanol, which creates a 607 μM DPPH stock solution. The solution was then diluted to ~10:55 with methanol until the spectrophotometer read 1.1 at 515 nm. The extracted methanol sample of 150 μL was combined with 2.85 mL of diluted DPPH in a scintillation vial, along with a blank which contained 150 μL of pure methanol (instead of methanol extract) with the diluted 2.85 mL DPPH. The samples reacted with each other for 15 min. After this time, the level of reduction was determined by the absorption at 515 nm in a plastic UV-spectrophotometeric cuvette. This reading is based on the activity of the sample after 15 min (initial antioxidant activity, AOAI), but the total reaction is a kinetic one, which continues for about 24 h until stabilization (stabilized antioxidant activity, AOAS). Each antioxidant compound reacts with the oxidizing substance at a given time; therefore, two readings were taken. The second reading was taken after 24 h, when the samples and the DPPH had stabilized. The first reading (after 15 min) represents an initial response, whereas the second represents a final response. It is currently unknown how long consumed antioxidants are functional; therefore, these readings may represent two responses.

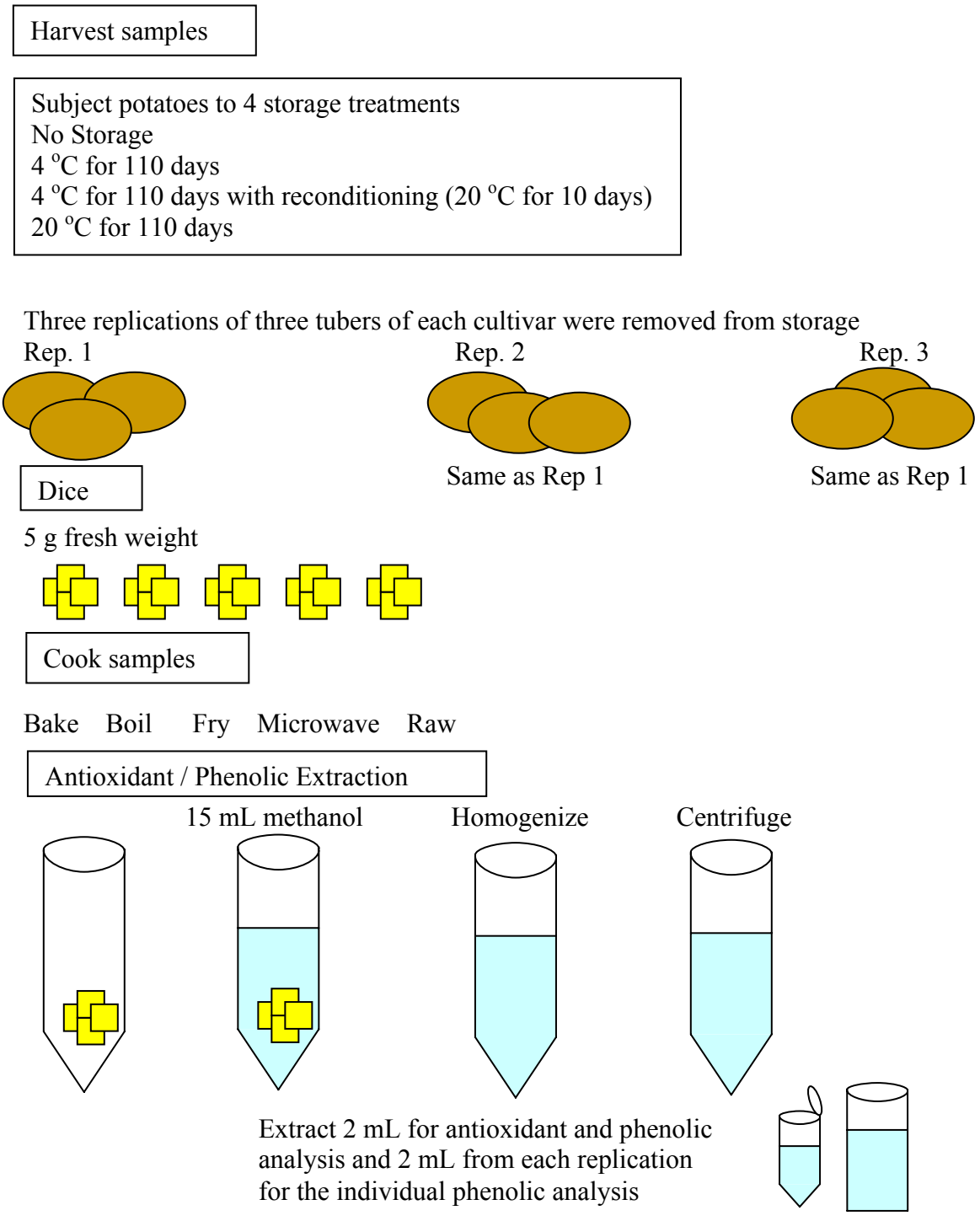

Fig. 4.2 Antioxidant/ phenolic extraction procedure for the factors storage, cultivar, and cooking method.

Absorptions are subtracted from the blank, a standard curve using a known antioxidant, trolox, (6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid) was prepared, and a regression curve was calculated to convert the antioxidant activity into trolox equivalents.

TOTAL PHENOLIC CONTENT. The Folin-Ciocalteau phenol method to determine phenolic content was first described by Swain and Hillis (1959) and modified by Singleton and Rossi (1965). This method, like the total antioxidant activity method, is a colorimetric reaction that is determined by absorbance. A 0.25 N Folin - Ciocalteau phenol reagent solution with nanopure water and a 1.0 N Na_2Co_3 solution with nanopure water were prepared. The extracted methanol sample of 150 μL was combined with 2.4 mL of nanopure water in a scintillation vial, along with a blank which contained 150 μL of pure methanol (instead of methanol extract) with 2.4 mL of nanopure water. The samples and blank reacted with 150 μL of the 0.25 N Folin - Ciocalteau phenol reagent solution for 3 min. Afterwards, 300 μL of the 1.0 N Na_2Co_3 solution were added to both the samples and blank. The reaction again is kinetic, and stabilization occurred after 1 h and 55 min. Data was taken at stabilization. Absorption was determined at 725 nm in plastic UV-spectrophotometeric cuvettes. The blank was read first, and the sample absorption was based on the cleared response of the blank. The phenolic content was determined by a prepared regression curve to chlorogenic acid equivalents.

HPLC ANALYSIS FOR INDIVIUAL PHENOLIC COMPOUNDS. Selected cultivars (Innovator, Russet Burbank, and Santana) analyzed for individual phenolic compounds and were chosen based on high quantifications on earlier studies (Chapter III). The extracted samples were concentrated under nitrogen gas or by using a heated speed vacuum centrifuge and filtered through a 0.45 μm syringe filter (Hale, 2003). A PC-operated Waters high performance liquid chromatograph was used to analyze individual phenolic compounds through spectra and retention time. The samples were analyzed using Waters Millennium 3.2 software, Waters 515 binary pump system (Waters 515), an autoinjector (Waters 717 plus), and photodiode detector (Waters 996), along with a column heater (SpectraPhysics SP8792) maintained at 40 ^{o}C (104 ^{o}F). A

4.6 x 150 mm, 5 μm, Atlantis C-18 reverse-phase column (Milford, MA) was used to separate phenolic compounds. The compounds analyzed included: 1) 5,7-Trihydroxyflavanone, 2) sinapic acid, 3) kampherol, 4) (-) epicatechin, 5) catechin, 6) quercetin dehydrate, 7) rutin hydrate, 8) protocatechuic acid, 9) salicylic acid, 10) myricetin, 11) syringic acid, 12) gallic acid, 13) vanillic acid, 14) t-cinnamic acid, 15) p-coumaric acid, 16) ferulic acid, 17) caffeic acid, and 18) chlorogenic acid; the standard compounds were obtained from Agros Organics (Pittsburgh, PA). Two filtered and de-gassed solution solvents were used for the phenolic extraction. "Solvent A" consisted of acetonitrile, and "solvent B" consisted of nanopure water and HCL adjusted to pH 2.3. The following gradient was used, (min/%A) 0:85, 5:85, 30:0, 35:0 (Hale, 2003).

STATISTICAL ANALYSIS. The field plot was a completely randomized design, with cultivar sample replications collected from three different blocks in the field. A multiple analysis of variance (MANOVA) general linear model was used to determine significant factors. The statistical model of the experiment was a full factorial design. The dependent variables included total carotenoid content, total phenolic content, total antioxidant activity initially (AOAI) (measurements taken after 15 min), and total antioxidant activity at stabilization (AOAS) (measurements taken after 24 h). The fixed factors included cultivar, cooking method, and storage treatment. Factor comparison was conducted using the post hoc multiple comparison methods of S-N-K tests. A test to measure the estimate of magnitude of effect or strength of association was also conducted. This test determines how strongly two or more variables are related, or how large the difference is between groups. The effect size is reported as eta squared values and is defined as the sums of squares of the effect of interest divided by the total sums of squares (Levine and Hullett, 2002). The analysis was conducted using the SPSS statistical package version 11.5.

Results

One of the undesirable results of storage is weight loss. Weight of each cultivar and each treatment was measured before and after storage. Percentage weight loss was

determined based on the original fresh weight of the tubers (Table 4.2). A cultivar with high weight loss lost a lot of water, becoming dehydrated. This may cause the antioxidant compounds to be more concentrated or may cause them to degrade because of a high amount of metabolic activity. The greatest weight loss occurred in the 110 days, 20 ^{o}C treatment. The cultivars ATX85404-8W, NDTX4930-5W, and Santana appeared to lose a high amount of weight across all storage treatments. The cultivars Atlantic, Innovator, Krantz, and Russet Burbank appeared to be less affected by storage treatment because of the lower percent weight loss. The cultivars with high weight loss have relatively thin skin, while those in the latter have thicker russet skin.

Table 4.2 Percent weight loss for each storage treatment and cultivar.

Cultivars	110 days at 4^{o}C	110 days at 4^{o}C plus 10 days reconditioning at 20 ^{o}C	110 days at 20 ^{o}C	Average
Atlantic	3.4	3.1	8.9	5.1
ATX85404-8W	6.5	6.5	11.1	8.0
Innovator	2.6	2.5	6.3	3.8
Krantz	3.8	3.9	6.3	4.7
NDTX4930-5W	5.0	5.7	9.4	6.7
Russet Burbank	3.3	3.3	3.0	3.2
Santana	6.1	5.8	13.0	8.3
Shepody	3.8	4.5	9.8	6.0
Average	4.3	4.4	8.5	5.7

STANDARD CURVE FOR LUTEIN. The linear regression equation to equate the spectrophotometric absorbance readings of the methanol extract at 445 nm into lutein equivalents was the following: 3028.6x + 8.1063, where x was the absorbance at 445 nm and y was the µg lutein equivalents per hundred grams fresh weight. The R^2 value of this equation was 0.9991.

The average amount of xanthophylls or lutein equivalents was 106 µg/100gfw. Analysis of variance indicated significant differences for cultivar ($p < 0.000$), cooking method ($p < 0.000$), the interaction of cultivar and cooking method ($p < 0.000$), the interaction between cultivar and storage treatment ($p < 0.000$), and the interaction between cooking method and storage treatment ($p < 0.000$). However, the three-way

interaction of cultivar by cooking method by storage treatment (p = 0.415) was not significant for the xanthophylls (Table 4.3).

The eta squared values for the following factors were cultivar, 30 %, cooking method, 3 %, storage treatment, 19 %, the interaction of cultivar and cooking method, 5 %, the interaction of cultivar and storage treatment, 11 %, the interaction between cooking method and storage treatment, 3 %, the interaction of cultivar, cooking method and storage treatment, 6 %, and error, 22 %.

Table 4.3 Analysis of variance for carotenoid (xanthophyll) content for the factors storage, cultivar, and cooking method, Dalhart 2003.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	529319.204 [z]	159	3329.052	6.941	.000
Intercept	5383373.309	1	5383373.309	11223.434	.000
Cultivar	201900.359	7	28842.908	60.133	.000
Cook	21770.531	4	5442.633	11.347	.000
Store	131364.240	3	43788.080	91.291	.000
Cultivar * Cook	34592.422	28	1235.444	2.576	.000
Cultivar * Store	75278.770	21	3584.703	7.474	.000
Cook * Store	22846.812	12	1903.901	3.969	.000
Cultivar * Cook * Store	41566.070	84	494.834	1.032	.415
Error	153489.516	320	479.655		
Total	6066182.028	480			
Corrected Total	682808.719	479			

[z] R^2 = .775 (Adjusted R^2 = .664)

There was significant variability among cultivars; Santana had the highest xanthophyll content of 137 µg/100gfw, while Atlantic had the lowest at 78 µg/100gfw, a range of 59 µg/100gfw (Table 4.4). This is in agreement with the results from McCook 2003 (Chapter III), where both Santana and Innovator were among the top three cultivars for xanthophyll content.

Table 4.4 Cultivar ranking for carotenoid (xanthophyll) content, Dalhart 2003.

Cultivar	Eq. Lutein (µg/100gfw)	
Santana	137	a[z]
Russet Burbank	127	b
Innovator	120	c
Krantz	112	c
Shepody	99	d
ATX85404-8W	95	d
NDTX4930-5W	78	e
Atlantic	78	e

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

There were significant differences among cooking methods (Table 4.5). Frying ranked highest in carotenoid content at 112 µg/100gfw, while boiling was lowest at 94 µg/100gfw, with a range of 18 µg/100gfw (Table 4.5). The range due to cooking method and the eta squared value for cooking were smaller than the antioxidant activity range and the phenolic range, indicating that xanthophyll content did not appear to be as affected by cooking method.

Table 4.5 Cooking method ranking for carotenoid (xanthophyll) content, Dalhart 2003.

Cooking method	Eq. Lutein (µg/100gfw)	
Fry	112	a[z]
Raw	111	a
Bake	107	a
Micro	104	a
Boil	94	b

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The analysis of variance results indicated a significant difference among the four storage treatments (Table 4.6). The range in carotenoid content of samples in the storage treatments was 45µg/100gfw. The highest ranking storage treatment was as 4 ^{o}C with reconditioning (124 µg/100gfw), while the no storage treatment was lowest at 79 µg/100gfw. The eta squared value for storage treatment (19 %) was much higher than

that for cooking method (3 %). Therefore, the effect of storage treatment on xanthophyll content was larger than that of cooking method.

Table 4.6 Storage method ranking for carotenoid (xanthophyll) content, Dalhart 2003.

Storage treatment	Eq. Lutein (µg/100gfw)	
4 °C, reconditioned	124	a[z]
4 °C	113	b
20 °C	108	b
None	79	c

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The following are the results of the interaction of cultivar and cooking method (Table 4.7). The highest carotenoid, xanthophyll (equivalents of lutein µg/100gfw), average values were in 'Russet Burbank', bake (151); 'Krantz', raw (141); 'Santana', microwave (140); 'Santana', raw (140); and 'Santana', fry (139). The lowest carotenoid, xanthophyll (equivalents of lutein µg/100gfw), average values were in 'Atlantic', boil (71); 'NDTX4930-5W', boil (72); 'NDTX4930-5W', microwave (72); 'NDTX4930-5W', bake (75); and 'Atlantic', bake (77). The over all range of this interaction was 80 µg/100gfw.

Table 4.7 Cultivar by cooking method interaction for carotenoid (xanthophyll) content, Dalhart 2003.

Cultivar and cooking method	Eq. Lutein (µg/100gfw)
Atlantic	
Bake	77
Boil	71
Fry	78
Micro	88
Raw	78
ATX85404-8W	
Bake	88
Boil	89
Fry	100
Micro	96
Raw	104

Table 4.7 (continued).

Cultivar and cooking method	Eq. Lutein (µg/100gfw)
Innovator	
Bake	113
Boil	92
Fry	138
Micro	129
Raw	126
Krantz	
Bake	101
Boil	95
Fry	122
Micro	101
Raw	141
NDTX4930-5W	
Bake	75
Boil	72
Fry	84
Micro	72
Raw	88
Russet Burbank	
Bake	151
Boil	111
Fry	133
Micro	115
Raw	127
Santana	
Bake	139
Boil	129
Fry	139
Micro	140
Raw	140
Shepody	
Bake	111
Boil	92
Fry	106
Micro	95
Raw	92

The interaction between cultivar and storage treatment (Table 4.8) was quite influential, accounting for 11 % of the total variability in the carotenoid (xanthophyll) content of the samples, with a range of 128 µg/100gfw. The greatest interactions (expressed as equivalents of lutein µg/100gfw) were in 'Santana', 4 ^{o}C with reconditioning (174); 'Innovator', 4 ^{o}C (159); 'Krantz', 4 ^{o}C with reconditioning (147); 'Russet Burbank', 4 ^{o}C with reconditioning (145); and 'Russet Burbank', 4 ^{o}C (140).

The smallest interactions (expressed as equivalents of lutein µg/100gfw) were found in 'NDTX4930-5W', no storage (46); 'Atlantic', no storage (52); 'Krantz', no storage (62); 'Shepody', no storage (78); and 'ATX85404-8W', no storage (80). The smallest interactions occurred in the no storage treatment, while the greatest interactions involved some type of storage treatment, either 4 ^{o}C or 4 ^{o}C with reconditioning. This further supports that storage results in increased xanthophyll content.

Table 4.8 Cultivar by storage treatment interaction for carotenoid (xanthophyll) content, Dalhart 2003.

Cultivar and storage treatment	Eq. Lutein (µg/100gfw)
Atlantic	
None	52
20 ^{o}C	86
4 ^{o}C	86
4 ^{o}C, reconditioned	88
ATX85404-8W	
None	80
20 ^{o}C	109
4 ^{o}C	81
4 ^{o}C, reconditioned	111
Innovator	
None	99
20 ^{o}C	105
4 ^{o}C	159
4 ^{o}C, reconditioned	115
Krantz	
None	62
20 ^{o}C	111
4 ^{o}C	128
4 ^{o}C, reconditioned	147
NDTX4930-5W	
None	46
20 ^{o}C	88
4 ^{o}C	92
4 ^{o}C, reconditioned	88
Russet Burbank	
None	97
20 ^{o}C	128
4 ^{o}C	140
4 ^{o}C, reconditioned	145
Santana	
None	118
20 ^{o}C	138
4 ^{o}C	119
4 ^{o}C, reconditioned	174

Table 4.8 (continued).

Cultivar and storage treatment	Eq. Lutein (µg/100gfw)
Shepody	
None	78
20 °C	100
4 °C	98
4 °C, reconditioned	121

The interaction between cooking method and storage treatment was significant ($p < 0.000$), but the interaction did not have a large influence (eta squared 3 %) (Table 4.9). The range for xanthophyll content was 70 µg/gfw, and the five highest interactions (expressed as equivalents of lutein µg/100gfw) were raw, 4 °C with reconditioning (135); fry, 4 °C (133); bake, 4 °C with reconditioning (131); fry, 4 °C with reconditioning (128); and microwave, 4 °C with reconditioning (122). The five lowest interactions (expressed as equivalents of lutein µg/100gfw) were boil, no storage (65); fry, no storage (80); bake, no storage (83); raw, no storage (83); and microwave, no storage (84). Again, five of the lowest interactions included the no storage treatment, while all five highest interactions included some type of storage treatment either 4 °C with reconditioning or 4 °C. This further supports that storage increases xanthophyll content.

Table 4.9 Cooking method by storage treatment interaction for carotenoid (xanthophyll) content, Dalhart 2003.

Cooking method and storage treatment	Eq. Lutein (µg/100gfw)
Bake	
None	83
20 °C	104
4 °C	109
4 °C, reconditioned	131
Boil	
None	65
20 °C	115
4 °C	94
4 °C, reconditioned	103

Table 4.9 (continued).

Cooking method and storage treatment	Eq. Lutein (µg/100gfw)
Fry	
None	80
20 ºC	108
4 ºC	133
4 ºC, reconditioned	128
Micro	
None	84
20 ºC	103
4 ºC	110
4 ºC, reconditioned	122
Raw	
None	83
20 ºC	112
4 ºC	118
4 ºC, reconditioned	135

DPPH ASSAY FOR TOTAL ANTIOXIDANT ACTIVITY - STANDARD CURVE FOR TROLOX. The linear regression equation to convert the spectrophotometric absorbance readings of the methanol extract and reduced DPPH at 515 nm into trolox equivalents was the following: $y = 891.69x$, where x was the delta absorption calculated from the subtraction of the sample from the blank of methanol and DPPH at 515 nm and y is the µg trolox equivalents per gram fresh weight. The R^2 value of this equation was 0.997.

Analysis of variance results indicated that all main effects (cultivar, cooking methods, and storage treatments) and the interaction of cultivar and storage treatment were significant factors for antioxidant activity (Table 4.10). Analysis of variance results for AOAI indicated that there were significant differences in cultivar ($p < 0.000$), cooking method ($p < 0.000$), and the interaction between cultivar and storage treatment ($p < 0.000$). There were no significant differences in the interaction of cultivar and cooking method ($p = 0.382$), cooking method and storage treatment ($p = 0.976$), and for the three-way interaction of cultivar by cooking method by storage treatment ($p = 0.415$) for AOAI. Analysis of variance results for AOAS suggest that there were significant differences in the factors of cultivar ($p < 0.000$), cooking method ($p < 0.000$), and the

interaction between cultivar and storage treatment ($p < 0.000$). There were no significant differences in the interactions of cultivar and cooking method ($p = 0.097$), cooking method and storage treatment ($p = 0.159$), and for the three-way interaction of cultivar by cooking method by storage treatment ($p = 0.978$) for AOAS. The average value for AOAI was 128 µg/ gfw equivalents of trolox, while the average value of AOAS was 305 µg/ gfw equivalents of trolox.

The eta squared values for AOAI were 24 % for cultivar, 16 % for cooking method, 2 % for storage method, 3 % for the interaction of cultivar and cooking method, 21 % for the interaction of cultivar and storage method, 1 % for the interaction of cooking method and storage treatment, 5 % for the interaction of cultivar, cooking method, and storage method, and 28 % for error. The eta squared values for AOAS were 18 % for cultivar, 17 % for cooking method, 4 % storage treatment, 3 % for the interaction of cultivar and cooking method, 24 % for the interaction of cultivar and storage method, 1 % for the interaction of cooking method and storage treatment, 5 % for the interaction of cultivar, cooking method and storage treatment, and 26 % for error. Both AOAI and AOAS were greatly influenced by cultivar, cooking method, and the interaction of cultivar and storage treatment, whereas carotenoid (xanthophyll) content was strongly influenced by cultivar, storage method and the interaction of cultivar and storage method.

Table 4.10 Analysis of variance results for antioxidant activity for the factors storage, cultivar, and cooking method, Dalhart 2003.

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	AOAI	2575361.063 [z]	159	16197.239	5.075	.000
	AOAS	7980596.324 [y]	159	50192.430	5.586	.000
Intercept	AOAI	7868118.373	1	7868118.373	2465.205	.000
	AOAS	44633987.270	1	44633987.270	4967.173	.000
Cultivar	AOAI	851539.331	7	121648.476	38.114	.000
	AOAS	1964755.143	7	280679.306	31.236	.000
Cook	AOAI	567407.738	4	141851.935	44.444	.000
	AOAS	1893151.845	4	473287.961	52.671	.000
Store	AOAI	84040.880	3	28013.627	8.777	.000
	AOAS	454951.186	3	151650.395	16.877	.000
Cultivar * Cook	AOAI	95032.893	28	3394.032	1.063	.382
	AOAS	348318.656	28	12439.952	1.384	.097
Cultivar * Store	AOAI	764328.055	21	36396.574	11.404	.000
	AOAS	2645095.195	21	125956.914	14.017	.000
Cook * Store	AOAI	26100.616	12	2175.051	.681	.769
	AOAS	152124.250	12	12677.021	1.411	.159
Cultivar * Cook * Store	AOAI	186911.549	84	2225.137	.697	.976
	AOAS	522200.049	84	6216.667	.692	.978
Error	AOAI	1021333.897	320	3191.668		
	AOAS	2875453.411	320	8985.792		
Total	AOAI	11464813.333	480			
	AOAS	55490037.004	480			
Corrected Total	AOAI	3596694.959	479			
	AOAS	10856049.735	479			

[z] $R^2 = .716$ (Adjusted $R^2 = .575$)
[y] $R^2 = .735$ (Adjusted $R^2 = .604$)

There was wide variability in antioxidant activity among cultivars (Table 4.11). The range for AOAI was 133 µg/gfw, with the highest cultivar Russet Burbank at 208 µg/gfw, and Shepody the lowest at 75 µg/gfw of equivalents of trolox. The range for AOAS was 215 µg/ gfw. The highest cultivar was again Russet Burbank at 420 µg/gfw and the lowest NDTX4930-5W at 205 µg/gfw of equivalents of trolox. Over all, the ranking of the cultivars for AOAI and AOAS were very similar except for the switching of last to cultivars (Shepody and NDTX4930-5W) between analyses.

Table 4.11 Cultivar ranking for antioxidant activity, Dalhart 2003.

Cultivar	AOAI[z] (µg/gfw)		Cultivar	AOAS[y] (µg/gfw)	
Russet Burbank	208	a[x]	Russet Burbank	420	a
Santana	169	b	Santana	353	b
Krantz	141	c	Krantz	325	bc
Atlantic	135	c	Atlantic	317	bc
ATX85404-8w	114	d	ATX85404-8w	309	bc
Innovator	103	d	Innovator	282	c
NDTX4930-5w	79	e	Shepody	227	d
Shepody	75	e	NDTX4930-5w	205	d

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[x] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

There were significant differences among cooking treatments (Table 4.12). The AOAI range was 86 µg/gfw. The cooking methods with the highest antioxidant activity were frying at 165 µg/gfw and microwaving at 162 µg/gfw, and the lowest was raw with 79 µg/gfw trolox equivalents. The AOAS range was 156 µg/gfw. The cooking methods with the highest antioxidant activity were microwaving at 374 µg/gfw and frying at 365 µg/gfw, while the lowest was raw at 218 µg/gfw trolox equivalents.

Table 4.12 Cooking method ranking for antioxidant activity, Dalhart 2003.

Cooking method	AOAI[z] (µg/gfw)		Cooking method	AOAS[y] (µg/gfw)	
Fry	165	a[x]	Micro	374	a
Micro	162	a	Fry	365	a
Bake	136	b	Bake	323	b
Boil	98	c	Boil	245	c
Raw	79	d	Raw	218	d

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[x] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Although storage influenced antioxidant activity less than carotenoid content, it was still a significant factor (Table 4.13). The AOAI range was 33 µg/gfw. The 4 ^{o}C storage treatment resulted in the highest equivalents of trolox with 151 µg/gfw, while 4

^{o}C with reconditioning was lowest at 118 µg/gfw. The AOAS range was 78 µg/gfw. The 4 ^{o}C storage treatment resulted in the highest equivalents of trolox at 355 µg/gfw, while the 20 ^{o}C treatment was the lowest at 277 µg/gfw. The AOAS ranking of storage treatments varied only slightly when paired with AOAI; however, the 4 ^{o}C storage treatment resulted in significantly higher AOAI and AOAS.

Table 4.13 Storage treatment ranking for antioxidant activity, Dalhart 2003.

Storage treatments	AOAI[z] (µg/gfw)		Storage treatments	AOAS[y] (µg/gfw)	
4 ^{o}C	151	a[x]	4 ^{o}C	355	a
20 ^{o}C	125	b	None	304	b
None	119	b	4 ^{o}C reconditioned	283	b
4 ^{o}C reconditioned	118	b	20 ^{o}C	277	b

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[x] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The interaction of cultivar and cooking method was not significant; however, the data contributes to better understanding the effects of the cultivar and cooking, which were significant. The over all range for AOAI was 235 µg/gfw (Table 4.14). The top five interactions for AOAI (eq. trolox µg/gfw) were in 'Russet Burbank', fry (271); 'Russet Burbank', microwave (261); 'Santana', microwave (217); 'Santana', fry (197); and 'Russet Burbank' bake (196). The lowest five interactions for AOAI (eq. trolox µg/gfw) were in 'Shepody', raw (36); 'Shepody', boil (37); 'NDTX4930-5W', raw (39); 'Innovator', raw (43); and 'NDTX4930-5W', boil (50). The over all range for AOAS was 387 µg/gfw. The top five interactions for AOAS (eq. trolox µg/gfw) were in 'Russet Burbank', microwave (538); 'Russet Burbank', fry (518); 'Santana', microwave (454); 'Krantz', microwave (412); and 'Russet Burbank', bake (411). The lowest five interactions for AOAS (eq. trolox µg/gfw) were in 'NDTX4930-5W', raw (151); 'Shepody', boil (153); 'NDTX4930-5W', boil (155); 'Shepody', raw, (157); and 'Innovator', raw (167). Over all, Russet Burbank and Santana appeared in the top

rankings more often than other cultivars. While microwaving and frying consistently ranked at the top among cooking methods.

Table 4.14 Cultivar by cooking method interaction for antioxidant activity, Dalhart 2003.

Cultivar and cooking method	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Atlantic		
Bake	139	302
Boil	138	300
Fry	164	365
Micro	145	370
Raw	87	250
ATX85404-8W		
Bake	126	332
Boil	107	300
Fry	141	374
Micro	129	318
Raw	68	224
Innovator		
Bake	112	304
Boil	56	194
Fry	151	370
Micro	153	375
Raw	43	167
Krantz		
Bake	167	374
Boil	87	228
Fry	159	355
Micro	186	412
Raw	105	257
NDTX4930-5W		
Bake	81	232
Boil	50	155
Fry	124	246
Micro	102	243
Raw	39	151
Russet Burbank		
Bake	196	411
Boil	177	346
Fry	271	518
Micro	261	538
Raw	135	286
Santana		
Bake	178	378
Boil	134	285
Fry	197	396
Micro	217	454
Raw	117	283

Table 4.14 (continued).

Cultivar and cooking method	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Shepody		
Bake	85	249
Boil	37	153
Fry	112	295
Micro	106	278
Raw	36	157

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox

The interaction between cultivar and storage method was quite strong, based on the eta squared values (Table 4.15). The over all range for AOAI (eq. trolox µg/gfw) was 240 µg/gfw. The highest interactions for AOAI (eq. trolox µg/gfw) were in 'Atlantic', 4 ^{o}C (276); 'Russet Burbank' 20 ^{o}C (267); 'Santana', 20 ^{o}C (231); 'Russet Burbank', 4 ^{o}C with reconditioning (207); and 'Russet Burbank', 4 ^{o}C (180). The lowest interactions for AOAI (eq. trolox µg/gfw) were in 'Atlantic', 4 ^{o}C with reconditioning (36); 'Shepody', 20 ^{o}C, (53); 'ATX85404-8W', 20 ^{o}C (64); 'Atlantic', 20 ^{o}C (66); and 'Shepody', no storage (69). The over all range for AOAS was 515 µg/gfw. The highest interactions for AOAS (eq. trolox µg/gfw) were in 'Atlantic', 4 ^{o}C (648); 'Russet Burbank', 4 ^{o}C with reconditioning (469); 'ATX85404-8W', 4 ^{o}C with reconditioning (443); 'Russet Burbank', no storage, (436); and 'Russet Burbank', 20 ^{o}C 392 µg/gfw. The lowest interactions for AOAS (eq. trolox µg/gfw) were in 'Atlantic', 4 ^{o}C with reconditioning (133); 'NDTX4930-5W', 20 ^{o}C (162); 'Atlantic', 20 ^{o}C (189); 'NDTX4930-5W', 4 ^{o}C (199); and 'Shepody', 4 ^{o}C with reconditioning (207). The cultivar Atlantic varied greatly with storage method, this describes how antioxidant activity is greatly dependent on storage treatment. Cold temperature storage may have caused 'Atlantic' to greatly increase in reducing sugars and this may have had a large affect on antioxidant activity.

Table 4.15 Cultivar by storage treatment interaction for antioxidant activity, Dalhart 2003.

Cultivar and storage treatment	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Atlantic		
None	160	300
20 °C	66	189
4 °C	276	648
4 °C reconditioned	36	133
ATX85404-8W		
None	102	271
20 °C	64	248
4 °C	166	443
4 °C reconditioned	124	277
Innovator		
None	87	306
20 °C	79	273
4 °C	98	229
4 °C reconditioned	148	320
Krantz		
None	131	307
20 °C	162	375
4 °C	156	351
4 °C reconditioned	114	269
NDTX4930-5W		
None	76	238
20 °C	75	162
4 °C	88	199
4 °C reconditioned	79	223
Russet Burbank		
None	178	436
20 °C	267	392
4 °C	180	382
4 °C reconditioned	207	469
Santana		
None	146	358
20 °C	231	360
4 °C	146	328
4 °C reconditioned	152	367
Shepody		
None	69	215
20 °C	53	220
4 °C	94	265
4 °C reconditioned	86	207

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox

The interaction of cooking method and storage treatment was not significant. However, the data facilitates understanding the effects of both cooking method and storage treatment which were significant. The over all range for AOAI was 134 µg/gfw.

The top five AOAI (eq. trolox µg/gfw) interactions were fry, 4 °C (196); microwave, 4 °C (180); fry, 20 °C (166); microwave, 4 °C with reconditioning (161); and microwave, 20 °C (157). The lowest five AOAI (eq. trolox µg/gfw) interactions were raw, 4 °C with reconditioning (62); raw, no storage (66); boil, 4 °C with reconditioning (81); boil, 20 °C (83); and raw, 20 °C (87).

The over all range for AOAS was 264 µg/gfw. The top five AOAS (eq. trolox µg/gfw) interactions were fry, 4 °C (449); microwave, 4 °C (429); microwave, no storage (378); microwave, 4 °C with reconditioning (361); and bake, 4 °C (350). The lowest five interactions for AOAS (eq. trolox µg/gfw) were raw, 4 °C with reconditioning (185); raw, no storage (205); boil, 20 °C (207); boil, 4 °C with reconditioning (208); and raw, 20 °C (221) (Table 4.16). The 4 °C storage treatment resulted in higher antioxidant activity for all cooking treatments. The cooking treatments of fry and microwave produced higher antioxidant activity than those of raw and boil. The combination of storage at 4 °C and frying produced a synergistic increase.

Table 4.16 Cooking method and storage treatment interaction for antioxidant activity, Dalhart 2003.

Cooking method and storage treatment	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Bake		
None	125	320
20 °C	131	305
4 °C	151	350
4 °C reconditioned	136	316
Boil		
None	104	280
20 °C	83	207
4 °C	125	287
4 °C reconditioned	81	208
Fry		
None	147	337
20 °C	166	328
4 °C	196	449
4 °C reconditioned	151	346
Micro		
None	152	378
20 °C	157	326
4 °C	180	429
4 °C reconditioned	161	361

Table 4.16 (continued).

Cooking method and storage treatment	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Raw		
None	66	205
20 °C	87	221
4 °C	101	262
4 °C reconditioned	62	185

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox

TOTAL PHENOLIC CONTENT. The linear regression equation to equate the spectrophotometric absorbance readings from the Folin test at 727 nm into chlorogenic acid equivalents was the following: $y = 0.5775x - 0.0279$, where x was the absorbance at 727 nm after zeroing the spectrophotometer with a blank lacking antioxidant extract but containing all other solutions, and y was the µg chlorogenic acid equivalents per gram fresh weight. The R^2 value of this equation was 0.970.

The analysis of variance revealed significant differences for all main effects, i.e., cultivar ($p < 0.000$), cooking method ($p < 0.000$), and storage treatment ($p < 0.000$), as well as for the two-factor interactions of cultivar and cooking method ($p < 0.000$); cultivar and storage ($p < 0.000$); and cooking method and storage treatment ($p < 0.000$). The over all average for phenolic content was 336 µg/gfw equivalents of chlorogenic acid (Table 4.17).

The eta squared values for each factor were 39 % for cultivar, 22 % for cooking method, 1 % for storage method, 4 % for the interaction of cultivar and cooking method, 12 % for the interaction of cultivar and storage method, 2 % for the interaction of cooking method and storage, 3 % for the three factor interaction of cultivar, cooking method and storage treatment and 16 % for error. The higher eta squared values for cultivar and cooking method indicate that these factors had a greater influence on phenolic levels than did storage treatment.

Table 4.17 Analysis of variance results for phenolic content for the factors storage, cultivar, and cooking method, Dalhart 2003.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	7054223.141[z]	159	44366.183	10.824	.000
Intercept	54306555.483	1	54306555.483	13249.188	.000
Cultivar	3302754.339	7	471822.048	115.111	.000
Cook	1808816.151	4	452204.038	110.324	.000
Store	107051.454	3	35683.818	8.706	.000
Cultivar * Cook	304691.839	28	10881.851	2.655	.000
Cultivar * Store	1032320.937	21	49158.140	11.993	.000
Cook * Store	172057.292	12	14338.108	3.498	.000
Cultivar * Cook * Store	326531.128	84	3887.275	.948	.606
Error	1311634.912	320	4098.859		
Total	62672413.536	480			
Corrected Total	8365858.053	479			

[z] R^2 = .843 (Adjusted R^2 = .765)

Cultivar had a strong effect on phenolic content, with an eta squared value of 39 %. As with antioxidant activity and carotenoid content, the cultivars Krantz, Russet Burbank, and Santana ranked higher than the other cultivars. The range among cultivars was 295 µg/gfw equivalents of chlorogenic acid, with Krantz the highest at 509 µg/gfw and NDTX4930-5W the lowest at 214 µg/gfw (Table 4.18).

Table 4.18 Cultivar ranking for phenolic content, Dalhart 2003.

Cultivar	Eq. Chlorogenic acid (µg/gfw)	
Krantz	509	a[z]
Russet Burbank	400	b
Santana	363	c
Innovator	318	d
Atlantic	307	de
Shepody	294	de
ATX85404-8W	286	e
NDTX4930-5W	214	f

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Cooking method significantly influenced phenolic content (Table 4.19). The range for cooking methods was 138 µg/gfw, with microwaving ranking highest at 396 µg/gfw and boiling lowest at 258 µg/gfw. These rankings are similar to those in earlier studies (Chapter III), where microwaving and frying were higher than raw and boiling, and baking was somewhere in between.

Table 4.19 Cooking method ranking for phenolic content, Dalhart 2003.

Cooking method	Eq. Chlorogenic acid (µg/gfw)	
Micro	396	a[z]
Fry	394	a
Bake	367	b
Raw	267	c
Boil	258	c

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Although storage alone had minimal influence, the interaction of cultivar and storage treatment was more important. The results of the main effect of storage method are shown in Table 4.20. The over all range was 40 µg/gfw, with 4 ^{o} C with reconditioning the highest, 361 µg/gfw, and 4 ^{o} C the lowest at 321 µg/gfw. The antioxidant activity was higher at 4 ^{o}C as compared to the other storage treatments, while phenolics were lowest at 4 ^{o}C. This discrepancy may not represent a large difference, because of the small influence that storage has on antioxidant activity and phenolic content.

Table 4.20 Storage treatment ranking for phenolic content, Dalhart 2003.

Storage treatments	Eq. Chlorogenic acid (µg/gfw)	
4 ^{o}C reconditioned	361	a[z]
None	335	b
20 ^{o}C	329	b
4 ^{o}C	321	b

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Although the interaction of cultivar and cooking method was significant, the influence of this interaction was smaller than that of the individual factors of cultivar and cooking method. The over all range of the interaction was 442 µg/gfw equivalents of chlorogenic acid, which indicated the large variability in the samples. The five highest interactions (eq. chlorogenic acid µg/gfw) were in 'Krantz', microwave (593); 'Krantz', bake (588); 'Krantz', fry (563); 'Russet Burbank', fry (502); and 'Russet Burbank', microwave (483). The five lowest interactions (eq. chlorogenic acid µg/gfw) were in 'NDTX4930-5W', boil (151); 'NDTX4930-5W', raw (177); 'Shepody', boil (184); 'ATX85404-8W', raw (230); and 'Innovator', boil (234) (Table 4.21). The influence of cultivar and cooking method are further demonstrated by this interaction, where some cultivars and certain cooking methods consistently rank higher than others.

Table 4.21 Cultivar by cooking method interaction for phenolic content, Dalhart 2003.

Cultivars and cooking methods	Eq. Chlorogenic acid (µg/gfw)
Atlantic	
Bake	307
Boil	288
Fry	361
Micro	336
Raw	242
ATX85404-8W	
Bake	301
Boil	247
Fry	330
Micro	321
Raw	230
Innovator	
Bake	343
Boil	234
Fry	385
Micro	389
Raw	238
Krantz	
Bake	588
Boil	373
Fry	563
Micro	593
Raw	427

Table 4.21 (continued).

Cultivars and cooking methods	Eq. Chlorogenic acid (µg/gfw)
NDTX4930-5W	
Bake	242
Boil	151
Fry	245
Micro	255
Raw	177
Russet Burbank	
Bake	432
Boil	300
Fry	502
Micro	483
Raw	282
Santana	
Bake	405
Boil	285
Fry	399
Micro	440
Raw	288
Shepody	
Bake	320
Boil	184
Fry	364
Micro	352
Raw	249

The interaction of cultivar and storage method (Table 4.22) had a greater effect on phenolics than the interaction of cultivar and cooking method (Table 4.21). The over all range was 359 µg/gfw. The cultivars with the highest phenolic content (eq. chlorogenic acid µg/gfw) under the different storage treatments were 'Krantz', 4 ^{o}C (542); 'Krantz', 4 ^{o}C with reconditioning (523); 'Krantz', no storage (489); 'Krantz', 20 ^{o}C (482); and 'Russet Burbank', 4 ^{o}C with reconditioning (468). The cultivars with the lowest phenolic content (eq. chlorogenic acid µg/gfw) under the different storage treatments were 'NDTX4930-5W', 4 ^{o}C (183); 'Atlantic', 20 ^{o}C (192); 'NDTX4930-5W', no storage (196); 'NDTX4930-5W', 20 ^{o}C (209); and 'Innovator', no storage (239). Innovator appeared to increase in phenolic content with storage, Atlantic appeared to decrease in phenolic content with storage, and Krantz appeared to increase with cold temperature storage.

Table 4.22 Cultivar by storage treatment interaction for phenolic content, Dalhart 2003.

Cultivar and storage treatment	Eq. Chlorogenic acid (µg/gfw)
Atlantic	
None	422
20 °C	192
4 °C	364
4 °C reconditioned	249
ATX85404-8W	
None	293
20 °C	246
4 °C	290
4 °C reconditioned	314
Innovator	
None	239
20 °C	349
4 °C	302
4 °C reconditioned	383
Krantz	
None	489
20 °C	482
4 °C	542
4 °C reconditioned	523
NDTX4930-5W	
None	196
20 °C	209
4 °C	183
4 °C reconditioned	269
Russet Burbank	
None	406
20 °C	432
4 °C	293
4 °C reconditioned	468
Santana	
None	350
20 °C	388
4 °C	341
4 °C reconditioned	375
Shepody	
None	285
20 °C	333
4 °C	252
4 °C reconditioned	305

The interaction of cooking method and storage treatment was significant, but it carried little influence (eta squared 2 %). The interaction described trends found in the main effects of cooking method and storage treatment. The over all range was 208 µg/gfw. The five combinations resulting in the greatest phenolic content (eq.

chlorogenic acid µg/gfw) were microwave, 4 °C with reconditioning (443); fry, no storage (425); microwave, 20 °C (406); fry, 20 °C (400); and microwave, no storage (389). The five lowest combinations resulting in the lowest phenolic content (eq. chlorogenic acid µg/gfw) were boil, 20 °C (235); raw, 20 °C (236); boil, no storage (247); raw, 4 °C (256); and boil, 4 °C (262) (Table 4.23). Again, the cooking methods of microwave and frying resulted in higher phenolic contents than the cooking methods of raw and boil.

Table 4.23 Cooking method by storage treatment interaction for phenolic content, Dalhart 2003.

Cooking method and storage treatment	Eq. Chlorogenic acid (µg/gfw)
Bake	
None	349
20 °C	367
4 °C	372
4 °C reconditioned	382
Boil	
None	247
20 °C	235
4 °C	262
4 °C reconditioned	288
Fry	
None	425
20 °C	400
4 °C	368
4 °C reconditioned	382
Micro	
None	389
20 °C	406
4 °C	347
4 °C reconditioned	443
Raw	
None	265
20 °C	236
4 °C	256
4 °C reconditioned	310

HPLC ANALYSIS FOR CAROTENOID COMPOUNDS. Sixty samples were analyzed for seven compounds, only six, violaxanthin, neoxanthin, antheraxanthin, lutein, zeaxanthin, and canthaxanthin, were found through either retention time or

spectra (Table 4.24). β-cryptoxanthin was not detected by HPLC. Lutein was the only compound detected by spectra. The cultivars chosen for analysis were Innovator, Russet Burbank and Santana. These cultivars were chosen due to their high levels of both carotenoids and phenolics. The analysis of variance indicated that there were no significant differences among cultivars in the total content of individual carotenoids based on either retention time or spectra. However, the cultivar Santana had the highest levels of neoxanthin, antheraxanthin, and canthaxanthin based on retention time, while the cultivar Innovator had the highest amount of lutein based on retention time, spectra, and the combination of retention time and spectra.

Table 4.24 Cultivar ranking for individual carotenoid compounds[z] (μg/100gfw), Dalhart 2003.

Cultivar	VIO	NEO	ANT	LUT	ZEA	CAN	Total-R	LUT-SP	LUT-RSP
Innovator	1 a[y]	1 a	0 b	9 a	1 a	1 a	14 a	1 a	1 a
Russet Burbank	0 a	0 a	0 b	7 a	1 a	0 a	8 a	0 a	0 a
Santana	0 a	2 a	5 a	0 b	0 a	2 a	9 a	0 a	0 a

[z]VIO : Violaxanthin content based on retention time
NEO : Neoxanthin content based on retention time
ANT : Antheraxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein content based on spectra
LUT-RSP : Lutein content based on retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Cooking method was also analyzed, and as with the cultivar main effect, analysis of variance indicated that there were no differences among cooking methods for total carotenoid content, although there were significant differences in lutein content based on retention time (Table 4.25). The baked samples were significantly higher in lutein content based on retention time than the raw samples. The total individual carotenoid content based on retention time was similar to that based on spectrophotometric methods described previously, except the spectrophotometric method ranked the raw samples

slightly higher. The raw samples were also higher in total individual carotenoid content based on retention time (Chapter III). This difference may be due to the small influence of cooking on carotenoid content.

Table 4.25 Cooking method ranking for individual carotenoid compounds[z] (µg/100gfw), Dalhart 2003.

Cooking methods	VIO	NEO	ANT	LUT	ZEA	CAN	Total-R	LUT-SP	LUT-RSP
Bake	0 a[y]	4 a	4 a	11 a	0 a	2 a	21 a	0 a	0 a
Boil	0 a	0 a	1 a	5 ab	0 a	0 a	6 a	0 a	0 a
Fry	0 a	0 a	1 a	3 ab	2 a	4 a	9 a	1 a	1 a
Micro	2 a	2 a	1 a	8 ab	0 a	0 a	13 a	0 a	0 a
Raw	0 a	0 a	0 a	0 b	2 a	1 a	2 a	1 a	1 a

[z]VIO : Violaxanthin content based on retention time
NEO : Neoxanthin content based on retention time
ANT : Antheraxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein content based on spectra
LUT-RSP : Lutein content based on retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

There were no significant differences in individual carotenoid content among storage treatments; however, the ranking further supports the spectrophotometric readings, where storage treatments ranked higher than no storage treatments in carotenoid content (Table 4.26). The only significant difference among storage treatments was with lutein based on retention time. Storage treatments ranked higher in lutein content than the no storage treatments.

Table 4.26 Storage treatment ranking for individual carotenoid compounds[z] (µg/100gfw), Dalhart 2003.

Storage treatments	VIO	NEO	ANT	LUT	ZEA	CAN	Total-R	LUT-SP	LUT-RSP
None	0 a[y]	0 a	1 a	0 b	0 a	1 a	2 a	0 a	0 a
20 °C	0 a	1 a	1 a	11 a	0 a	1 a	15 a	0 a	0 a
4 °C	2 a	1 a	2 a	9 ab	1 a	1 a	16 a	1 a	1 a
4 °C reconditioned	0 a	2 a	2 a	2 b	1 a	1 a	9 a	1 a	1 a

[z]VIO : Violaxanthin content based on retention time
NEO : Neoxanthin content based on retention time
ANT : Antheraxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein content based on spectra
LUT-RSP : Lutein content based on retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

HPLC ANALYSIS FOR PHENOLIC COMPOUNDS. Although there were 18 compounds in the library, only 13 were found in the samples tested. Unlike results for the carotenoid compounds, there were significant differences in a number of phenolic compounds. The same three cultivars were chosen for analysis, Innovator, Russet Burbank, and Santana (Table 4.27). The samples contained high levels of chlorogenic acid, caffeic acid, gallic acid, protocatechuic acid, and catechin. Over all, the cultivar Santana appeared to have a greater amount of total individual phenolic compounds than Innovator and Russet Burbank, but the difference was not significant. However, there were significant differences in chlorogenic acid, caffeic acid, gallic acid, rutin hydrate, sinapic acid, quercetin dihydrate, and protocatechuic acid.

Table 4.27 Cultivar ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, Dalhart 2003.

Cultivar	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
Innovator	43	48	11	238	28	40	10	5	48	22	12	215	14	734
	b[y]	b	a	ab	b	c	a	a	b	a	a	a	a	a
Russet	45	50	8	255	35	72	11	1	80	22	12	227	16	834
Burbank	b	a	a	a	a	a	a	c	a	a	a	a	a	a
Santana	62	51	10	227	26	54	10	3	89	21	12	364	16	945
	a	a	a	b	b	b	a	b	a	a	a	a	a	a

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Chlorogenic acid and caffeic acid were the only compounds detected based on spectra (Table 4.28). Analysis indicated there were significant differences for both compounds. 'Santana' ranked highest in chlorogenic acid based on spectra and the combination of retention time and spectra, while 'Innovator' ranked highest in caffeic acid based on spectra and the combination of retention time and spectra.

Table 4.28 Cultivar ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both spectra and retention time, Dalhart 2003.

Cultivar	CH-SP	CA-SP	Total-SP	CH-RSP	CA-RSP	Total-RSP
Innovator	74 b[y]	194 a	268 b	38 ab	48 a	86 a
Russet	191 a	169 a	360 a	27 b	28 b	55 b
Burbank						
Santana	202 a	98 b	300 b	54 a	5 c	59 b

[z]CH-SP : Chlorogenic acid based on spectra
CA-SP : Caffeic acid based on spectra
Total-SP : the addition of all measured phenolics based on spectra
CH-RSP : Chlorogenic acid based on retention time and spectra
CA-RSP : Caffeic acid based on retention time and spectra
Total-RSP : the addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Analysis of variance revealed that there were significant differences in phenolic content among cooking methods (Table 4.29). A greater number compounds were significantly affected by cooking method than by cultivar. Over all, the raw samples ranked in the highest level of significance for only five compounds, boiling ranked in the highest level of significance for six compounds, baking was listed in the highest level of significance for nine compounds, frying was listed in the highest level of significance for eleven compounds, and microwaving was listed in the highest level of significance for twelve compounds. Only quercetin dihydrate content appeared to be highest in raw samples. In Chapter III, quercetin dihydrate also ranked higher in raw samples as compared to cooked samples. Apparently, quercetin dihydrate is more affected by cooking than the other phenolic compounds.

Table 4.29 Cooking method ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, Dalhart 2003.

Cooking method	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
Bake	52	49	10	273	31	62	10	3	87	21	13	225	16	852
	b[y]	c	a	a	ab	a	ab	b	a	a	a	a	bc	a
Boil	36	48	10	180	25	51	9	3	59	21	12	222	13	688
	c	c	a	c	b	ab	ab	b	b	a	a	a	c	a
Fry	70	52	9	265	33	62	11	1	83	21	13	461	19	1099
	a	b	a	a	ab	a	ab	b	a	a	a	a	ab	a
Micro	79	54	8	269	36	61	14	2	76	23	13	213	20	868
	a	a	a	a	a	a	a	b	ab	a	a	a	a	a
Raw	13	45	11	213	24	41	7	7	58	22	9	222	9	682
	a	d	a	b	b	b	b	a	b	a	b	a	d	a

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The HPLC results based on spectra and the combination of retention time and spectra were similar to the results based solely on retention time. Based on the combination of retention time and spectra there were significant differences among cooking treatments for all compounds, except, caffeic acid. Microwaved samples ranked highest in content of all compounds (Table 4.30).

Table 4.30 Cooking method ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both spectra and retention time, Dalhart 2003.

Cooking method	CH-SP	CA-SP	Total-SP	CH-RSP	CA-RSP	Total-RSP
Bake	195 a[y]	150 ab	346 ab	39 b	20 a	59 bc
Boil	135 a	174 ab	309 b	29 b	28 a	57 bc
Fry	178 a	170 ab	347 ab	52 b	24 a	76 b
Micro	213 a	202 a	415 a	76 a	37 a	112 a
Raw	56 b	74 b	130 c	2 c	27 a	29 c

[z]CH-SP : Chlorogenic acid based on spectra
CA-SP : Caffeic acid based on spectra
Total-SP : the addition of all measured phenolics based on spectra
CH-RSP : Chlorogenic acid based on retention time and spectra
CA-RSP : Caffeic acid based on retention time and spectra
Total-RSP : the addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The content of nine phenolic compounds was significantly different among storage treatments based on retention time (Table 4.31). The no storage treatment ranked in the highest level of significance for ten compounds, the 20 ^{o}C storage ranked highest for seven, the 4 ^{o}C storage ranked highest for five, while the 4 ^{o}C with reconditioning storage ranked highest for ten compounds. The 4 ^{o}C storage treatment with reconditioning was significantly higher in phenolic content based on the Folin method, described previously. The HPLC phenolic results based on retention time further supports the validity of the total phenolic content results based on the Folin method.

Table 4.31 Storage treatment ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, Dalhart 2003.

Storage method	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
None	60	65	13	270	26	42	11	1	79	27	13	485	18	1110
	b[x]	a	a	a	a	c	a	b	a	a	a	a	a	a
20 °C	31	43	8	232	30	60	9	4	79	19	12	200	12	738
	c	c	b	bc	a	ab	a	a	a	b	a	a	b	b
4 °C	38	44	9	212	31	54	11	4	59	21	12	188	13	693
	c	c	b	c	a	b	a	a	b	b	a	a	b	b
4 °C reconditioned	71	48	9	247	32	66	10	4	73	20	12	202	18	809
	a	b	b	ab	a	a	a	a	ab	b	a	a	a	b

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

There were significant differences in phenolic content based on spectra and the combination of retention time and spectra among storage treatments (Table 4.32). The no storage treatment ranked highest in phenolic content based on spectra, while 4 °C with reconditioning ranked highest in phenolic content based on the combination of retention time and spectra.

Table 4.32 Storage treatment ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both spectra and retention time, Dalhart 2003.

Storage method	CH-SP	CA-SP	Total-SP	CH-RSP	CA-RSP	Total-RSP
None	236 a[x]	205 a	441 a	41 b	34 a	75 ab
20 °C	113 b	137 ab	250 b	23 b	26 a	48 b
4 °C	89 b	171 ab	260 b	30 b	29 a	59 ab
4 °C reconditioned	184 b	103 b	287 b	64 a	19 a	84 a

Table 4.32 (continued).

[z]CH-SP	: Chlorogenic acid based on spectra	CH-RSP	: Chlorogenic acid based on retention time and spectra
CA-SP	: Caffeic acid based on spectra		
Total-SP	: the addition of all measured phenolics based on spectra	CA-RSP	: Caffeic acid based on retention time and spectra
		Total-RSP	: the addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Discussion and Conclusion

As in the McCook 2003 study (Chapter III), wide variability was found among cultivars for carotenoid content, antioxidant activity and phenolic content. 'Santana' had the highest carotenoid content, while 'Atlantic' had the lowest. The cultivar with the highest AOAI was Russet Burbank and the lowest was Shepody. The cultivar with the highest AOAS was again Russet Burbank, while the lowest was NDTX4930-5W. The cultivar with the highest phenolic content was Krantz and the lowest was NDTX4930-5W.

Over all, cooking method results were also similar to the McCook 2003 study (Chapter III). The raw carotenoid samples were in the highest level of significance in both studies. These findings support Bianchini and Penteado's (1998) study which stated that carotenoids in pepper decreased with cooking, but contradicted Dietz et al. (1988); Granado et al. (1992); Klein and Kurilich (2000); and Van den Berg et al. (2000) where carotenoids in cooked products were more easily extracted and increased in content. Antioxidant activity, total phenolic content and individual phenolic compounds (except for quercetin dihydrate) were higher with the cooking methods of microwaving, frying and baking than with raw and boiling. This supported Amakura et al. (2000); Dewanto et al. (2002); and Zafrilla et al. (2001) who concluded that antioxidant activity and phenolic content increased with cooking. This may be due to the release of phenolics bound by the cell matrix, resulting in increased free phenolics. Crozier et al. (1997) and Häkkinen et al. (2000) reported a decrease in quercetin in cooked products,

supporting the findings of the present investigation. Over all, cooking had a greater influence on antioxidant activity and phenolic content, than on carotenoid content.

Generally, some type of storage treatment seemed to result in equal or greater total carotenoid content, individual carotenoid content, antioxidant activity, total phenolic content, and individual phenolic content, than the no storage treatment. In contrast, a number of studies reported a loss of antioxidant compounds in storage studies. Craft and Wise (1993) reported carotenoid content decreased, while Percival et al. (2000) and Häkkinen et al. (2000) reported losses in chlorogenic acid, quercetin, myricetin, and kaempferol contents with storage. Other studies reported an increase in carotenoids (Bhushan and Thomas, 1990), chlorogenic acid (Friedman, 1997) and ellagic acid content (Zafrilla et al., 2001) with storage. Friedman (1997) and Craft and Wise (1993) reported interactions with storage temperature and other conditions in levels of antioxidant compounds. The effect of storage on antioxidant compounds appears to differ among food products, time of storage, temperature, humidity, and light level of storage. Storage time should be considered an influential factor; future studies should evaluate antioxidant compounds over numerous time periods during storage.

CHAPTER V
THE EFFECTS OF LOW DOSE GAMMA- IRRADIATION, STORAGE, CULTIVAR, AND COOKING METHOD ON CAROTENOIDS, ANTIOXIDANT ACTIVITY, AND PHENOLICS IN POTATO

Synopsis

The consumption of processed potato products has increased. With this increase, there has been a demand for stored potato to ensure production of processed goods throughout the year. One of the major storage problems with potato is sprouting. Low-dose gamma- irradiation is a proven method to prevent sprouting, and may become more popular in the future. Past research has shown that there are significant levels of antioxidants, phenolics and carotenoids among cultivars and advanced selections in the Texas Potato Variety Development Program. However, it is unknown how these photochemical levels are affected by the processing methods of low-dose gamma-irradiation, storage treatments, and cooking methods. The objective of this experiment was to study the effects of low-dose gamma- irradiation (0, 75, 150 Gy), storage treatments (no storage, and 4 ^{o}C for 110 days), and cooking methods (no cooking, microwaving, boiling, baking, and frying) on carotenoid content, antioxidant activity, and phenolic content in eight named cultivars and advanced selections. Whole tubers were subjected to one of three irradiation doses, one of four storage treatments, then diced. A five-gram sample of the diced tubers was subjected to one of five cooking methods and frozen until extraction and quantification of phytochemicals. Carotenoid (xanthophyll) content was determined via absorbance at 445 nm. Individual carotenoid compounds were quantified via HPLC identification based on retention time, spectra, and the combination of retention time and spectra corresponding to standards. Antioxidant activity was determined by the DPPH method, and the kinetic reaction was quantified at two times, initially and at stabilization. Phenolic content was determined by the Folin-Ciocalteau method and individual phenolic compounds were quantified via HPLC identification based on retention time, spectra, and the combination of retention time and spectra corresponding to standards. The cultivars Santana, Innovator, Russet

Burbank, and Krantz ranked high in all analyses. Raw samples ranked significantly higher in carotenoid content. This ranking contradicted the HPLC results for individual carotenoid content, where boiled and raw samples ranked lower than the other cooked samples. The cooking methods of raw and boiled also ranked significantly lower than the other cooking methods in both antioxidant activity and phenolic content, and the boiled and raw samples also ranked lower in total phenolic content via HPLC. Carotenoid content was greater with storage than without storage, although HPLC total carotenoid content was not significantly affected by storage, but no storage method ranked higher. For antioxidant activity and total phenolic content via the Folin method no storage ranked significantly greater than storage; however, the storage treatment ranked significantly higher via HPLC quantification of total phenolics. Samples exposed to irradiation were significantly higher in carotenoid content than those not exposed, while exposure to irradiation was not a significant factor for total carotenoid content via HPLC quantification. The 0 and 150 Gy doses were similar for antioxidant activity, whereas samples exposed to irradiation significantly increased in phenolic content via the Folin method. HPLC quantification of total phenolic content only revealed significant differences based on spectra readings, while the 0 and 75 Gy doses ranked significantly higher than the 150 Gy dose. Numerous interactions were determined to be significant, most notable was the interaction between storage and irradiation for antioxidant activity, where higher dose samples (75, and 150 Gy) ranked higher before storage then lower after storage, as compared to the 0 Gy dose.

Introduction

Use of processed vegetables, especially potato products, has increased over the years, and with this increase there is a greater need for stored potatoes to ensure production of processed goods throughout non-harvesting seasons. One of the major storage problems with potato is sprouting. Sprouting causes loss of weight, quality, market value, and results in increased production of glycoalkaloids. Potatoes generally are dormant for a certain amount of time after harvest, and can only be induced to sprout by external factors. When dormancy is over, however, sprouting will occur

automatically and can only be suppressed by external artificial means. Therefore, the goal of storage is to maintain the potato in a state of dormancy. Current sprout control measures include low temperature storage (4-10 C), chemical sprout inhibitors (chlorpropham, CIPC; maleic hydrazide, MH; tecnazene, TCNB), and low dose irradiation (75-200 Gy). About 5% of potato production in the United States is lost due to sprouting (Mondy and Gosselin, 1989; Ogawa and Uritani, 1970; Rastovski et al., 1987; Saour and Makee, 2002; Thomas, 1984).

The use of low temperature storage, either by itself or in combination with chemical sprout inhibitors, is by far the most popular means of sprout inhibition, but this may not be true in the future. Other means of sprout inhibition are being investigated, especially for market and processing potatoes in tropical areas, due to the difficulty and the long term cost of cold storage facilities (Thomas et al. 1978). Another added concern for potato storage is that the chemicals used for sprout inhibition may eventually be phased out due to environmental concerns, health concerns, or added issuing costs to companies. Currently, many organophosphate pesticides are being phased out due to health concerns. Another phase-out that is causing wide concern for trade is methyl bromide, which is a widely used fumigant. The Montreal Protocol signed by the United States and 182 other countries calls for the reduction in use of chemicals that cause ozone depletion, which includes methyl bromide. This fumigant has been a principal chemical in postharvest quarantine treatments for many fruits and vegetables. Food irradiation is now being examined as a possible alternative to chemical quarantine treatments.

Food irradiation is a proposed means of reducing food losses due to microbial or insect spoilage, extending shelf life of foods, improving the hygienic quality of foods, and reducing the level of chemicals on food products. As of 2002, there were 20 gamma (^{60} Co) commercial irradiation facilities and four x-ray, electron beam facilities in the United States (Kume et al., 2002). There is only one facility in Shihoro, Hokkaido, Japan, that irradiates potatoes for the inhibition of sprouting. This site irradiates 15,000 tons / year and has an economic crop worth of $16M (Kume et al., 2002).

Many are reluctant to consume products that have been irradiated due to unknown chemical or physical changes that could be caused by exposure to ionizing radiation. In 1976, the World Health Organization (WHO) approved the marketing of irradiated potatoes, and in 1987 the WHO broadly determined that food irradiation is safe. In 1993, over thirty countries approved of irradiation for food items, and this process is now replacing many banned chemicals.

Sprout inhibition via irradiation is caused by changes in the metabolism of hormones, namely auxins, that induce sprouting in potatoes. It is also hypothesized that proteins synthesized after irradiation may act as repressors of certain enzymes that are needed for sprouting. Past studies on the chemical changes in irradiated products include amino acid changes and enzyme changes. Jaarma (1966) determined there was an association with sprout inhibition in irradiated tubers and proline accumulation, while Kodenchery and Nair (1972) reported an increase in the following free amino acids: aspartic acid, asparagines, threonine, serine, alanine, isoleucine, leucine, lysine, and arginine, and a decrease in glutamic acid, praline, methionine, and phenylalanine. The amino acid changes may effect levels of the PAL (phenylalanine ammonia lyase) enzyme, which is responsible for the production of many phenolic compounds. Pendharker and Nair (1975) reported a dose dependent increase in PAL activity in irradiated tubers. There have been numerous studies involving the effect of irradiation on carotenoids; however, results have differed based on different food products (World Health Organization, 1994). Craft and Wise (1993) and Thomas and Joshi (1977) reported a loss of carotenoids in potatoes with storage, while Bhushan and Thomas (1990) and Janave and Thomas (1979) reported an increase in carotenoids with ambient storage. Craft and Wise (1993) reported a decrease in carotenes, and a simultaneous increase in xanthophylls in potatoes exposed to gamma- irradiation. Patil et al. (1999) and Penner and Fromm (1972) both reported an immediate increase in phenolic content (quercetin and chlorogenic acid) followed by a decrease and leveling of phenolic content in irradiated products. Antioxidant activity may be affected by changes in the levels of phenolics or carotenoids, and possibly changes in Maillard reaction products (MRPs) in

irradiated, cooked potatoes. Leszczyński et al. (1992) determined that irradiated stored (4, 7, and 13 ^{o}C) tubers were higher in sucrose sugar content. Higher amounts of sugars may cause greater levels of MRPs, which may lead to a loss in quality of processed potato products, although Aoki (1983) reported that irradiated potatoes stored for eight months at both 5 ^{o}C and 7 ^{o}C produced better chips than those that were not irradiated.

Thomas (1984) reported that there are a number of factors affecting sprout inhibition via irradiation. These factors include: dose rate, cultivar, storage temperature, and time after harvest for irradiation. One important factor involved is that a higher dose is needed for sprout inhibition at higher storage temperatures, and a lower dose at colder temperatures (McKinney, 1971). Irradiation does not usually act alone in postharvest processing. Some type of storage method and duration is always combined with irradiation treatments. The postharvest factor of cooking always accompanies potatoes used for food consumption.

Significant levels of antioxidants, phenolics and carotenoids within cultivars and advanced breeding lines in the Texas Potato Variety Development Program have been reported (Hale, 2003; Al-Saikhan, 1994, 2000). It is unknown how these photochemical levels are affected by processing with low-dose gamma- irradiation, storage, and cooking and their interactions. The objectives of this investigation were to determine the effects of low-dose gamma- irradiation, storage treatments, and cooking methods on total carotenoid content, individual carotenoid content, total phenolic content, individual phenolic content, and total antioxidant activity in eight named cultivars and advanced selections.

Materials and Methods

HARVEST LOCATION. Planting and harvesting was conducted near, Dalhart, Texas, located on the border of Dallam county, in the northwest corner of the Texas Panhandle.

PLANT MATERIAL. Named processing cultivars and advanced selections were harvested in October 2003, and eight popular processing cultivars (Atlantic, ATX85404-8W, Innovator, Krantz, NDTX4930-5W, Russet Burbank, Santana, and Shepody) were selected for this study (Table 5.1).

Table 5.1 Characteristics of potato cultivars and advanced selections used in low-dose gamma- irradiation, storage, and cooking studies.

Cultivar	Shape	Skin Color / Flesh Color	Utilization	Maturity
Atlantic	Oval to round	White, Buff / White	Chipping, Boiling, and Baking	Early to Medium
ATX85404-8W	Oval	White / White	Chipping	Medium to Late
Innovator	Oblong to long	Russet / Yellow	Baking and French fries	Early to Medium
Krantz	Oblong	Russet / White	Boiling, Baking and French fries	Medium
NDTX4930-5W	Oval to long	Light buff / White	Chipping and French fries	Early to Medium
Russet Burbank	Long	Russet / White	Baking and Fry Processing	Late
Santana	Oval to long	White / Yellow	Fry Processing	Early to Medium
Shepody	Long	Buff / White	Boiling, Baking, and French fries	Medium

Three different processing methods were involved in this study, gamma-irradiation, storage treatment, and cooking method. Each potato sample was first subjected to a one of three doses (0, 75, or 150 Gy) of irradiation, then to one of two storage treatments (no storage, or 4 ^{o}C for 110 days), then to one of five cooking methods (raw, boiling, microwave cooking, baking, and frying).

GAMMA- IRRADIATION TREATMENT. The potatoes were transported to the USDA/APHIS Moore Air Field Base in Mission, Texas facility. At the Mission site, the allotted irradiated samples were subjected to gamma- irradiation via the source Cesium-137. Doses were determined based on a pre-calculated dose per time rate per irradiator. The dose per time rates were calculated based on the degradation of the irradiation source. The dose rate was 0.638 Gy per second. Tubers were exposed to irradiation and then transported to College Station for storage and cooking.

STORAGE TREATMENTS. Two different storage treatments were conducted. The no storage treatment allowed analysis of fresh harvested samples, while stored potatoes were held for 110 days at 4 ^{o}C.

SAMPLE PREPARATION. Three potatoes from each field replication were diced with a manual vegetable dicer (The Redco Insta Cut 3.5, Lincoln Foodservice, Fort Wayne, IN). The size of the diced samples was roughly 0.64 cm. The diced potatoes were mixed to obtain a randomized sample. A 5 g sample was used for each cooking treatment. Once diced, samples were placed in extraction tubes and frozen at -18 ^{o}C (0 ^{o}F) until further extraction and/or cooking.

COOKING METHODS. Four cooking methods and a control of no cooking or raw were used. Processing times and temperatures were based on the optimum times and required temperatures to cook the average sample. This was determined based on the texture and feel of the potato sample. The uncooked sample had a starchy texture that is firm and sticky, while cooked samples had an interior that was mealy and/or powdery. The raw samples remained frozen at -18 ^{o}C (0 ^{o}F) until extraction.

Microwave. Six samples were cooked in their tubes for 2.5 min on high with a microwave (model MW8985W, Emerson, St. Louis, MO). After one minute, the

cooking process was stopped to mix the contents. After cooking for the second minute, the cooking process was stopped to rotate the sample, then cooking continued for another thirty seconds. Cooked samples were then frozen at -18 ^{o}C (0 ^{o}F) until extraction.

Boil. Water was brought to a boil using a stove range (Montgomery Ward, Cedar Rapids, IA). Ten mL of nanopure, autoclaved water was added to each sample in the plastic extraction tubes. Samples were cooked for 25 min in boiling water. After cooking, the leachate was drawn out and discarded. The cooked potato sample was patted dry and frozen at -18 ^{o}C (0 ^{o}F) until extraction.

Bake. A gas oven (Montgomery Ward, Cedar Rapids, IA) was brought to 204 ^{o}C (400 ^{o}F). One-hundred-sixty samples, in glass tubes were cooked for 15 min. Foil was used to cover the tubes to prevent water loss. After cooking, the samples were removed from the glass test tubes and placed back into the plastic tubes for extraction. Cooked samples were then frozen at -18 ^{o}C (0 ^{o}F) until extraction.

Fry. Canola oil was brought 191 ^{o}C (375 ^{o}F) in a mini fryer (Rival CF250 Cool Touch Deep Fryer, El Paso, TX). Potato samples were placed in tea balls. The cooking time, which began once the tea ball entered the oil, was one min. After cooking, the samples were removed from the tea ball and placed on paper towels to cool, and placed back into plastic extraction tubes. Cooked samples were then frozen at -18 ^{o}C (0 ^{o}F) until extraction.

EXTRACTION OF CAROTENOIDS. Due to low carotene content found in Chapter III, only the xanthophylls were analyzed. The xanthophylls were extracted with methanol (plus 1 g/ L of BHT for stabilization). This extraction procedure was used to quantify the total carotenoid content based on the content of xanthophylls, and the individual carotenoid content via HPLC. Twenty-five mL of methanol plus BHT was added to a 5 g sample of diced potato. This mixture was then homogenized with an ultra turrax tissumizer from Tekmar (Cincinnati, OH). Samples and solvent were stored at -20 ^{o}C (-4 ^{o}C) for at least 12 h to ensure that the solvent extracted all carotenoids. Samples were then placed in a J-17 rotor at 17,000 rpm for 20 min in a refrigerated

centrifuge (Beckman model J2-21, Fullerton, CA). Two mL of the methanol were extracted and saved for analysis of total carotenoids, and 2 mL of each replication (total 6 mL) were used for HPLC analysis on select cultivars (Fig. 5.1). The extracted samples were stored at -29 ^{o}C (-20 ^{o}F).

HPLC ANALYSIS FOR INDIVIUAL CAROTENOID COMPOUNDS. Selected cultivars that were analyzed for individual carotenoid compounds were chosen based on high quantifications on all tests. The extracted samples were concentrated under nitrogen gas and filtered through a 0.45 μm syringe filter (Hale, 2003). The samples were resuspended in 0.5 mL ethanol and 0.5 mL nanopure water. A PC-operated Waters high performance liquid chromatograph was used to analyze individual carotenoid compounds through spectra and retention time. The samples were analyzed using Waters Millennium 3.2 software, a Waters 515 binary pump system (Waters 515), an autoinjector (Waters 717 plus), and a photodiode detector (Waters 996), along with a column heater (SpectraPhysics SP8792) maintained at 35 ^{o}C. A 4.6 x 250 mm, 5 μm, YMC Carotenoid Column (C-30 reverse phase) (Waters, Milford, MA) was used to separate the carotenoid compounds. The compounds analyzed and used to create a library included: 1) violaxanthin (CaroteNature, Lupsingen, Switzerland), 2) neoxanthin, 3) antheraxanthin (CaroteNature, Lupsingen, Switzerland), 4) β-cryptoxanthin (Hoffman La Roche, Basel, Switzerland), 5) canthaxanthin (Hoffman La Roche, Basel, Switzerland), 6) zeaxanthin (Hoffman La Roche, Basel, Switzerland), and 7) lutein (Hoffman La Roche, Basel, Switzerland). Two filtered and de-gassed solution solvents were used for carotenoid extraction: “solvent A” consisted of methanol, water, and triethylamine (90:10:0.1), while “solvent B” consisted of methanol, MTBE, and triethylamine (6:90:0.1). The gradient for the analysis was (min/ %A) 0/99, 8/99, 8/99, 45/0, 50/0, and 53/99 (Breithaupt and Barmedi, 2002; Hale, 2003).

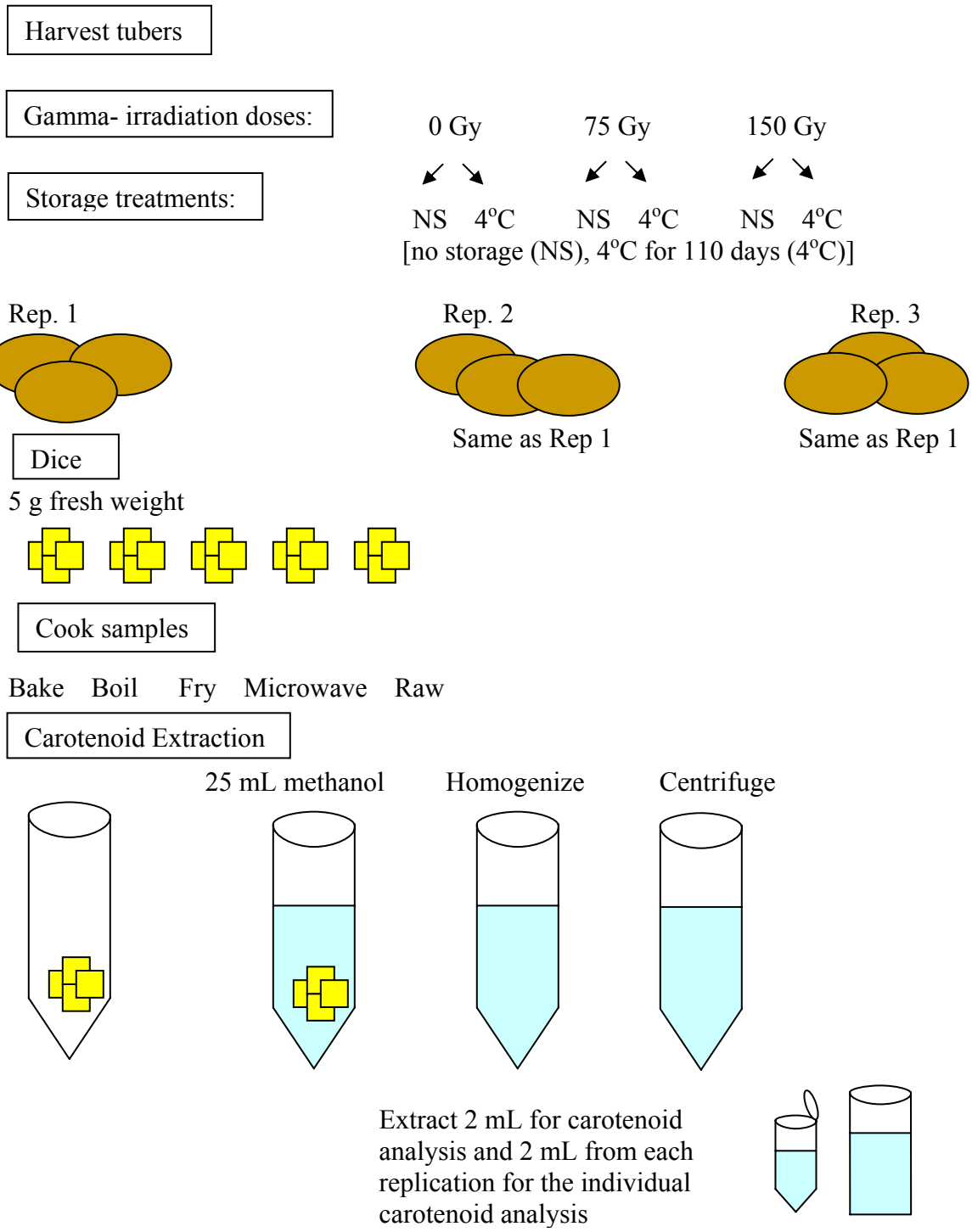

Fig. 5.1 Carotenoid extraction procedure for the factors cultivar, gamma- irradiation, storage, and cooking method.

EXTRACTION OF PHENOLICS AND TOTAL ANTIOXIDANT ACTIVITY. The same extraction procedure was conducted for total phenolic content, individual phenolic content, and total antioxidant content. Fifteen mL of methanol was added to a 5 g sample of diced potato. This mixture was then homogenized with an ultra turrax tissumizer from Tekmar (Cincinnati, Ohio). After homogenizing, samples were placed in a J-17 rotor at 17,000 rpm for 20 min in a refrigerated centrifuge (Beckman model J2-21). Two mL of the methanol extract were saved in snap-cap tubes for analysis of total antioxidant activity and total phenolic content. Selected cultivars (Innovator, Russet Burbank, and Santana) were chosen for individual phenolics analysis, and 6 mL of the methanol extracts were saved in glass vials (Fig. 5.2). The extracted samples were stored at -29 ^{o}C (-20 ^{o}F).

DPPH ASSAY FOR TOTAL ANTIOXIDANT ACTIVITY. Total antioxidant activity was analyzed using DPPH (1,1 Diphenyl-2 picrylhydrazyl), a colorimetric assay first described by Brand-Williams et al. (1995). DPPH is a stable radical which causes oxidation and can be reduced by natural antioxidants, reducing the oxidizing power of DPPH. While non-reduced DPPH is dark purple, reduction shifts the color from dark purple to lighter purple to light yellow. This decrease in color and reduction power can be measured at 515 nm. The reduction was correlated to absorbance, the lower the absorbance, the greater the antioxidant activity of the sample.

The DPPH solution was diluted by dissolving 24 mg DPPH in 100 mL methanol, which created a 607 μM DPPH stock solution. The solution was then diluted to ~10:55 with methanol until the spectrophotometer read 1.1 at 515 nm. The extracted methanol sample of 150 μL was combined with 2.85 mL of diluted DPPH in a scintillation vial, along with a blank which contained 150 μL of pure methanol (instead of methanol extract) with the diluted 2.85 mL DPPH. The samples reacted with each other for 15 min. After this time, the level of reduction was determined by absorption at 515 nm in a plastic UV-spectrophotometeric cuvette.

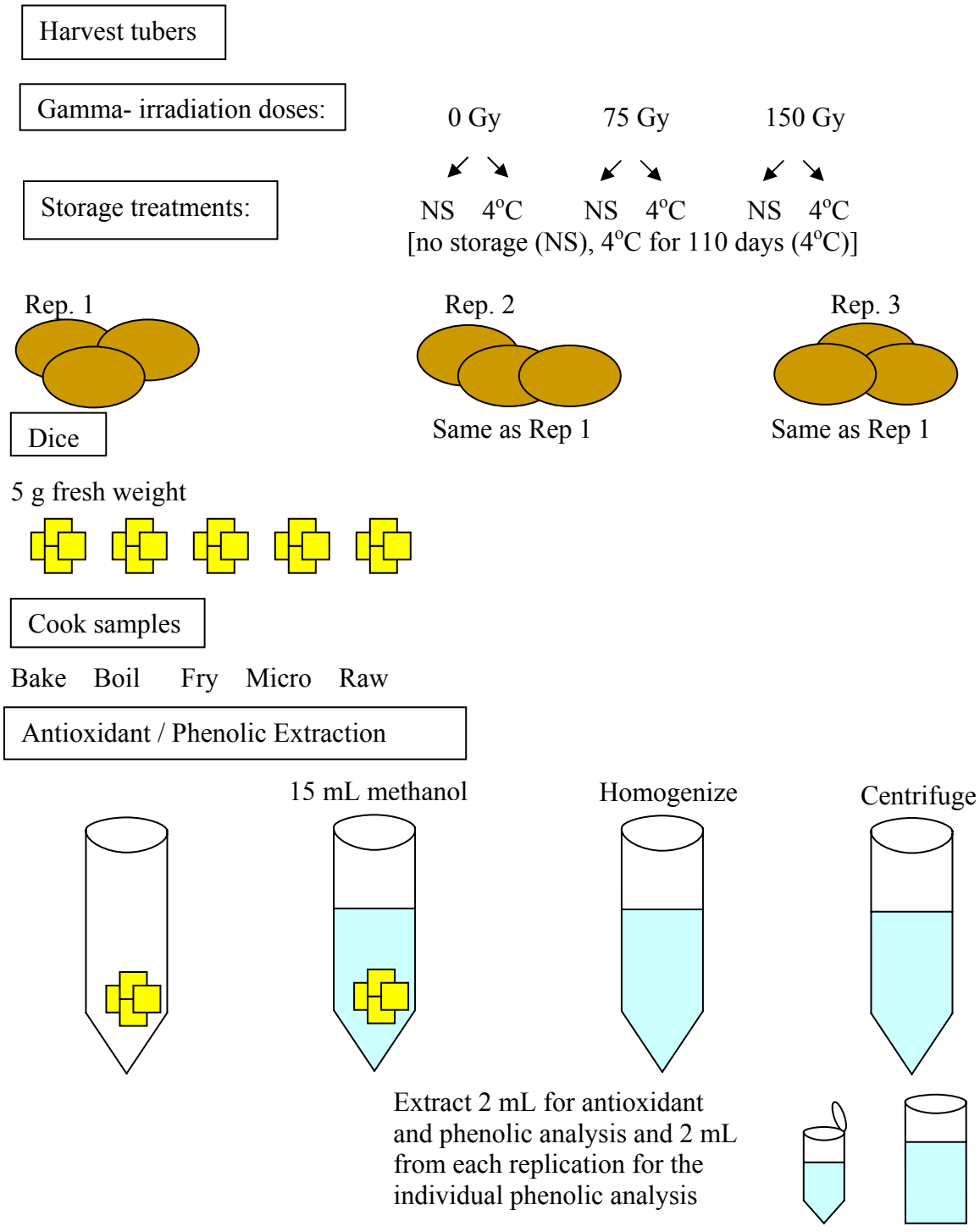

Fig. 5.2 Antioxidant/ phenolic extraction procedure for the factors cultivar, gamma-irradiation, storage, and cooking method.

This reading is based on the activity of the sample after 15 min. (initial antioxidant activity, AOAI), but the total reaction is a kinetic one, which continues for about 24 h until stabilization (stabilized antioxidant activity, AOAS). Each antioxidant compound reacts with the oxidizing substance at a given time. Therefore, two readings were taken. The second reading was taken after 24 h, when the samples and the DPPH had stabilized. The first reading (after 15 min) represents an initial response, whereas the second represents a final response. It is currently unknown how long consumed antioxidants are functional; therefore, these readings may represent two responses. Absorptions were subtracted from the blank. A standard curve using a known antioxidant, trolox (6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid) was prepared, and a regression curve was calculated to convert the antioxidant activity into trolox equivalents.

TOTAL PHENOLIC CONTENT. The Folin -Ciocalteau method to determine phenolic content was first described by Swain and Hillis (1959) and later modified by Singleton and Rossi (1965). This method, like the total antioxidant activity method, is a colorimetric reaction that is determined by absorbance. A 0.25 N Folin - Ciocalteau phenol reagent solution with nanopure water and a 1.0 N Na_2Co_3 solution with nanopure water was prepared. The extracted methanol sample of 150 μL was combined with 2.4 mL of nanopure water in a scintillation vial, along with a blank which contained 150 μL of pure methanol (instead of methanol extract) with 2.4 mL of nanopure water. The samples and blank reacted with 150 μL of the 0.25 N Folin - Ciocalteau phenol reagent solution for 3 min. Afterwards, 300 μL of the 1.0 N Na_2Co_3 solution were added to both the samples and blank. The reaction again was kinetic, and stabilization occurred after 1 h and 55 min. Data was taken at stabilization. Absorption was determined at 725 nm in plastic UV-spectrophotometeric cuvettes. The blank was read first, and the sample absorption was based on the cleared response of the blank. The phenolic content was determined by a prepared regression curve to chlorogenic acid equivalents.

HPLC ANALYSIS FOR INDIVIDUAL PHENOLIC COMPOUNDS. Selected cultivars (Innovator, Russet Burbank, and Santana) analyzed for individual phenolic compounds were chosen based on high quantifications on all tests. The extracted samples were concentrated under nitrogen gas or by using a heated speed vacuum centrifuge and filtered through a 0.45 μm syringe filter (Hale, 2003). A PC-operated Waters high performance liquid chromatograph was used to analyze individual phenolic compounds through spectra and retention time. The samples were analyzed using Waters Millennium 3.2 software, a Waters 515 binary pump system (Waters 515), an autoinjector (Waters 717 plus), and a photodiode detector (Waters 996), along with a column heater (SpectraPhysics SP8792) maintained at 40 ^{o}C (104 ^{o}F). A 4.6 x 150 mm, 5 μm, Atlantis C-18 reverse-phase column (Milford, MA) was used to separate phenolic compounds. The compounds analyzed included: 1) 5,7-Trihydroxyflavanone, 2) sinapic acid, 3) kampherol, 4) (-) epicatechin, 5) catechin, 6) quercetin dehydrate, 7) rutin hydrate, 8) protocatechuic acid, 9) salicylic acid, 10) myricetin, 11) syringic acid, 12) gallic acid, 13) vanillic acid, 14) t-cinnamic acid, 15) p-coumaric acid, 16) ferulic acid, 17) caffeic acid, and 18) chlorogenic acid; the standard compounds were obtained from Agros Organics (Pittsburgh, PA). Two filtered and de-gassed solution solvents were used for the phenolic extraction, "solvent A" consisted of acetonitrile, and "solvent B" consisted of nanopure water and HCL adjusted to pH 2.3. The following gradient was used, (min/%A) 0:85, 5:85, 30:0, 35:0 (Hale, 2003).

STATISTICAL ANALYSIS. The field plot was a completely randomized design, with tuber sample replications collected from three different blocks. A multiple analysis of variance (MANOVA) general linear model was used to determine significant factors. The statistical model of the experiment was a full factorial design. The dependent variables included total carotenoid content, total phenolic content, total antioxidant activity initially (AOAI) (measurements taken after 15 min), and total antioxidant activity stabilization (AOAS) (measurements taken after 24 h). The fixed factors included cultivar, cooking method, and storage treatment. Factor comparison

was conducted using the post hoc multiple comparison methods of S-N-K tests. A test to measure the estimate of magnitude of effect or strength of association was also conducted. This test determines how strongly two or more variables are related, or the magnitude of the difference between groups. The effect size is reported as eta squared values and is defined as the sums of squares of the effect of interest divided by the total sums of squares (Levine and Hullett, 2002). The analysis was conducted using the SPSS statistical package version 11.5.

Results

One of the undesirable effects of storage is weight loss. Weights of each cultivar and each treatment were measured before and after storage. Percentage weight loss was determined based on the original fresh weight of the tubers (Table 5.2). A cultivar with high weight loss lost a lot of water and became dehydrated. This loss of water may cause the antioxidant compounds to be more concentrated or may cause the antioxidant compounds to degrade because of a high amount of metabolic activity. Dehydration may also induce stress which has been linked to increased phenolic levels. Some thin-skined cultivars such as Santana, ATX85404-8W and NDTX4930-5W all had a weight loss that averaged over 6 % of the original weight, while other russet-skinned cultivars, such as Russet Burbank and Innovator had much lower weight loss percentages, averaging about 3 %. Irradiation dose did not appear to affect weight loss.

Table 5.2 Weight loss of the cultivars and irradiation doses over 110 days at 4°C, Dalhart 2003.

Cultivar	0 Gy	75 Gy	150 Gy	Average
Atlantic	3.4	3.3	3.9	3.5
ATX85404-8W	6.5	5.8	6.7	6.3
Innovator	2.6	2.9	3.2	2.9
Krantz	3.8	3.0	3.4	3.4
NDTX4930-5W	5.0	6.3	7.1	6.1
Russet Burbank	3.3	2.7	3.1	3.0
Santana	6.1	6.6	6.5	6.4
Shepody	3.8	3.8	3.9	3.8
Average	4.3	4.3	4.7	4.4

STANDARD CURVE FOR LUTEIN. The linear regression equation to equate the spectrophotometric absorbance readings of the methanol extract at 445 nm into lutein equivalents was the following: 3028.6x + 8.1063, where x was the absorbance at 445 nm and y was the µg lutein equivalents per hundred grams fresh weight. The R^2 value of this equation was 0.9991.

The average amount of xanthophylls (lutein equivalents) was 120 µg/100gfw. Analysis of variance (Table 5.3) indicated that there were significant differences in cultivar ($p < 0.000$), cooking method ($p < 0.000$), storage treatment ($p < 0.000$), irradiation dose ($p < 0.000$), the interaction of cultivar and cooking method ($p < 0.000$), the interaction of cultivar and storage treatment ($p < 0.000$), the interaction of cooking method and storage treatment ($p = 0.001$), the interaction of cultivar and irradiation dose ($p < 0.000$), the interaction of cooking method and irradiation dose ($p < 0.000$), the interaction of cultivar, storage treatment and irradiation dose ($p < 0.000$), and the interaction of cooking method, storage treatment and irradiation dose ($p < 0.000$). There were no significant differences for the interaction of storage treatment and irradiation dose ($p = 0.128$), the three-factor interaction of cultivar by cooking method by storage treatment ($p = 0.823$), the interaction of cultivar, cooking method and irradiation dose ($p = 0.311$), and the four-way interaction of cultivar, irradiation dose, storage treatment, and cooking method ($p = 0.421$) for the xanthophylls.

The eta squared values were the following: cultivar, 17 %; cooking method, 6 %; storage treatment, 12 %; irradiation dose, 17 %; the interaction of cultivar and cooking method, 3 %; the interaction of cultivar and storage treatment, 2 %; the interaction between cooking method and storage treatment, 1 %; the interaction of cultivar and irradiation dose, 5 %; the interaction of cooking method and irradiation dose, 4 %; the interaction of storage and irradiation dose, 0 %; the interaction of cultivar, cooking method and storage treatment, 1 %; the interaction of cultivar, cooking method and irradiation dose, 3 %; the interaction of cultivar, storage, and irradiation dose, 4 %; the interaction of cooking method, storage treatment, and

irradiation dose, 2 %; the interaction of cultivar, cooking method, storage treatment and irradiation dosage, 3 %; and error, 21 %.

Table 5.3 Analysis of variance results for carotenoid (xanthophyll) content for the factors gamma-irradiation, storage, cultivar, and cooking method, Dalhart 2003.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	1000401.722[z]	239	4185.781	7.515	.000
Intercept	10381882.061	1	10381882.061	18638.149	.000
Cultivar	214649.057	7	30664.151	55.050	.000
Cook	74908.728	4	18727.182	33.620	.000
Store	154329.284	1	154329.284	277.061	.000
Irrdose	221442.476	2	110721.238	198.773	.000
Cultivar * Cook	34355.759	28	1226.991	2.203	.000
Cultivar * Store	26059.326	7	3722.761	6.683	.000
Cook * Store	11084.293	4	2771.073	4.975	.001
Cultivar * Cook * Store	11671.441	28	416.837	.748	.823
Cultivar * Irrdose	59408.729	14	4243.481	7.618	.000
Cook * Irrdose	47141.454	8	5892.682	10.579	.000
Cultivar * Cook * Irrdose	34023.580	56	607.564	1.091	.311
Store * Irrdose	2297.991	2	1148.996	2.063	.128
Cultivar * Store * Irrdose	55725.101	14	3980.364	7.146	.000
Cook * Store * Irrdose	21183.007	8	2647.876	4.754	.000
Cultivar * Cook * Store * Irrdose	32121.496	56	573.598	1.030	.421
Error	267371.148	480	557.023		
Total	11649654.931	720			
Corrected Total	1267772.870	719			

[z] $R^2 = .789$ (Adjusted $R^2 = .684$)

The analysis of variance determined that there were significant differences among cultivars. 'Santana' had the highest xanthophyll content at 141 µg/100gfw, while Atlantic had the lowest at 93 µg/100gfw, a range of 48 µg/100gfw (Table 5.4). This data is comparable to that from past studies. Previous cooking and storage studies in Dalhart 2003 (Chapter IV) and McCook 2003 (Chapter III) both included Santana and Innovator among the top three cultivars, while the cultivar Atlantic remained at the low end in xanthophyll content.

Table 5.4 Cultivar ranking for carotenoid (xanthophyll) content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar	Eq. Lutein (µg/100gfw)	
Santana	141	a[z]
Innovator	140	a
Russet Burbank	140	a
Krantz	118	b
ATX85404-8W	114	b
NDTX4930-5W	110	bc
Shepody	105	c
Atlantic	93	d

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The ANOVA results indicated significant differences among cooking methods (Table 5.5). In this study, the raw samples ranked highest at 139 µg/100gfw, while the boiled sample ranked lowest at 112 µg/100gfw, resulting in a range of 27 µg/100gfw. Raw samples from the Dalhart 2003 (Chapter IV) and the McCook 2003 (Chapter III) studies also ranked in the first level of significance, while the boiled samples were the lowest.

Table 5.5 Cooking method ranking for carotenoid (xanthophyll) content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cooking method	Eq. Lutein (µg/100gfw)	
Raw	139	a[z]
Bake	123	b
Fry	114	c
Micro	112	c
Boil	112	c

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Significant differences were found between the two storage treatments (Table 5.6). As with past experiments, the storage treatment was higher in xanthophyll content than no storage. Storage had a large effect on carotenoid content, as supported by an eta squared of 12 %, which is almost as large as the main factor of cultivar at 17 %. The range for xanthophyll content was 30 µg/100gfw.

Table 5.6 Storage method ranking for (xanthophyll) carotenoid content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Storage treatment	Eq. Lutein (µg/100gfw)	
4 °C	135	a[z]
No Storage	105	b

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

There were significant differences among irradiation doses; based on the S-N-K post hoc test (Table 5.7). Over all, the higher irradiation doses of 75 and 150 Gy produced greater amounts of carotenoids than no irradiation. The 75 Gy dosage resulted in the highest xanthophyll contents at 137 µg/100gfw, while the lowest was the 0 Gy at 96 µg/100gfw, creating a range of 41 µg/100gfw. Irradiation dose had a very high eta squared value of 17 %, the same magnitude of strength as cultivar.

Table 5.7 Irradiation dosage ranking for (xanthophyll) carotenoid content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Irradiation dose	Eq. Lutein (µg/100gfw)	
75 Gy	137	a[z]
150 Gy	128	b
0 Gy	96	c

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The interaction of cultivar and cooking method was significant in determining xanthophyll content. The interactions with the highest xanthophylls in equivalents of lutein (µg/100gfw) were 'Santana', raw (166); 'Russet Burbank', bake (163); 'Innovator', raw (154); 'Innovator', microwave (151); and 'Krantz', raw (147). The interactions with the lowest xanthophyll content were 'Atlantic', fry (86); 'Atlantic', microwave (87); 'Atlantic', boil (91); 'Atlantic', bake (95); and 'NDTX4930-5W', microwave (96) (Table 5.8). The range of this interaction was 80 µg/100gfw.

Table 5.8 Cultivar by cooking method interaction for (xanthophyll) carotenoid content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar and cooking method	Eq. Lutein (µg/100gfw)
Atlantic	
Bake	95
Boil	91
Fry	86
Micro	87
Raw	105
ATX85404-8W	
Bake	116
Boil	111
Fry	107
Micro	107
Raw	128
Innovator	
Bake	144
Boil	119
Fry	135
Micro	151
Raw	154
Krantz	
Bake	112
Boil	108
Fry	116
Micro	106
Raw	147
NDTX4930-5W	
Bake	110
Boil	105
Fry	99
Micro	96
Raw	140
Russet Burbank	
Bake	163
Boil	135
Fry	135
Micro	121
Raw	146
Santana	
Bake	144
Boil	129
Fry	137
Micro	131
Raw	166
Shepody	
Bake	101
Boil	99
Fry	98
Micro	99
Raw	126

This interaction between cultivar and storage treatment did not have the same magnitude as in the storage study from Dalhart 2003 (Chapter IV), but was still a significant factor (Table 5.9). The highest interactions in equivalents of lutein (µg/100gfw) were 'Innovator', 4 °C (163); 'Russet Burbank', 4 °C (152); 'Santana', 4 °C (148); 'Krantz', 4 °C (142); and 'Santana', no storage (135). The lowest interactions were 'Atlantic', no storage (75); 'Krantz', no storage (93); 'Shepody', no storage (95); 'NDTX4930-5W', no storage (97); and 'ATX85404-8W', no storage (102). The range for this interaction was 88 µg/100gfw. Most of the highest interactions included a storage treatment, while the five lowest did not include any storage treatment. The addition of storage appeared to have a synergistic effect on cultivars that are already ranked high.

Table 5.9 Cultivar by storage treatment interaction for carotenoid (xanthophyll) content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar and storage treatment	Eq. Lutein (µg/100gfw)
Atlantic	
No Storage	75
4 °C	111
ATX85404-8W	
No Storage	102
4 °C	126
Innovator	
No Storage	118
4 °C	163
Krantz	
No Storage	93
4 °C	142
NDTX4930-5W	
No Storage	97
4 °C	123
Russet Burbank	
No Storage	128
4 °C	152
Santana	
No Storage	135
4 °C	148

Table 5.9 (continued).

Cultivar and storage treatment	Eq. Lutein (µg/100gfw)
Shepody	
No Storage	95
4 °C	114

Although the interaction between cooking method and storage treatment (Table 5.10) had a very small magnitude of strength (eta squared is 1 %), the interaction was still significant ($p = 0.001$). The top three interactions in equivalents of lutein (µg/100gfw) were raw, 4 °C storage (152); bake, 4 °C storage (138); and fry, 4 °C storage (134). The lowest interactions were fry, no storage (94); microwave, no storage (96); and boil, no storage (104). The over all range for this interaction was 58 µg/100gfw. Again, a general trend can be seen where the storage treatment had a positive synergistic effect on the cooking methods that retained or produced the most carotenoids.

Table 5.10 Cooking method by storage treatment interaction for carotenoid (xanthophyll) content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cooking method and storage treatment	Eq. Lutein (µg/100gfw)
Bake	
No Storage	108
4 °C	138
Boil	
No Storage	104
4 °C	120
Fry	
No Storage	94
4 °C	134
Micro	
No Storage	96
4 °C	129
Raw	
No Storage	126
4 °C	152

The interaction of cultivar and irradiation treatment had the largest magnitude of strength (eta squared 5 %) as compared to all other interactions, and is a significant factor (Table 5.11). The irradiated samples appeared to rank higher than the non-irradiated samples, but the differences between the 75 Gy and 150 Gy were less striking. This is where the significance of the interaction is seen. In some cultivars, the 75 Gy resulted in higher xanthophylls, while in other cultivars, the 150 Gy had higher xanthophyll content. This may be due to the small difference in dosage, or possibly a physiological reason why certain cultivars perform better at lower or higher dosages. The top five interactions in equivalents of lutein (µg/100gfw) were 'Santana', 75 Gy (169); 'Russet Burbank', 75 Gy (155); 'Innovator', 75 Gy and 150 Gy, and 'Russet Burbank', 150 Gy all at 146. The lowest five interactions were 'Atlantic', 0 Gy (69); 'NDTX4930-5W', 0 Gy (69); 'ATX85404-8W', 0 Gy (80); 'Shepody', 0 Gy (88); and 'Krantz', 0 Gy (94). The range for this interaction was 100 µg/100gfw.

Table 5.11 Cultivar by irradiation dose interaction for carotenoid (xanthophyll) content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar and irradiation dose	Eq. Lutein (µg/100gfw)
Atlantic	
0 Gy	69
75 Gy	107
150 Gy	103
ATX85404-8W	
0 Gy	80
75 Gy	120
150 Gy	141
Innovator	
0 Gy	129
75 Gy	146
150 Gy	146
Krantz	
0 Gy	94
75 Gy	134
150 Gy	124
NDTX4930-5W	
0 Gy	69
75 Gy	133
150 Gy	128

Table 5.11 (continued).

Cultivar and irradiation dose	Eq. Lutein (µg/100gfw)
Russet Burbank	
0 Gy	118
75 Gy	155
150 Gy	146
Santana	
0 Gy	119
75 Gy	169
150 Gy	136
Shepody	
0 Gy	88
75 Gy	129
150 Gy	97

The interaction between cooking method and irradiation treatment had a magnitude of strength, eta squared value of 4 % and was a significant factor (Table 5.12). In all of the cooking methods, the effect of 75 Gy is greater than 150 Gy, and all 150 Gy treatments were greater than the 0 Gy. The irradiation treatment seemed to synergistically increase carotenoid content when combined with cooking methods. The highest interactions in equivalents of lutein (µg/100gfw) were raw, 75 Gy (164); raw, 150 Gy (152); bake, 75 Gy (140); boil, 75 Gy (134); and bake, 150 Gy (133). The lowest interactions were boil, 0 Gy (79); bake, 0 Gy (96); microwave, 0 Gy, (97); raw, 0 Gy (100); and fry, 0 Gy (107). The range for this interaction was 85 µg/100gfw.

Table 5.12 Cooking method by irradiation dose interaction for (xanthophyll) carotenoid content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cooking method and irradiation dose	Eq. Lutein (µg/100gfw)
Bake	
0 Gy	96
75 Gy	140
150 Gy	133
Boil	
0 Gy	79
75 Gy	134
150 Gy	124
Fry	
0 Gy	107
75 Gy	122
150 Gy	114

Table 5.12 (continued)

Cooking method and irradiation dose	Eq. Lutein (µg/100gfw)
Micro	
0 Gy	97
75 Gy	125
150 Gy	116
Raw	
0 Gy	100
75 Gy	164
150 Gy	152

The two-factor interaction of storage method and irradiation dose was not significant, while the two three-factor interactions were significant: the interaction of cultivar, storage treatment, and irradiation dose; and the interaction of cooking method, storage treatment, and irradiation dose.

The interaction of cultivar, storage method, and irradiation dosage is shown in Table 5.13, and some of the synergistic increases and decreases in carotenoid content per cultivar based on storage and irradiation treatment can be seen. Certain cultivars (Innovator, Santana, Russet Burbank) consistently ranked higher than others (Atlantic, Shepody, and NDTX4930-5W). The storage treatment usually ranked higher than no storage, and 75 and 150 Gy dosage ranked higher than 0 Gy. The physical appearance of the stored, irradiated potatoes as compared to the stored, non-irradiated potatoes was strikingly different. As predicted, the irradiated potatoes appeared fresh, were plump, and the sprouts were dormant. The non-irradiated potatoes were shriveled, sprouted and did not look as fresh when removed from storage. The added stress of sprouting and dehydration on the non-irradiated potatoes could have caused a possible increase in carotenoids, but this was not found. The stored, irradiated, potatoes contained a larger amount of carotenoids as compared to the non-irradiated, stored potatoes. The five highest interactions in equivalents of lutein (µg/100gfw) were 'Santana', 4 ^{o}C, 75 Gy (192); 'ATX85404-8W', 4 ^{o}C, 150 Gy (174); 'Innovator', 4 ^{o}C, 75 Gy (169); 'Russet Burbank', 4 ^{o}C, 75 Gy (164); and 'Innovator', 4 ^{o}C, 150 Gy (161). The five lowest interactions were 'NDTX4930-5W', no storage, 0 Gy (46); 'Atlantic', no storage, 0 Gy

(52); ‘Krantz’, no storage, 0 Gy (62); ‘Atlantic’, no storage, 0 Gy (79); and ‘Shepody’, no storage, 0 Gy (79). The range for this interaction was 146 µg/100gfw.

Table 5.13 Cultivar by storage treatment by irradiation dose interaction for carotenoid (xanthophyll) content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar	Storage treatment	Irradiation dose	Eq. Lutein (µg/100gfw)
Atlantic			
	No Storage	0 Gy	52
		75 Gy	79
		150 Gy	94
	4 ºC	0 Gy	86
		75 Gy	135
		150 Gy	112
ATX85404-8W			
	No Storage	0 Gy	80
		75 Gy	118
		150 Gy	108
	4 ºC	0 Gy	81
		75 Gy	122
		150 Gy	174
Innovator			
	No Storage	0 Gy	99
		75 Gy	124
		150 Gy	132
	4 ºC	0 Gy	159
		75 Gy	169
		150 Gy	161
Krantz			
	No Storage	0 Gy	62
		75 Gy	122
		150 Gy	95
	4 ºC	0 Gy	128
		75 Gy	146
		150 Gy	153
NDTX4930-5W			
	No Storage	0 Gy	46
		75 Gy	125
		150 Gy	121
	4 ºC	0 Gy	91
		75 Gy	141
		150 Gy	135
Russet Burbank			
	No Storage	0 Gy	97
		75 Gy	146
		150 Gy	141

Table 5.13 (continued).

Cultivar	Storage treatment	Irradiation dose	Eq. Lutein (µg/100gfw)
	4 °C	0 Gy	140
		75 Gy	164
		150 Gy	151
Santana			
	No Storage	0 Gy	118
		75 Gy	147
		150 Gy	140
	4 °C	0 Gy	119
		75 Gy	192
		150 Gy	132
Shepody			
	No Storage	0 Gy	79
		75 Gy	119
		150 Gy	89
	4 °C	0 Gy	98
		75 Gy	140
		150 Gy	104

The other significant interaction for the carotenoids was between cooking method, storage treatment, and irradiation dosage (Table 5.14). In this interaction, certain trends are noticeable, but significant interactions suggested that not all carotenoid content in cooking methods and storage methods were increased by a similar amount with the added irradiation treatment. Again, with some cooking and storage methods, the 75 Gy produced a larger carotenoid level, while with other cooking methods and storage methods, the 150 Gy produces a larger carotenoid level. The five highest ranking interactions in equivalents of lutein (µg/100gfw) were raw, 4 °C 75 Gy (178); raw, 4 °C, 150 Gy (161); bake, 4 °C, 150 Gy (157); raw, no storage, 75 Gy (150); and bake, 4 °C, 75 Gy (149). The five lowest ranking interactions were boil, no storage, 0 Gy (65); fry, no storage, 0 Gy (80); bake, no storage, 0 Gy (83); raw, no storage, 0 Gy (83); and microwave, no storage, 0 Gy (84). The range for this interaction was 113 µg/100gfw.

Table 5.14 Cooking method by storage treatment by irradiation dose interaction for carotenoid (xanthophyll) content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cooking method	Storage treatment	Irradiation dose	Eq. Lutein (µg/100gfw)
Bake			
	No Storage	0 Gy	83
		75 Gy	131
		150 Gy	109
	4 °C	0 Gy	109
		75 Gy	149
		150 Gy	157
Boil			
	No Storage	0 Gy	65
		75 Gy	120
		150 Gy	128
	4 °C	0 Gy	94
		75 Gy	148
		150 Gy	119
Fry			
	No Storage	0 Gy	80
		75 Gy	104
		150 Gy	98
	4 °C	0 Gy	133
		75 Gy	139
		150 Gy	129
Micro			
	No Storage	0 Gy	84
		75 Gy	106
		150 Gy	97
	4 °C	0 Gy	110
		75 Gy	143
		150 Gy	134
Raw			
	No Storage	0 Gy	83
		75 Gy	150
		150 Gy	144
	4 °C	0 Gy	118
		75 Gy	178
		150 Gy	161

DPPH ASSAY FOR TOTAL ANTIOXIDANT ACTIVITY - STANDARD CURVE FOR TROLOX. The linear regression equation to convert the spectrophotometric absorbance readings of the methanol extract and reduced DPPH at 515 nm into trolox equivalents was the following: $y = 891.69x$, where x is the absorbance at 515 nm and y was the µg trolox equivalents per gram fresh weight. The R^2 value of this equation was 0.997.

The average antioxidant activity reported as trolox equivalents was 123 µg/gfw for AOAI and 312 µg/gfw for AOAS. The analysis of variance test for AOAI indicated that there were significant differences in cultivar ($p < 0.000$), cooking method ($p < 0.000$), storage method ($p = 0.035$), irradiation dose ($p < 0.000$), the interactions of cultivar and cooking method ($p = 0.012$), cultivar and storage treatment ($p < 0.000$), cultivar and irradiation dose ($p < 0.000$), cooking method and irradiation dose ($p = 0.044$), storage and irradiation dose ($p < 0.000$), and cultivar, storage treatment, and irradiation dose ($p < 0.000$). There were no significant differences in the interactions of cooking method and storage treatment ($p = 0.241$), cultivar, cooking method, and storage treatment ($p = 0.324$), cultivar, cooking method, and irradiation dosage ($p = 0.377$), cooking method, storage treatment, and irradiation dosage ($p = 0.608$), and cultivar, cooking method, storage treatment, and irradiation dosage ($p = 0.160$) (Table 5.15). The factor's magnitude of strength (eta squared values) were cultivar, 8 %; cooking method, 19 %; storage treatment, 0 %; irradiation dose, 2 %; the interactions of cultivar and cooking method, 3 %; cultivar and storage treatment, 2 %; cooking method and storage treatment, 0 %; cultivar, cooking method, and storage treatment, 2 %; cultivar and irradiation dose, 9 %; cooking method and irradiation dose, 1 %; cultivar, cooking method, and irradiation dose, 4 %; storage treatment and irradiation dose, 3 %; cultivar, storage treatment and irradiation dose, 9 %; cooking method, storage treatment and irradiation dose, 0 %; cultivar, cooking method, storage treatment, and irradiation dose, 4 %; and error, 32 %.

The analysis of variance for AOAS indicated that there were significant differences in cultivar ($p < 0.000$), cooking method ($p < 0.000$), irradiation dose ($p < 0.000$), the interactions of cultivar and cooking method ($p < 0.000$), cultivar and storage ($p < 0.000$), cooking method and storage treatment ($p = 0.049$), cultivar and irradiation dose ($p < 0.000$), cooking method and irradiation dose ($p = 0.011$), storage treatment and irradiation dose ($p < 0.000$), and cooking method, storage treatment, and irradiation dose ($p < 0.000$). There were no significant differences in storage ($p = 0.627$), the interactions of cultivar, cooking method, and storage treatment ($p = 0.492$),

cultivar, cooking method, and irradiation dose (p = 0.991), cooking method, storage treatment, and irradiation dose (p = 0.450), and cultivar, cooking method, storage treatment, and irradiation dose (p = 0.761) (Table 5.15). The factor's magnitude of strength (eta squared values) were cultivar, 10 %; cooking method, 23 %; storage treatment, 0 %; irradiation dose, 1 %; the interactions of cultivar and cooking method, 4 %; cultivar and storage treatment, 5 %; cooking method and storage treatment, 1 %; cultivar, cooking method, and storage treatment, 8 %; cultivar and irradiation dose, 11 %; cooking method and irradiation dose, 1 %; cultivar, cooking method, and irradiation dose, 2 %; storage treatment and irradiation dose, 2 %; cultivar, storage treatment and irradiation dose, 9 %; cooking method, storage treatment, and irradiation dose, 0 %; cultivar, cooking method, storage treatment, and irradiation dose, 3 %; and error 27 %.

Table 5.15 Analysis of variance for antioxidant activity for the factors gamma- irradiation, storage, cultivar, and cooking method, Dalhart 2003.

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	AOAI	3145005.710 [z]	240	13104.190	4.264	.000
	AOAS	9706359.585 [y]	240	40443.165	5.324	.000
Intercept	AOAI	2782204.497	1	2782204.497	905.351	.000
	AOAS	17024084.304	1	17024084.304	2240.963	.000
Cultivar	AOAI	346805.003	7	49543.572	16.122	.000
	AOAS	1286148.458	7	183735.494	24.186	.000
Cook	AOAI	864617.942	4	216154.486	70.338	.000
	AOAS	3112917.436	4	778229.359	102.442	.000
Store	AOAI	13713.875	1	13713.875	4.463	.035
	AOAS	1791.478	1	1791.478	.236	.627
Irrdose	AOAI	88149.358	3	29383.119	9.562	.000
	AOAS	174751.426	3	58250.475	7.668	.000
Cultivar * Cook	AOAI	149069.515	28	5323.911	1.732	.012
	AOAS	487165.016	28	17398.751	2.290	.000
Cultivar * Store	AOAI	83101.811	7	11871.687	3.863	.000
	AOAS	605405.098	7	86486.443	11.385	.000
Cook * Store	AOAI	16912.770	4	4228.193	1.376	.241
	AOAS	72877.445	4	18219.361	2.398	.049
Cultivar * Cook * Store	AOAI	95252.788	28	3401.885	1.107	.324
	AOAS	209183.945	28	7470.855	.983	.492

Table 5.15 (continued).

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Cultivar * Irrdose	AOAI	433002.748	14	30928.768	10.064	.000
	AOAS	1448892.392	14	103492.314	13.623	.000
Cook * Irrdose	AOAI	49295.454	8	6161.932	2.005	.044
	AOAS	152382.109	8	19047.764	2.507	.011
Cultivar * Cook * Irrdose	AOAI	181224.291	56	3236.148	1.053	.377
	AOAS	254592.162	56	4546.289	.598	.991
Store * Irrdose	AOAI	159881.205	2	79940.602	26.013	.000
	AOAS	269430.808	2	134715.404	17.733	.000
Cultivar * Store * Irrdose	AOAI	429678.500	14	30691.321	9.987	.000
	AOAS	1190883.272	14	85063.091	11.197	.000
Cook * Store * Irrdose	AOAI	19523.732	8	2440.466	.794	.608
	AOAS	59599.568	8	7449.946	.981	.450
Cultivar * Cook * Store * Irrdose	AOAI	206897.474	56	3694.598	1.202	.160
	AOAS	363994.078	56	6499.894	.856	.761
Error	AOAI	1471998.529	479	3073.066		
	AOAS	3638853.742	479	7596.772		
Total	AOAI	15482105.317	720			
	AOAS	83513809.616	720			
Corrected Total	AOAI	4617004.239	719			
	AOAS	13345213.326	719			

[z] R^2 = .681 (Adjusted R^2 = .521)
[y] R^2 = .727 (Adjusted R^2 = .591)

The analysis of variance results suggested significant difference for cultivar. Table 5.16 reports the mean separation and ranking of cultivars. The ranking for AOAI was slightly different as compared to past experiments. The cultivar Atlantic was in the highest bracket in the AOAI study. The AOAS ranking was similar to that from the McCook 2003 harvest (Chapter III) and the Dalhart 2003 (Chapter IV) storage study. This may be due to the numerous postharvest treatments which caused changes in levels of the antioxidant compounds. These changes may have been due to the kinetics of the reduction of DPPH by the antioxidants. The range for AOAI was 72 µg/gfw; Russet Burbank was the highest ranking cultivar, with 158 µg/gfw, and NDTX4930-5W was the lowest, with 90 µg/gfw equivalents of trolox. The range for AOAS was

139 µg/gfw, with Russet Burbank again the highest cultivar at 384 µg/gfw and NDTX4930-5W was again the lowest at 245 µg/gfw equivalents of trolox. The two tests ranking AOAI and AOAS were similar, with minor ranking changes in certain cultivars, e.g. Atlantic, Shepody and ATX85404-8W.

Table 5.16 Cultivar ranking for antioxidant activity, Dalhart 2003 study involving the factors gamma-irradiation, storage, cultivar, and cooking method.

Cultivar	AOAI[z] (µg/gfw)		Cultivar	AOAS[y] (µg/gfw)	
Russet Burbank	158	a[x]	Russet Burbank	384	a
Atlantic	140	ab	Krantz	351	b
Krantz	140	ab	Santana	335	b
Santana	136	b	Atlantic	325	b
Innovator	109	c	Innovator	297	c
Shepody	107	c	ATX85404-8W	290	c
ATX85404-8W	104	c	Shepody	270	cd
NDTX4930-5W	90	c	NDTX4930-5W	245	d

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[x] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Cooking methods were significantly different, and magnitude of strength (eta squared) indicated that cooking method was the strongest factor in this study. In both AOAI and AOAS, the cooking treatments were ranked the same (Table 5.17). This ranking was also similar to two past studies, McCook 2003 (Chapter III) and Dalhart 2003 (Chapter IV). The microwaved samples in both tests had the highest antioxidant activity, with 163 µg/gfw for AOAI and 392 µg/gfw equivalents of trolox for AOAS. The raw samples had the lowest antioxidant activity in both tests and all antioxidant tests in the past two studies. The ranges were 85 and 162 µg/gfw for AOAI and AOAS, respectively. Texture changes in starch of the cooked samples may have helped release some of the bound antioxidants and/ or chemical changes, such as Maillard reactions, may have produced an increase in antioxidants in cooked samples.

Table 5.17 Cooking method ranking for antioxidant activity, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cooking method	AOAI[z] (µg/gfw)		Cooking method	AOAS[y] (µg/gfw)	
Micro	163	a[x]	Micro	392	a
Fry	152	a	Fry	363	b
Bake	135	b	Bake	336	c
Boil	86	c	Boil	240	d
Raw	78	c	Raw	230	d

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[x] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Storage was not a significant factor for AOAS, but it was significant for AOAI. Storage as a main factor has a very small magnitude of strength in both antioxidant activity tests, as eta squared values were 0 % for both. Both rankings indicated that the no storage treatment was higher in antioxidant activity than the storage treatment of 4°C for 110 days, with ranges of 9 µg/gfw for AOAI and 3 µg/gfw for AOAS (Table 5.18). Although the main effect of storage was not significant, the two-way interactions of cultivar and storage treatment, cooking method and storage treatment for AOAS, irradiation dose and storage treatment, and the three-factor interaction of cultivar, storage treatment, and irradiation dose were all significant. Therefore, the storage treatment had a larger effect on antioxidant activity when combined with other factors.

Table 5.18 Storage treatment ranking for antioxidant activity, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Storage treatment	AOAI[z] (µg/gfw)		Storage treatment	AOAS[y] (µg/gfw)	
No Storage	127	a[x]	No Storage	314	a
4 °C	118	b	4 °C	311	a

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[x] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Irradiation dose was a significant factor (Table 5.19). In both antioxidant activity tests, the 0 and 150 Gy were significantly greater than the 75 Gy. The highest ranking dose for AOAI was 0 Gy with 135µg/gfw and the lowest was 75 Gy at 109 µg/gfw, a range of 26 µg/gfw. The highest ranking dose for AOAS was 0 Gy with 330 µg/gfw, and the lowest was 75 Gy at 293 µg/gfw, a range of 37 µg/gfw. The storage effect may be better described by examination of some of the interactions, especially the interaction of cultivar, storage treatment and irradiation dose. A trend is seen with that interaction, where storage increases the antioxidant activity of the 0 Gy samples, but caused a decrease in antioxidant activity in irradiated samples. Consequently, the 0 Gy dose was highest in the main-effect, because the higher irradiation dosages were averaged with lower values from storage.

Table 5.19 Irradiation dose ranking for antioxidant activity, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Irradiation dose	AOAI[z] (µg/gfw)		Irradiation dose	AOAS[y] (µg/gfw)	
0 Gy	135	a[x]	0 Gy	330	a
150 Gy	125	a	150 Gy	314	a
75 Gy	109	b	75 Gy	293	b

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[x] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The interaction of cultivar and cooking treatment is presented in Table 5.20. Both main effects, cultivar and cooking method, were significant. The highest interactions for AOAI (eq. trolox µg/gfw) were 'Russet Burbank', microwave (239); 'Russet Burbank', fry (204); 'Krantz', microwave (198); 'Atlantic', fry (170); and 'Santana', microwave (170). The lowest interactions for AOAI (eq. trolox µg/gfw) were 'NDTX4930-5W', raw (48); 'NDTX4930-5W', boil (55); 'Innovator', raw (57); 'Shepody', boil (61); and 'Innovator', boil (64). The range for AOAI was 191 µg/gfw. The highest interactions for AOAS (eq. trolox µg/gfw) were 'Russet Burbank', microwave (548); 'Krantz', microwave (467); 'Russet Burbank', fry (452); 'Santana',

microwave (404); and 'Krantz', bake (402). The lowest interactions for AOAS (eq. trolox µg/gfw) were 'NDTX4930-5W', raw (183); 'NDTX4930-5W', boil (185); 'Shepody', boil (186); 'Shepody', raw (196); and 'Innovator', raw (201). The range for AOAS was 365 µg/gfw. Cultivars that ranked high in the main-effect of cultivar ranked high in this interaction. The cooking methods that ranked high in the main-effect of cooking also ranked high in this interaction as well. This interaction was significant because the cooking methods did not consistently affect all cultivars similarly.

Table 5.20 Cultivar by cooking method interaction for antioxidant activity, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar and cooking method	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Atlantic		
Bake	143	314
Boil	126	289
Fry	170	376
Micro	158	378
Raw	104	270
ATX85404-8W		
Bake	118	309
Boil	75	237
Fry	134	351
Micro	125	324
Raw	66	230
Innovator		
Bake	140	354
Boil	64	209
Fry	133	343
Micro	154	379
Raw	57	201
Krantz		
Bake	164	402
Boil	82	234
Fry	164	384
Micro	198	467
Raw	89	268
NDTX4930-5W		
Bake	97	261
Boil	55	185
Fry	123	293
Micro	126	304
Raw	48	183

Table 5.20 (continued).

Cultivar and cooking method	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Russet Burbank		
Bake	148	371
Boil	114	293
Fry	204	452
Micro	239	548
Raw	84	253
Santana		
Bake	150	366
Boil	112	287
Fry	168	382
Micro	170	404
Raw	79	236
Shepody		
Bake	120	313
Boil	61	186
Fry	124	320
Micro	133	334
Raw	96	196

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox

The interaction of cultivar and storage method is shown in Table 5.21. The no storage treatment resulted in higher antioxidant activity than the storage treatment of 4^{o}C for 110 days, with minor exceptions such as with the cultivars Krantz and Russet Burbank for AOAS. It is suggested that the main effect storage treatment is lower in Table 5.18 due to the decrease in antioxidant activity in irradiated, stored potatoes. The irradiated, stored tubers did not experience the added stress of shriveling and sprouting, which might account for lower values when compared to non-irradiated stored tubers. The non-irradiated, stored tubers experienced a concentration effect of their solids due to dehydration, and the additional stress may have caused an induction of phenolics. The highest interactions for AOAI (eq. trolox µg/gfw) were 'Russet Burbank', no storage (161); 'Krantz', 4 ^{o}C (159); 'Santana', no storage (157); 'Russet Burbank', 4 ^{o}C (154); and 'Atlantic', no storage (144). The lowest interactions for AOAI (eq. trolox µg/gfw) were 'NDTX4930-5W', 4 ^{o}C (81); 'NDTX4930-5W', no storage (99); 'Shepody', 4 ^{o}C (100); 'Innovator', 4 ^{o}C (100); and 'ATX85404-8W', 4 ^{o}C (102). The range for AOAI was 80 µg/gfw. The highest interactions for AOAS (eq.

trolox µg/gfw) were 'Russet Burbank', 4 °C (400); 'Krantz', 4 °C (395); 'Santana', no storage (368); 'Russet Burbank', no storage (368); and 'Atlantic', 4 °C (361). The lowest interactions for AOAS (eq. trolox µg/gfw) were 'NDTX4930-5W', 4 °C (230); 'Shepody', 4 °C (246); 'NDTX4930-5W', no storage (260); 'ATX85404-8W', no storage (269); and 'Innovator', 4 °C (279). The range for AOAS was 170 µg/gfw.

Table 5.21 Cultivar by storage treatment interaction for antioxidant activity, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar and storage treatment	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Atlantic		
No Storage	144	290
4 °C	136	361
ATX85404-8W		
No Storage	105	269
4 °C	102	312
Innovator		
No Storage	119	315
4 °C	100	279
Krantz		
No Storage	120	307
4 °C	159	395
NDTX4930-5W		
No Storage	99	260
4 °C	81	230
Russet Burbank		
No Storage	161	368
4 °C	154	400
Santana		
No Storage	157	376
4 °C	115	293
Shepody		
No Storage	114	293
4 °C	100	246

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox

The interaction of cooking method and storage treatment was significant for AOAS, but not for AOAI. The magnitude of strength was small for both tests of antioxidant activity. The no storage treatment tended to produce higher antioxidant activity than the storage treatment, with the exception of AOAS fry samples and both

AOAI and AOAS raw samples. Clearly, the raw and boil treatments resulted in the lowest antioxidant activity (Table 5.22). The top three interactions for AOAI (eq. trolox µg/gfw) were microwave, no storage (172); microwave, 4 ^{o}C (154); and fry, no storage (153). The four lowest interactions for AOAI (eq. trolox µg/gfw) were raw, no storage (74); boil, 4 ^{o}C (78); raw, 4 ^{o}C (81); and boil, no storage (94). The range for AOAI was 98 µg/gfw. The highest three interaction for AOAS (eq. trolox µg/gfw) were microwave, no storage (400); microwave, 4 ^{o}C (385); and fry, 4 ^{o}C (375). The four lowest interactions for AOAS (eq. trolox µg/gfw) were raw, no storage (221); boil, 4 ^{o}C (230); raw, 4 ^{o}C (239); and boil, no storage (250). The range for AOAS was 179 µg/gfw.

Table 5.22 Cooking method by storage treatment interaction for antioxidant activity, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cooking method and storage treatment	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Bake		
No Storage	143	348
4 ^{o}C	127	324
Boil		
No Storage	94	250
4 ^{o}C	78	230
Fry		
No Storage	153	351
4 ^{o}C	152	375
Micro		
No Storage	172	400
4 ^{o}C	154	385
Raw		
No Storage	74	221
4 ^{o}C	81	239

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox

The interaction between cultivar and irradiation treatment is presented in Table 5.23. This interaction produced significant differences in both AOAI and AOAS, and had a relatively high magnitude of strength for both AOAI and AOAS (eta squared 9 % and 11 %, respectively). The cultivars Innovator, Krantz (for AOAS), NDTX4930-

5W, and Shepody had higher antioxidant activity for irradiated samples than non-irradiated samples. Irradiated samples of the cultivars, Innovator, NDTX4930-5W and Shepody experienced a greater loss in weight as compared to non-irradiated samples. This may explain why some cultivars had higher antioxidant activity in irradiated than in non-irradiated samples. These samples may have been subjected to more stress from dehydration, which may have induced antioxidant activity. The highest interactions for AOAI (eq. trolox µg/gfw) were 'Atlantic', 0 Gy (218); 'Russet Burbank', 0 Gy (179); 'Russet Burbank', 150 Gy (161); 'Krantz', 150 Gy (147); and 'Santana', 0 Gy (146). The five lowest interactions for AOAI (eq. trolox µg/gfw) were 'Atlantic', 75 Gy (66); 'ATX85404-8W', 75 Gy (74); 'Shepody', 0 Gy (82); 'NDTX4930-5W', 0 Gy (82); and 'NDTX4930-5W', 150 Gy (91). The range for AOAI was 152 µg/gfw. The five highest interactions for AOAS (eq. trolox µg/gfw) were 'Atlantic', 0 Gy (474); 'Russet Burbank', 0 Gy (409); 'Krantz', 150 Gy (391); 'Russet Burbank', 150 Gy (380); and 'Russet Burbank', 75 Gy (363). The five lowest interactions for AOAI (eq. trolox µg/gfw) were 'Atlantic', 75 Gy (210); 'NDTX4930-5W', 0 Gy (218); 'ATX85404-8W', 75 Gy (234); 'NDTX4930-5W', 75 Gy (239); and 'Shepody', 0 Gy (240). The range for AOAS was 264 µg/gfw.

Table 5.23 Cultivar by irradiation dose interaction for antioxidant activity, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar and irradiation dose	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Atlantic		
0 Gy	218	474
75 Gy	66	210
150 Gy	137	292
ATX85404-8W		
0 Gy	134	357
75 Gy	74	234
150 Gy	103	280
Innovator		
0 Gy	93	267
75 Gy	130	321
150 Gy	106	303

Table 5.23 (continued).

Cultivar and irradiation dose	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Krantz		
0 Gy	143	329
75 Gy	127	333
150 Gy	147	391
NDTX4930-5W		
0 Gy	82	218
75 Gy	96	239
150 Gy	91	278
Russet Burbank		
0 Gy	179	409
75 Gy	134	363
150 Gy	161	380
Santana		
0 Gy	146	343
75 Gy	118	327
150 Gy	144	334
Shepody		
0 Gy	82	240
75 Gy	126	312
150 Gy	112	257

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox

The interaction of cooking method and irradiation dose is presented in Table 5.24. Both AOAI and AOAS were significant but with low magnitudes of strength. The highest interactions for AOAI (eq. trolox µg/gfw) were microwave, 150 Gy (174); fry, 0 Gy (171); microwave, 0 Gy (166); microwave, 75 Gy (149); and fry, 75 Gy (144). The lowest interactions for AOAI (eq. trolox µg/gfw) were boil, 75 Gy (62); raw, 75 Gy (66); boil, 150 Gy (82); raw, 0 Gy (83); and raw, 150 Gy (85). The range for AOAI was 112 µg/gfw. The highest interactions for AOAS (eq. trolox µg/gfw) were microwave, 150 Gy (408); microwave, 0 Gy (403); fry, 0 Gy (393); microwave, 75 Gy (365); and bake, 150 Gy (358). The lowest interactions for AOAS (eq. trolox µg/gfw) were boil, 75 Gy (204); raw, 150 Gy (227); raw, 75 Gy (229); raw, 0 Gy (233); and boil, 150 Gy (233). The range for AOAS was 204 µg/gfw. Cooking method appeared to exert more influence in this interaction.

Table 5.24 Cooking method by irradiation dose interaction for antioxidant activity, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cooking methods and irradiation doses	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Bake		
0 Gy	138	335
75 Gy	123	317
150 Gy	143	358
Boil		
0 Gy	114	283
75 Gy	62	204
150 Gy	82	233
Fry		
0 Gy	171	393
75 Gy	144	348
150 Gy	141	346
Micro		
0 Gy	166	403
75 Gy	149	365
150 Gy	174	408
Raw		
0 Gy	83	233
75 Gy	66	229
150 Gy	85	227

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox

In the interaction between storage treatment and irradiation dose, the no storage, irradiated samples had higher antioxidant activity than the non-irradiated samples, while in the storage treatments, the irradiated samples were lower than the non-irradiated samples (Table 5.25). This interaction may provide further evidence that non-stored, irradiated samples have higher antioxidant activity but, over time, it decreases to a lower point. The highest interaction for AOAI was 4 ^{o}C, 0 Gy (151), while the lowest was 4 ^{o}C, 75 Gy (89), a range of 62 µg/gfw. The highest interaction for AOAS was 4 ^{o}C, 0 Gy (355), while the lowest was 4 ^{o}C, 75 Gy (278), a range of 77 µg/gfw.

Table 5.25 Storage treatment by irradiation dose interaction for antioxidant activity, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Storage treatment and irradiation dose	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
None		
0 Gy	119	304
75 Gy	128	307
150 Gy	135	330
4 °C		
0 Gy	151	355
75 Gy	89	278
150 Gy	115	298

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox

The significant three-factor interaction of cultivar, storage and irradiation dose had a relatively high magnitude of strength for both AOAI and AOAS (eta square 9 % for both) (Table 5.26). This interaction shows the effect on antioxidant activity of irradiated samples over time. The decrease of antioxidant activity in stored, irradiated samples may be caused by changes in metabolism, although further study should be conducted to determine when and how this decrease occurs. The highest interactions for AOAI (eq. trolox µg/gfw) were 'Atlantic', 4 °C, 0 Gy (276); 'Santana', no storage, 150 Gy (208); 'Krantz', 4 °C, 150 Gy (201); 'Atlantic', no storage, 150 Gy (198); and 'Russet Burbank', no storage, 150 Gy (183). The lowest interactions for AOAI (eq. trolox µg/gfw) were 'NDTX4930-5W', 4 °C, 75 Gy (45); 'ATX85404-8W', 4 °C, 75 Gy (51); 'Atlantic', 4 °C, 75 Gy (57); 'Shepody', no storage, 0 Gy (69); and 'NDTX4930-5W', no storage, 150 Gy (74). The range for AOAI was 231µg/gfw. The highest interactions for AOAS (eq. trolox µg/gfw) were 'Atlantic', 4 °C, 0 Gy (648); 'Krantz', 4 °C, 150 Gy (480); 'ATX85404-8W', 4 °C, 0 Gy (443); 'Russet Burbank', no storage, 0 Gy (436); and 'Santana', no storage 150 Gy (424). The lowest interactions for AOAS (eq. trolox µg/gfw) were 'Atlantic', 4 °C, 75 Gy (199); 'NDTX4930-5W', 4 °C, 0 Gy (199); 'Shepody', 4 °C, 150 Gy (206); 'NDTX4930-5W', 4 °C, 75 Gy (210); and 'Shepody', no storage, 0 Gy (215). The range for AOAS was 449 µg/gfw. The cultivar Atlantic when non-irradiated and stored at 4 °C had the highest antioxidant activity, and when exposed to 75 Gy and stored had the lowest

antioxidant activity. This indicates how storage and irradiation can greatly affect antioxidant activity.

Table 5.26 Cultivar by storage treatment by irradiation dose interaction for antioxidant activity, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar	Storage treatment	Irradiation dose	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Atlantic				
	No Storage	0 Gy	160	300
		75 Gy	74	222
		150 Gy	198	349
	4 °C	0 Gy	276	648
		75 Gy	57	199
		150 Gy	75	235
ATX85404-8W				
	No Storage	0 Gy	102	271
		75 Gy	96	249
		150 Gy	116	286
	4 °C	0 Gy	166	443
		75 Gy	51	219
		150 Gy	89	274
Innovator				
	No Storage	0 Gy	87	306
		75 Gy	176	350
		150 Gy	94	290
	4 °C	0 Gy	98	229
		75 Gy	84	293
		150 Gy	118	315
Krantz				
	No Storage	0 Gy	131	307
		75 Gy	134	313
		150 Gy	94	301
	4 °C	0 Gy	156	351
		75 Gy	120	353
		150 Gy	201	480
NDTX4930-5W				
	No Storage	0 Gy	76	238
		75 Gy	146	269
		150 Gy	74	272
	4 °C	0 Gy	88	199
		75 Gy	45	210
		150 Gy	108	283
Russet Burbank				
	No Storage	0 Gy	178	436
		75 Gy	123	353
		150 Gy	183	411
	4 °C	0 Gy	180	382
		75 Gy	145	372
		150 Gy	139	349

Table 5.26 (continued).

Cultivar	Storage treatment	Irradiation dose	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
Santana				
	No Storage	0 Gy	146	358
		75 Gy	116	347
		150 Gy	208	424
	4 °C	0 Gy	146	328
		75 Gy	119	307
		150 Gy	80	245
Shepody				
	No Storage	0 Gy	69	215
		75 Gy	161	356
		150 Gy	111	309
	4 °C	0 Gy	94	265
		75 Gy	92	268
		150 Gy	114	206

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox

TOTAL PHENOLIC CONTENT. The linear regression equation to equate the spectrophotometric absorbance readings from the Folin test at 727 nm into chlorogenic acid equivalents was $y = 0.5775x - 0.0279$, where x is the absorbance at 727 nm after zeroing the spectrophotometer with a blank lacking antioxidant extract but containing all other solutions, and y is the µg chlorogenic acid equivalents per gram fresh weight. The R^2 value of this equation was 0.970.

The average amount of phenolics was 371 µg/gfw chlorogenic acid equivalents. There were significant differences in phenolic acid content for cultivar ($p < 0.000$), cooking method ($p < 0.000$), storage treatment ($p < 0.000$), irradiation dose ($p < 0.000$), the interactions of cultivar and cooking method ($p < 0.000$), cultivar and storage treatment ($p < 0.000$), cultivar and irradiation dose ($p < 0.000$), cooking method and irradiation dose ($p < 0.000$), storage treatment and irradiation dose, and cultivar, storage treatment and irradiation dose ($p < 0.000$) (Table 5.27). There were no significant differences in phenolics for the interactions of cooking method and storage treatment ($p = 0.101$); cultivar, cooking method, and storage treatment ($p = 0.232$); cultivar, cooking method, and irradiation dose ($p = 0.979$); cooking method, storage

treatment, and irradiation dose (p = 0.082); and cultivar, cooking method, storage treatment, and irradiation dose (p = 0.221).

The factor's magnitude of strength (eta squared values) for individual factors were cultivar, 34 %; cooking method, 24 %; storage treatment, 1 %; irradiation dose, 5 %; and the interactions of cultivar and cooking method, 3 %; cultivar and storage treatment, 2 %; cooking method and storage treatment, 0 %; cultivar, cooking method, and storage treatment, 1 %; cultivar and irradiation dose, 7 %; cooking method and irradiation dose, 1 %; cultivar, cooking method, and irradiation dose, 1 %; storage treatment and irradiation dose, 1 %; cultivar, storage treatment and irradiation dose, 3 %; cooking method, storage treatment and irradiation dose, 0 %; cultivar, cooking method, storage treatment and irradiation dose, 2 %; and error at 15 %.

Table 5.27 Analysis of variance results for phenolic content for the factors gamma- irradiation, storage, cultivar, and cooking method, Dalhart 2003.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	11870815.894 [z]	239	49668.686	11.351	.000
Intercept	99002529.525	1	99002529.525	22624.789	.000
Cultivar	4773212.372	7	681887.482	155.830	.000
Cook	3304487.460	4	826121.865	188.791	.000
Store	96353.798	1	96353.798	22.019	.000
Irrdose	672872.328	2	336436.164	76.885	.000
Cultivar * Cook	398610.515	28	14236.090	3.253	.000
Cultivar * Store	239197.256	7	34171.037	7.809	.000
Cook * Store	34123.879	4	8530.970	1.950	.101
Cultivar * Cook * Store	145919.009	28	5211.393	1.191	.232
Cultivar * Irrdose	1005049.580	14	71789.256	16.406	.000
Cook * Irrdose	192017.954	8	24002.244	5.485	.000
Cultivar * Cook * Irrdose	157263.426	56	2808.275	.642	.979
Store * Irrdose	109622.445	2	54811.223	12.526	.000
Cultivar * Store * Irrdose	398281.925	14	28448.709	6.501	.000
Cook * Store * Irrdose	61816.785	8	7727.098	1.766	.082
Cultivar * Cook * Store * Irrdose	281987.162	56	5035.485	1.151	.221

Table 5.27 (continued).

Source	Type III sum of squares	df	Mean square	F	Sig.
Error	2100404.734	480	4375.843		
Total	112973750.153	720			
Corrected Total	13971220.628	719			

[z] $R^2 = .850$ (Adjusted $R^2 = .775$)

There were significant differences in phenolics among cultivars. The magnitude of strength of cultivar (34 %) was the highest of all factors. The ranking was also very similar to earlier studies (Chapters III and IV). The cultivar Krantz ranked highest in phenolics, but it was lower than Russet Burbank in antioxidant activity (Table 5.28). Each cultivar was significantly different from the others, with the exception of Shepody and Atlantic. The range for cultivars was 279 µg/gfw equivalents of chlorogenic acid, with Krantz at 544 µg/gfw and NDTX4930-5W at 265 µg/gfw.

Table 5.28 Cultivar ranking for phenolic content, Dalhart 2003 study involving the factors gamma-irradiation, storage, cultivar, and cooking method.

Cultivar	Eq. Chlorogenic acid (µg/gfw)	
Krantz	544	a[z]
Santana	418	b
Russet Burbank	397	c
Innovator	377	d
Shepody	343	e
Atlantic	337	e
ATX85404-8W	287	f
NDTX4930-5W	265	g

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Cooking methods also resulted in significant differences. The magnitude of strength was 24 %, which is the second largest eta square value behind cultivar. The mean separation and ranking (Table 5.29) was similar to earlier storage studies in Dalhart 2003 (Chapter IV) and McCook 2003 (Chapter III). The microwave cooking

method produced the greatest amount of phenolics at 444 µg/gfw, while boiling produced the lowest at 278 µg/gfw, a range of 166 µg/gfw. The cooking methods microwaving, frying, and baking consistently were at the top of the ranking for both phenolics and antioxidant activity. This consistency in ranking is probably related to the strong correlation between the phenolic acid compounds and their antioxidant activity.

Table 5.29 Cooking method ranking for phenolic content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cooking method	Eq. Chlorogenic acid (µg/gfw)	
Micro	444	a[z]
Fry	418	b
Bake	413	b
Raw	301	c
Boil	278	d

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Storage treatment was a significant factor, although the strength of this factor alone is weak (eta square 1 %). As with antioxidant activity, the non-stored samples had a larger amount of phenolics than the stored samples (Table 5.30). Further examination of the interactions aids in understanding the complete relationship. Most cultivars experienced a decrease in phenolic levels through storage, except for Innovator and Krantz. Both of these cultivars are ranked very high in antioxidant activity and phenolics. Over all, there was a significant decrease in measurable phenolics with storage. This decrease may be related to the degree of weight loss which is related to the tuber skin thickness. The range between the two storage treatments was 24 µg/gfw.

Table 5.30 Storage treatments ranking for phenolic content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Storage treatment	Eq. Chlorogenic acid (µg/gfw)	
No Storage	383	a[z]
4 °C	359	b

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at P ≤ 0.05.

Mean separation results indicate that the irradiation dosages were significantly different. The two irradiated samples ranked higher than the non-irradiated samples (Table 5.31). Most cultivars experienced an increase in phenolic acids with irradiation. The highest irradiation dose was 150 Gy with 397 µg/gfw, and the lowest was 0 Gy with 328 µg/gfw, creating a range of 69 µg/gfw.

Table 5.31 Irradiation doses ranking for phenolic content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Irradiation dose	Eq. Chlorogenic acid (µg/gfw)	
150 Gy	397	a[z]
75 Gy	387	a
0 Gy	328	b

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at P ≤ 0.05.

The interaction between cultivar and cooking method accounted for 3 % of the magnitude of strength of the regression, and the interaction was significant (Table 5.32). The microwave cooking method ranked highest for five cultivars, while frying and baking ranked highest for two and one cultivar, respectively. The highest phenolic (eq. chlorogenic acid µg/gfw) interactions were 'Krantz', microwave (631); 'Krantz', bake (627); 'Krantz', fry (587); 'Russet Burbank', microwave (522); and 'Santana', microwave (501). The lowest phenolic interactions were 'NDTX4930-5W', boil (201); 'ATX85404-8W', boil (218); 'ATX85404-8W', raw (236); 'NDTX4930-5W', raw (236); and 'Shepody', boil (237). The range was 430 µg/gfw. Cooking method did not

cause the same effect on the phenolic content of each cultivar, hence the interaction. There is a notable trend where certain cooking methods are ranked similarly.

Table 5.32 Cultivar by cooking method interaction for phenolic content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar and cooking method	Eq. Chlorogenic acid (µg/gfw)
Atlantic	
Bake	343
Boil	288
Fry	395
Micro	377
Raw	280
ATX85404-8W	
Bake	315
Boil	218
Fry	325
Micro	340
Raw	236
Innovator	
Bake	436
Boil	269
Fry	428
Micro	455
Raw	296
Krantz	
Bake	627
Boil	395
Fry	587
Micro	631
Raw	478
NDTX4930-5W	
Bake	281
Boil	201
Fry	305
Micro	317
Raw	220
Russet Burbank	
Bake	423
Boil	281
Fry	471
Micro	522
Raw	287
Santana	
Bake	474
Boil	334
Fry	456
Micro	501
Raw	326

Table 5.32 (continued).

Cultivar and cooking method	Eq. Chlorogenic acid (µg/gfw)
Shepody	
Bake	404
Boil	237
Fry	381
Micro	410
Raw	282

The interaction between cultivar and storage treatment is presented in Table 5.33. Again certain trends can be seen, where the treatment of no storage ranked higher among all cultivars, except for Innovator and Krantz. Both cultivars have a slightly yellow flesh, which could be related to certain individual phenolic compounds that might not be affected by storage. Also, both cultivars experienced a low amount of weight loss through storage, which also may be related to their skin thickness and / or higher phenolic content in storage. Also, the difference between the stored and non-stored samples of these two cultivars is relatively small and may not be significant. The highest phenolic (eq. chlorogenic acid µg/gfw) interactions were 'Krantz', 4 ^{o}C (552); 'Krantz', no storage (535); 'Santana', no storage (450); 'Russet Burbank', no storage (423); and 'Innovator', 4 ^{o}C (392). The lowest phenolic interactions were 'NDTX4930-5W', 4 ^{o}C (263); 'NDTX4930-5W', no storage (267); 'ATX85404-8W', 4 ^{o}C (285); 'ATX85404-8W', no storage (288); and 'Shepody', 4 ^{o}C (305). The range for this interaction was 289 µg/gfw.

Table 5.33 Cultivar by storage treatment interaction for phenolic content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar and storage treatment	Eq. Chlorogenic acid (µg/gfw)
Atlantic	
No Storage	354
4 ^{o}C	319
ATX85404-8W	
No Storage	288
4 ^{o}C	285
Innovator	
No Storage	361
4 ^{o}C	392

Table 5.33 (continued).

Cultivar and storage treatment	Eq. Chlorogenic acid (µg/gfw)
Krantz	
No Storage	535
4 °C	552
NDTX4930-5W	
No Storage	267
4 °C	263
Russet Burbank	
No Storage	423
4 °C	370
Santana	
No Storage	450
4 °C	386
Shepody	
No Storage	380
4 °C	305

The interaction between cultivar and irradiation dose was significant and was the most influential (highest eta square, 7 %) interaction. All cultivars had higher amounts of phenolics with the additional treatment of irradiation, except for Atlantic and ATX85404-8W (Table 5.34). Further interactions may explain this phenomenon. The highest phenolic (eq. chlorogenic acid µg/gfw) interactions were for 'Krantz', 150 Gy (576); 'Krantz', 75 Gy (539); 'Krantz', 0 Gy (516); 'Shepody', 75 Gy (473); and 'Innovator', 150 Gy (439). The lowest phenolic (eq. chlorogenic acid µg/gfw) interactions were 'NDTX4930-5W', 0 Gy (189); 'Innovator', 0 Gy (270); 'NDTX4930-5W', 75 Gy (285); 'ATX85404-8W', 75 Gy (285); and 'ATX85404-8W', 150 Gy (285). The range for this interaction was 387 µg/gfw.

Table 5.34 Cultivar by irradiation dose interaction for phenolic content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar and irradiation dose	Eq. Chlorogenic acid (µg/gfw)
Atlantic	
0 Gy	393
75 Gy	291
150 Gy	326
ATX85404-8W	
0 Gy	292
75 Gy	285
150 Gy	285
Innovator	
0 Gy	270
75 Gy	421
150 Gy	439
Krantz	
0 Gy	516
75 Gy	539
150 Gy	576
NDTX4930-5W	
0 Gy	189
75 Gy	285
150 Gy	321
Russet Burbank	
0 Gy	350
75 Gy	401
150 Gy	439
Santana	
0 Gy	346
75 Gy	401
150 Gy	439
Shepody	
0 Gy	346
75 Gy	473
150 Gy	436

The interaction between cooking method and irradiation dose had a small magnitude of strength (eta square, 1 %), but it was still significant (Table 5.35). Phenolic content in each cooking treatment increased with irradiation, although no particular dose appeared to increase content most, hence the significant interaction. The greatest phenolic (eq. chlorogenic acid µg/gfw) interactions were microwave, 150 Gy, (502); microwave, 75 Gy (463); bake, 150 Gy (447); fry, 75 Gy (432); and bake, 75 Gy (430). The smallest phenolic (eq. chlorogenic acid µg/gfw) interactions were

boil, 0 Gy (254); raw, 0 Gy (261); boil, 75 Gy (282); boil, 150 Gy (298); and raw, 150 Gy (312). The range was 248 μg/gfw.

Table 5.35 Cooking method by irradiation dose interaction for phenolic content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cooking method and irradiation dose	Eq. Chlorogenic acid (μg/gfw)
Bake	
0 Gy	361
75 Gy	430
150 Gy	447
Boil	
0 Gy	254
75 Gy	282
150 Gy	298
Fry	
0 Gy	396
75 Gy	432
150 Gy	427
Micro	
0 Gy	368
75 Gy	463
150 Gy	502
Raw	
0 Gy	261
75 Gy	330
150 Gy	312

The interactions between storage treatment and irradiation dose also had a small magnitude of strength (eta square, 1 %), but again were significant (Table 5.36). The irradiation treated samples tended to be higher in phenolics than non-irradiated samples. The largest interaction was no storage, 150 Gy at 425 μg/gfw; and the lowest was 4 ^{o}C, 0 Gy at 321 μg/gfw, with a range of 104 μg/gfw.

Table 5.36 Storage treatment by irradiation dose interaction for phenolic content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Storage treatment and irradiation dose	Eq. Chlorogenic acid (µg/gfw)
No storage	
0 Gy	335
75 Gy	387
150 Gy	425
4 °C	
0 Gy	321
75 Gy	388
150 Gy	369

The only significant three-factor interaction was that for cultivar, storage treatment, and irradiation dose. A trend was noted for antioxidant activity, where the non-stored, irradiated samples had higher antioxidant activity than the non-stored, non-irradiated samples. This trend reversed once the samples were stored for 110 days at 4°C, when the non-irradiated samples were much higher than the irradiated samples (Table 5.37). This trend does not seem to apply for phenolic levels. Generally, non-stored samples tended to have a higher phenolic level, except in the cultivars Innovator and Krantz. Also, all cultivars except Atlantic, experienced an increase in phenolic levels. The highest phenolic (eq. chlorogenic acid µg/gfw) interactions were 'Krantz', 4°C, 150 Gy (577); 'Krantz', no storage, 150 Gy (576); 'Krantz', 4°C, 0 Gy (542); 'Krantz', no storage, 75 Gy (540); and 'Krantz', 4°C, 75 Gy (538). The lowest phenolic (eq. chlorogenic acid µg/gfw) interactions were 'NDTX4930-5W', 4°C, 0 Gy (183); 'NDTX4930-5W', no storage, 0 Gy (196); 'Innovator', no storage, 0 Gy (239); 'NDTX4930-5W', no storage, 75 Gy (246); and 'Shepody', 4°C, 0 Gy (252). The range for this interaction was 394 µg/gfw. Clearly, 'Krantz' ranks highest in phenolic content, while 'NDTX4930-5W' and 'ATX85404-8W' ranks lowest.

Table 5.37 Cultivar by storage treatment by irradiation dose interaction for phenolic content, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar	Storage treatment	Irradiation dose	Eq. Chlorogenic acid (µg/gfw)
Atlantic			
	No Storage	0 Gy	422
		75 Gy	255
		150 Gy	384
	4 ºC	0 Gy	364
		75 Gy	326
		150 Gy	269
ATX85404-8W			
	No Storage	0 Gy	294
		75 Gy	297
		150 Gy	275
	4 ºC	0 Gy	290
		75 Gy	272
		150 Gy	295
Innovator			
	No Storage	0 Gy	239
		75 Gy	439
		150 Gy	407
	4 ºC	0 Gy	302
		75 Gy	403
		150 Gy	472
Krantz			
	No Storage	0 Gy	489
		75 Gy	540
		150 Gy	576
	4 ºC	0 Gy	542
		75 Gy	538
		150 Gy	577
NDTX4930-5W			
	No Storage	0 Gy	196
		75 Gy	246
		150 Gy	359
	4 ºC	0 Gy	183
		75 Gy	324
		150 Gy	282
Russet Burbank			
	No Storage	0 Gy	406
		75 Gy	375
		150 Gy	488
	4 ºC	0 Gy	293
		75 Gy	427
		150 Gy	391
Santana			
	No Storage	0 Gy	350
		75 Gy	505
		150 Gy	495
	4 ºC	0 Gy	341
		75 Gy	441
		150 Gy	377

Table 5.37 (continued).

Cultivar	Storage treatment	Irradiation dose	Eq. Chlorogenic acid (µg/gfw)
Shepody			
	No Storage	0 Gy	285
		75 Gy	436
		150 Gy	420
	4 °C	0 Gy	252
		75 Gy	375
		150 Gy	288

HPLC ANALYSIS FOR CAROTENOID COMPOUNDS. Ninety samples were analyzed for seven specific carotenoid compounds via HPLC. Only six compounds, violaxanthin, neoxanthin, antheraxanthin, lutein, zeaxanthin, and canthaxanthin, were found though retention time and spectra or retention time only. The cultivars chosen were Innovator, Russet Burbank and Santana because of their high content of these compounds, in this study and others. There were no significant differences in total carotenoid content among cultivars. However, the cultivar Innovator had the highest total carotenoid content, 13 µg/100gfw, while Santana had the lowest, 7 µg/100gfw (Table 5.38). The most prominent compound was lutein, and there were significant differences among cultivars. 'Innovator' had the most lutein, 9 µg/100gfw, while 'Santana' had none. There were also significant differences among cultivars in antheraxanthin content. 'Santana' had the most antheraxanthin, 3 µg/100gfw, while 'Innovator' had none. Matching of individual carotenoids via HPLC based on spectra identification and the combination of retention time and spectra identification is also presented in Table 5.38. The only carotenoid compound that was matched to the spectra was lutein. 'Innovator' had the greatest lutein content, with 3 µg/100gfw based on spectra. The only compound to match both retention time and spectra was also lutein.

Table 5.38 Cultivar ranking for individual carotenoid compounds[z] (µg/100 gfw), Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar	VIO	NEO	ANT	LUT	ZEA	CAN	Total-R	LUT-SP	LUT-RSP
Innovator	1 a[y]	1 a	0 b	9 a	1 a	1 ab	13 a	3 a	3 a
Russet Burbank	0 a	0 a	1 ab	7 a	0 a	0 b	8 a	0 b	0 b
Santana	0 a	1 a	3 a	0 b	0 a	3 a	7 a	0 b	0 b

[z]VIO : Violaxanthin content based on retention time
NEO : Neoxanthin content based on retention time
ANT : Antheraxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

As with cultivar, there were no significant differences among cooking methods for total carotenoid content (Table 5.39). Frying resulted in the most total carotenoids at 13 µg/100gfw, while the lowest was found in raw samples at 4 µg/100gfw. In the McCook 2003 (Chapter III) and Dalhart 2003 (Chapter IV) studies the raw samples had the most total carotenoids. In the Dalhart 2003 (Chapter IV) storage study, baking ranked the highest for total carotenoids. Over all, cooking has little effect on individual carotenoid levels. Measurements of individual carotenoids based on spectra and the combination of spectra and retention time were similar to the results based on retention time alone. Although not significant ($p = 0.512$ and $p = 0.436$ for both spectra and spectra and retention time, respectively), the raw and boiled samples had the lowest carotenoid content while the baked and microwaved were highest.

Table 5.39 Cooking method ranking for individual carotenoid compounds[z] (µg/100gfw), Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cooking method	VIO	NEO	ANT	LUT	ZEA	CAN	Total-R	LUT-SP	LUT-RSP
Bake	0 a[y]	0 a	2 a	7 a	1 a	2 a	12 a	2 a	2 a
Boil	0 a	0 a	1 a	3 a	0 a	1 a	5 a	0 a	0 a
Fry	0 a	1 a	1 a	7 a	1 a	3 a	13 a	1 a	0 a
Micro	2 a	1 a	1 a	7 a	0 a	1 a	12 a	2 a	2 a
Raw	0 a	0 a	1 a	2 a	0 a	1 a	4 a	0 a	0 a

[z]VIO : Violaxanthin content based on retention time
NEO : Neoxanthin content based on retention time
ANT : Antheraxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The effects of storage treatment on carotenoid compounds are presented in Table 5.40. Although the spectrophotometric method for carotenoids in this study determined that storage had a significant effect on carotenoid levels, the HPLC data based on retention time were less conclusive.

Table 5.40 Storage treatment ranking for individual carotenoid compounds[z] (µg/100gfw), Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cooking method	VIO	NEO	ANT	LUT	ZEA	CAN	Total-R	LUT-SP	LUT-RSP
No Storage	0 a[y]	0 a	1 a	7 a	0 a	2 a	10 a	2 a	2 a
4 °C	1 a	1 a	1 a	4 a	0 a	1 a	9 a	0 a	0 a

[z]VIO : Violaxanthin content based on retention time
NEO : Neoxanthin content based on retention time
ANT : Antheraxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Irradiation dose did not affect individual carotenoid compounds (Table 5.41). The 150 Gy treatment ranked highest in total carotenoid levels, while 75 Gy was the lowest. Lutein levels were the highest of all carotenoids.

Table 5.41 Irradiation dose ranking for individual carotenoid compounds[z] (µg/100gfw), Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Irradiation dose	VIO	NEO	ANT	LUT	ZEA	CAN	Total-R	LUT-SP	LUT-RSP
0 Gy	1 a[y]	1 a	2 a	4 a	1 a	1 a	10 a	1 a	0 a
75 Gy	0 a	0 a	2 a	4 a	1 a	1 a	8 a	0 a	0 a
150 Gy	0 a	1 a	0 a	8 a	0 a	2 a	10 a	3 a	3 a

[z]VIO : Violaxanthin content based on retention time
NEO : Neoxanthin content based on retention time
ANT : Antheraxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Only lutein ($p = 0.004$) and total carotenoids ($p = 0.023$) interacted significantly with the interaction of cultivar, storage and irradiation dose (ANOVA table not shown). The results of this interaction with lutein and total carotenoid content based on retention time are presented in Table 5.42. The other compounds were omitted from Table 5.42 due to lack of significance.

Table 5.42 The interaction of cultivar, storage and irradiation treatment for individual carotenoid compounds (µg/100gfw) based on retention time, Dalhart 2003 study involving the factors gamma-irradiation, storage, cultivar, and cooking method.

Cultivar	Storage	Irradiation dose	Lutein	Total
Innovator				
	No Storage	0	0	0
		75	0	6
		150	28	28

Table 5.42 (continued).

Cultivar	Storage	Irradiation dose	Lutein	Total
	4 °C	0	13	30
		75	13	13
		150	0	0
Russet Burbank				
	No Storage	0	0	0
		75	13	13
		150	18	18
	4 °C	0	13	13
		75	0	0
		150	0	5
Santana				
	No Storage	0	0	6
		75	0	0
		150	0	11
	4 °C	0	0	7
		75	0	11
		150	0	4

HPLC ANALYSIS FOR PHENOLIC COMPOUNDS. Ninety-two samples were analyzed for phenolic content. Although there were 18 compounds in the library used for analyses, only 13 were found in the samples tested. Unlike the carotenoid compounds, there were more significant differences in the phenolic compounds. Of the three cultivars evaluated, Innovator appeared to have the greatest amount of total phenolics via HPLC analysis based on retention time; however the difference was not significant (Table 5.43).

Table 5.43 Cultivar ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
Innovator	49	55	11	241	28	48	11	3	69	24	12	336	16	902
	a[y]	a	a	a	a	b	a	a	c	a	a	a	a	a
Russet	44	54	9	248	35	63	11	1	107	19	13	255	16	874
Burbank	a	a	a	a	a	a	a	b	a	b	a	a	a	a
Santana	38	48	11	262	33	45	9	4	94	20	12	224	14	816
	a	b	a	a	a	b	a	a	b	b	a	a	b	a

Table 5.43 (continued).

[z]CH : Chlorogenic acid	QU : Quercetin dihydrate
CA : Caffeic acid	PR : Protocatechuic acid
CI : t-Cinnamic acid	MY : Myricetin
GA : Gallic acid	PC : p-Coumaric acid
RU : Rutin hydrate	CT : Catechin
SI : Sinapic acid	VA : Vanillic acid
EP : Epicatechin	Total : the addition of all measured phenolics

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Analysis of phenolic content based on spectra and retention time only produced data for two compounds, chlorogenic acid and caffeic acid (Table 5.44). Results of the total spectra data and the total retention time and spectra are also included. Innovator ranked high among the cultivars.

Table 5.44 Cultivar ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both retention time and spectra, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cultivar	CH-SP	CA-SP	Total-SP	CH-RSP	CA-RSP	Total-RSP
Innovator	181 a[y]	153 a	334 a	39 a	27 a	66 a
Russet Burbank	175 a	167 a	342 a	32 a	21 a	53 ab
Santana	110 b	174 a	283 a	10 b	31 a	40 b

[z] CH-SP : Chlorogenic acid based on spectra	CH-RSP : Chlorogenic acid based on retention time and spectra
CA-SP : Caffeic acid based on spectra	CA-RSP : Caffeic acid based on retention time and spectra
Total-SP : the addition of all measured phenolics based on spectra	Total-RSP : the addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Tuber samples from each cooking method were analyzed for phenolic content via HPLC based on retention time (Table 5.45). Mean separation revealed some significant differences among cooking methods. The fry samples tended to have the highest phenolic content, and raw samples had the lowest via HPLC; this correlates to the results obtained in the Dalhart 2003 (Chapter IV) storage study. Over all, phenolic

compounds increased with cooking. The exceptions were quercetin dihydrate and t-cinnamic acid, where the raw samples ranked equally high or higher than the cooked samples.

Table 5.45 Cooking method ranking for individual phenolic compounds (µg/gfw), Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cooking method	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
Bake	51	53	11	284	36	60	11	2	102	21	13	245	16	905
	b[y]	a	a	a	a	a	ab	ab	a	a	a	a	a	a
Boil	29	51	10	207	27	48	9	3	79	21	11	242	13	750
	c	a	a	b	a	a	b	a	ab	a	ab	a	b	a
Fry	50	53	10	268	32	52	11	1	98	21	13	396	16	1020
	b	a	a	a	a	a	ab	b	ab	a	a	a	a	a
Micro	75	56	9	273	35	56	13	2	95	22	13	238	19	906
	a	a	a	a	a	a	a	ab	ab	a	a	a	a	a
Raw	13	49	11	221	29	43	8	4	77	21	10	237	10	739
	d	a	a	b	a	a	b	a	b	a	b	a	c	a

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The results of the spectra and the combination of spectra and retention time data for cooking methods are presented in Table 5.46. Microwaving produced the greatest amount of phenolics, while the raw method produced the least amount of phenolics for both chlorogenic acid and caffeic acid.

Table 5.46 Cooking method ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both retention time and spectra, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Cooking method	CH-SP		CA-SP		Total-SP		CH-RSP		CA-RSP		Total-RSP	
Bake	173	ab[y]	212	a	385	a	30	b	25	a	55	b
Boil	125	bc	169	a	294	b	17	bc	25	a	43	bc
Fry	150	bc	202	a	352	ab	30	b	29	a	59	b
Micro	225	a	180	a	406	a	56	a	25	a	81	a
Raw	100	c	62	b	162	c	1	c	30	a	31	c

[z] CH-SP : Chlorogenic acid based on spectra
CA-SP : Caffeic acid based on spectra
Total-SP : the addition of all measured phenolics based on spectra
CH-RSP : Chlorogenic acid based on retention time and spectra
CA-RSP : Caffeic acid based on retention time and spectra
Total-RSP : the addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

There were no significant differences in individual phenolic compound content among storage treatments via the HPLC retention time (Table 5.47). All individual compounds were higher with storage except for t-cinnamic acid, myricetin, and p-coumaric acid, which ranked the same for both storages, and epicatechin which ranked higher but not significantly with no storage.

Table 5.47 Storage treatment ranking for individual phenolic compounds (µg/gfw), Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Storage treatment	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
No Storage	36 b[y]	52 a	10 a	231 b	30 a	49 a	11 a	2 b	81 b	21 a	12 a	237 a	14 b	785 b
4 °C	51 a	53 a	10 a	270 a	33 a	55 a	10 a	3 a	99 a	21 a	12 a	307 a	16 a	943 a

[z] CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The effect of storage method on individual phenolic compounds based spectra and the combination of spectra and retention time is presented in Table 5.48. The 4°C storage treatment produced a greater, but not significantly greater, amount of phenolic compound based on spectra, except for chlorogenic acid.

Table 5.48 Storage method ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both retention time and spectra, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Storage treatment	CH-SP	CA-SP	Total-SP	CH-RSP	CA-RSP	Total-RSP
No Storage	157 a[y]	155 a	312 a	22 a	24 a	45 b
4 °C	153 a	175 a	328 a	32 a	30 a	62 a

[z] CH-SP : Chlorogenic acid based on spectra
CA-SP : Caffeic acid based on spectra
Total-SP : the addition of all measured phenolics based on spectra
CH-RSP : Chlorogenic acid based on retention time and spectra
CA-RSP : Caffeic acid based on retention time and spectra
Total-RSP : the addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The effect of different irradiation doses on individual phenolic compound content via HPLC is presented in Table 5.49. There were no significant differences in total phenolic levels. This does not support the significant differences reported in the Folin assay performed on the same samples, which indicated that the irradiated samples were significantly higher than the 0 Gy. The 75 Gy dose ranked the highest or among the highest in ten compounds, excluding only sinapic acid, protocatechuic acid, and t-cinnamic acid; however, there were no significant differences among compounds.

Table 5.49 Irradiation dose ranking for individual phenolic compounds (µg/gfw), Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Irradiation dose	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
0 Gy	44	52	9	252	33	57	10	2	102	20	13	247	15	856
	a[y]	a	a	a	a	a	a	a	a	a	a	a	ab	a
75 Gy	50	54	10	256	33	48	11	3	100	22	13	334	16	951
	a	a	a	a	a	a	a	a	a	a	a	a	a	a
150 Gy	37	50	11	243	30	51	10	3	68	21	11	234	14	786
	a	a	a	a	a	a	a	a	b	a	b	a	b	a

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics
[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The ranking of irradiation doses based on spectra and the combination of spectra and retention time is presented in Table 5.50. Spectra identification data ranked 150 Gy below the other doses, due in part to the lower levels of caffeic acid in the irradiated samples.

Table 5.50 Irradiation dose ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both retention time and spectra, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Irradiation dose	CH-SP	CA-SP	Total-SP	CH-RSP	CA-RSP	Total-RSP
0 Gy	148 a[y]	211 a	358 a	27 a	34 a	61 a
75 Gy	184 a	152 b	336 a	32 a	22 b	55 a
150 Gy	133 a	132 b	266 b	21 a	24 b	46 a

[z] CH-SP : Chlorogenic acid based on spectra
CA-SP : Caffeic acid based on spectra
Total-SP : the addition of all measured phenolics based on spectra
CH-RSP : Chlorogenic acid based on retention time and spectra
CA-RSP : Caffeic acid based on retention time and spectra
Total-RSP : the addition of all measured phenolics based on spectra and retention time
[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The interaction between storage and irradiation dose revealed an interesting trend for antioxidant activity, where irradiated samples were higher than the non-irradiated samples for the non-stored samples, whereas the irradiated samples were much lower than the non-irradiated samples for the stored samples. This trend was not seen in the phenolics assay, and neither was it seen in the following interaction (Table 5.51). Stored samples appeared to have a greater amount of phenolics, which contradicts the Folin assay performed. Storage had a much greater influence in this interaction, while irradiation dose had less of an affect. The increase of phenolics during storage seems to be related to protocatechuic acid and catechin.

Table 5.51 Irradiation dose ranking for individual phenolic compounds[z] (µg/gfw), Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Storage Treatment	Irr. (Gy)	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
No Storage															
	0	41	55	10	236	25	44	10	1	94	20	13	264	14	828
	75	35	49	9	215	32	48	12	3	75	21	13	231	14	756
	150	32	51	11	242	35	56	11	1	74	21	11	237	15	796
4°C															
	0	47	49	8	269	40	70	11	2	110	19	12	229	15	883
	75	65	59	11	298	34	47	10	3	126	23	14	454	19	1162
	150	41	50	11	244	24	48	9	4	62	22	11	237	13	783

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics

The effect of irradiation dose and storage method on phenolic content based on spectra identification and the combination of spectra and retention time is presented in Table 5.52. The 0 Gy dosage ranked highest in all dependent variables for the non-

storage treatment, but this trend was less prominent in the stored samples. Chlorogenic acid increased in the 75 Gy stored samples based on spectra and increased in both the 75 and 150 Gy stored sample based on the combination of retention time and spectra identification.

Table 5.52 Irradiation dose ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both retention time and spectra, Dalhart 2003 study involving the factors gamma- irradiation, storage, cultivar, and cooking method.

Storage Treatment	Irrad.Dose (Gy)	CH-SP	CA-SP	Total-SP	CH-RSP	CA-RSP	Total-RSP
No Storage							
	0	169	212	381	30	24	55
	75	136	115	251	26	22	48
	150	168	134	303	7	23	31
4°C							
	0	127	209	336	23	43	67
	75	231	190	421	39	23	61
	150	100	126	227	35	24	59

[z] CH-SP : Chlorogenic acid based on spectra
CA-SP : Caffeic acid based on spectra
Total-SP : the addition of all measured phenolics based on spectra
CH-RSP : Chlorogenic acid based on retention time and spectra
CA-RSP : Caffeic acid based on retention time and spectra
Total-RSP : the addition of all measured phenolics based on spectra and retention time

Discussion and Conclusion

This study investigated the effects of several postharvest procedures on the antioxidant content of selected potato cultivars. The goal was not to increase or decrease the antioxidant levels, just to report the effect of these processes. Even gamma- irradiation, which many have hypothesized would have a great effect on secondary metabolites, only caused a slight change in antioxidant content. This supports Kader (1986) who stated that irradiation technology will not solve all postharvest problems, rather it should be only considered a supplement in postharvest procedures to preserve the original quality of products, and Kilcast (1994) who stated

that irradiation processing will only be accepted if limited changes, especially organoleptic changes, occur.

Cultivars that ranked high in total carotenoids were Santana, Innovator, and Russet Burbank. Individual carotenoid ranking placed Santana lower than the other cultivars, but there were no significant differences. These cultivars also ranked high in antioxidant activity, along with Krantz, and Atlantic. The cultivars Krantz, Santana, Russet Burbank, and Innovator ranked high in phenolic content as well. Innovator ranked highest, but not significantly so, in individual phenolic content.

The cooking method of raw was significantly higher in carotenoids via absorbance. However, the cooking methods of fry, microwave, baking, and boiling ranked higher than raw samples based on individual carotenoid content. This latter ranking was similar to past measurements of carotenoid content. Bianchini and Penteado (1998) reported raw pepper samples had more carotenoid content than cooked samples, while Boileau et al. (1999), Dietz et al. (1988), and Van den Berg et al. (2000) reported increased amounts of carotenoids with cooking methods. Differences in the results may be due to different methods and the particular compounds quantified, eg. spectrophotometric methods may be quantifying different compounds than the HPLC method. The cooking methods of microwave, fry, and baking ranked high in antioxidant activity, total phenolic content, and individual phenolic content. Cooking also increased antioxidant activity and phenolic content in studies conducted by Amakura et al. (2000) and Zafrilla et al. (2001).

Storage resulted in significantly higher total carotenoid content, while no storage ranked higher, but not significantly, in individual carotenoids. Past studies (Chapter IV) have shown that storage had a positive effect on carotenoids. The effect of carotenoid content appears to be contingent on the temperature and length of storage (Craft and Wise, 1993; and Klein and Kurilich, 2000). No storage was significantly greater than storage in both antioxidant assays and the phenolic content assay, but storage was slightly higher, but not significantly, in individual phenolics than non-storage via HPLC. There were significant interactions with other factors involved in

storage, and this could explain some of the differences in ranking. Past studies have reported that different phenolic compounds will react differently with prolonged storage, and the effects of storage depend on storage conditions (Awald and de Jager, 2000; Häkkinen et al., 2000; Friedman, 1997; Percival et al., 2000; and Zafrilla et al., 2001).

In total carotenoid content, irradiated samples were significantly greater than non-irradiated samples. The 150 Gy samples ranked highest in individual carotenoid content, but not significantly higher. Craft and Wise, 1993 reported that zanthophyll content rose with irradiation, while carotene content decreased. In both antioxidant activity tests, the 0 and 150 Gy dose ranked significantly higher than the 75 Gy. The total phenolic content analysis ranked 150 and 75 Gy significantly higher than the 0 Gy dose. The individual phenolic content based on retention time ranked 75 Gy dose the highest, but not significantly, than the rest, while spectra and the combination of retention time and spectra ranked the 0 Gy dose the highest due to lack of caffeic acid found in the irradiated samples. This may be due to an alteration in the spectra of caffeic acid with irradiation processing.

There were also some important interaction trends that should be noted for operations with multiple postharvest procedures. Most notable was the trend for the interaction of storage and irradiation dose to affect antioxidant activity. Non-stored, irradiated potatoes ranked higher than non-stored, non-irradiated potatoes; stored, non-irradiated potatoes ranked higher than stored, irradiated potatoes. This may be due to a loss of weight from dehydration, in stored, non-irradiated potatoes due to sprouting, while, stored, irradiated potatoes did not dehydrate because of lack of sprout production. Patil, et al. (1999); and Penner and Fromm (1972) reported a significant interaction with storage time and irradiation dose in phenolic content, and this interaction should be investigated further.

Future tests should further examine the effects of storage and ionizing irradiation on carotenoid content, antioxidant activity, and phenolics in potato. The effects of storage may be explained if more storage times were evaluated. The

interaction between storage time and irradiation dose is significant for a number of antioxidant compounds, and further tests may explain contradictions.

CHAPTER VI
THE EFFECTS OF LOW-DOSE GAMMA-IONIZING IRRADIATION AND STORAGE TIME ON CAROTENOIDS, ANTIOXIDANT ACTIVITY, AND PHENOLICS IN THE POTATO CULTIVAR ATLANTIC

Synopsis

Potatoes are stored to ensure a continuous supply; however, losses due to shrinkage and sprouting can be large. It is believed that low-dose ionizing irradiation will become more prominent for sprout inhibition due to the increasingly higher operating costs of low-temperature storage and possible phase-out of chemical sprout inhibitors. The effects of storage and gamma-ionizing irradiation on carotenoid content, antioxidant activity, and phenolic content were analyzed using the potato cultivar Atlantic. Tubers were subjected to 0, 75, and 200 Gy gamma- irradiation doses, stored at 20 ^{o}C, and analyzed after 0, 10, 20, 75, and 110 days. Carotenoid (xanthophyll) content was determined via absorbance at 445 nm. Individual carotenoid compounds were quantified via HPLC identification based on retention time, spectra, and the combination of retention time and spectra corresponding to standards. Antioxidant activity was determined by the DPPH method and the kinetic reaction was quantified at two times, initially and at stabilization. Phenolic content was determined by the Folin-Ciocalteau method and individual phenolic compounds were quantified via HPLC identification based on retention time, spectra, and the combination of retention time and spectra corresponding to standards. Total carotenoid content via spectrophotometry decreased over time. Total carotenoid content via HPLC quantification based on retention time ranked 0, 75 and 110 days similarly, while total carotenoid content via HPLC quantification based on spectra and the combination of spectra and retention time generally decreased. Antioxidant activity ranked significantly higher at 0 and 110 days, and is believed to be caused by a general decrease in antioxidant activity over time, but due to dehydration, a late stage increase occurred at 110 days. Phenolic content generally increased with storage, and this was supported by HPLC quantification via retention time. HPLC quantification via spectra

and the combination of spectra and retention time showed a decrease in phenolic content. The 0 and 200 Gy dose ranked similar in total carotenoid content, while irradiation did not significantly affect total carotenoid content via HPLC quantification. The 200 and 75 Gy doses ranked higher than the 0 Gy dose. Irradiation dose was again not a significant factor influencing antioxidant activity, phenolic content via the Folin method or total phenolic content via HPLC quantification, but higher doses ranked higher than the 0 Gy control. Storage exerted a much greater influence on carotenoid content, antioxidant activity, and phenolic content than the low-dose gamma-irradiation treatment.

Introduction

Over 34 countries have conducted experiments on sprout inhibition in potatoes by irradiation. Sprout inhibition by irradiation was first identified by Sparrow and Christensen in 1954, who discovered that weight loss and sprouting was reduced with a 1.5 to 20 krep dose of gamma- irradiation. Sprouting is undesirable in potatoes due to loss of quality and the bitter, toxic production of the glycoalkaloid solanine found in the sprouts and greening tissue of light exposed potato. The 5 % loss in the United States due to sprouting is much lower than in less modernized countries, where it is roughly 20 %, due to the use of a number of sprout inhibiting compound chemicals (USDA, 1965; and Thomas et al., 1978). There are some disadvantages to the continued use of chemical methods to control sprouting. Application of chloro-isopropyl carbamate (CIPC) and Tetrachoronitrobenzene (Fusarex) require an airtight warehouse with circulation for the mist chemicals to prevent sprouting in potatoes. This type of secure, air-tight storage facility is rare in some developing and less modernized countries. The other main chemical used for sprout inhibition, maleic hydrazide (MH), must be applied in the field precisely at the right time, or sprouting will not be controlled.

Currently, there are a number of possible reasons why irradiation inhibits sprouting. Factors that may be involved include change in state of cellular colloids,

alkalization of cellular sap in the meristematic tissue, and suppression of nucleic acid synthesis, oxidative enzyme activity, and respiration (Metlitsky et al., 1957).

The source of gamma-radiation is a radioisotope, usually Co-60 or Cs-137, which is unstable and becomes stable by emitting a β-particle and two photons of gamma radiation. Radioactivity is the emission of radiation, and occurs because atomic nuclei are unstable and emit radiation to form new nuclei as they seek to become stable, then becomes new atoms (Panel on Gamma and Electron Irradiation, 2002). Gamma- irradiation is a penetrating energy, although it loses energy by passing through very dense materials such as lead, concrete, and lots of water, a process referred to as attenuation. For most irradiated foods, however, the dose of gamma-irradiation is considered consistent throughout the product.

There are some known chemical changes that are effected by irradiation. Irradiation initiates the normal process of autoxidation of fats; irradiation of proteins that have sulfur may cause a slight breakdown of the amino acids; irradiation can break high-molecular-weight carbohydrates into smaller units, which can cause some fruit to soften; and there is some loss of vitamins such as vitamins C and B_1 (Kilcast, 1995). Most of these chemical changes are a result of exposures to high dosages. Thomas (1984) stated that most of the studies involving low-dose gamma- irradiation indicate that vitamin C is stable during and after exposure. He determined that there was a reduction in vitamin C during the early storage period following irradiation; however, the amount of the vitamin after prolonged storage is reported to be comparable, or even greater than, the non-irradiated tubers stored under identical conditions.

Some studies have reported the effect of low-dose gamma- irradiation on phytochemicals. Janave and Thomas (1979) reported that there was an increase in carotenoid content in non-irradiated potatoes during storage at ambient temperatures (25, 30^{o} C) and a smaller increase at lower temperatures (2, 4, and 15^{o}C). Potatoes exposed to an irradiation dose of 10 krad for sprout inhibition decreased in carotenoid content during storage.

Pendharker and Nair (1975) reported a dose-dependent (2-500 krad with gamma-rays) increase in PAL activity (the enzyme responsible for phenolic formation) in the cortex tissues. They noted two types of activation, one immediately following irradiation and the other occurring during the storage process. Likewise, Penner and Fromm (1972) reported that the content of chlorogenic acid, a phenolic, in irradiated potatoes immediately rises after irradiation and returns to normal within several weeks. Bergers (1981) also noted a time-dependent change in phenolic content with irradiated potatoes. There was a pronounced increase in several specific phenolic compounds, especially β-glycoside of scopoletin, and chlorogenic acid with irradiation and storage.

Significant levels of antioxidants, phenolics and carotenoids within cultivars and advanced breeding lines in the Texas Potato Variety Development Program have been reported (Hale, 2003; Al-Saikhan, 1994, 2000). The effects of cooking, storage, and/ or low-dose gamma- irradiation on 17 cultivars have been investigated (Chapter III, IV, and V); however, there remain unanswered questions regarding the effect of low-dose gamma- irradiation and storage on prominent phytochemicals found in potato. It is unknown how these photochemical levels are affected by low-dose gamma- irradiation, storage time, as well as the interactions of gamma- irradiation dose and storage. The objectives of this experiment were to study the effects of low-dose gamma- irradiation (0, 75, 200 Gy) and storage treatments (no storage, 20 ^{o}C for 10 days, 20 ^{o}C for 20 days, 20 ^{o}C for 75 days, at 20 ^{o}C for 110 days) on total carotenoid content, individual carotenoid content, total phenolic content, individual phenolic content, and total antioxidant activity in the cultivar Atlantic. Cooking was removed from this experiment, so as not to confound the effects of low-dose gamma- irradiation and storage. By eliminating cooking, there will be no production of Maillard reaction products and fewer possible interactions. The long-term objective of this study is to provide the Texas Potato Variety Development Program and the potato industry with information about the effects low-dose gamma- irradiation and storage effects on the cultivar Atlantic.

Materials and Methods

HARVEST LOCATION. Planting and harvesting was conducted near McCook, Texas, located near the Mexican border in the Lower Rio Grande Valley, 30 miles northwest of McAllen in west central Hidalgo County.

PLANT MATERIAL. The early market chipping cultivar Atlantic was used in this study. Atlantic is a standard for potato chip quality in the United States and also is a good cultivar for other cooking processes such as boiling, baking, and French fries.

Two different processing methods were involved, gamma- irradiation and storage. Each potato sample was subjected to one of three doses (0, 75, or 200 Gy) of irradiation, followed by storage at 20 ^{o}C for one of five storage times (0, 10, 20, 75 and 110 days).

GAMMA- IRRADIATION TREATMENT. Three tubers, one for each dose, were surrounded with four alanine dosimeter pellets (Bruker, Billerica, MA) and those allotted for 75 and 200 Gy were also surrounded with two alanine dosimeter films (Kodak, Rochester, NY). The potatoes were transported to the nearby USDA/APHIS Moore Air Field Base in Mission, Texas. At the Mission site, the allotted irradiated samples were subjected to gamma- irradiation via the Cesium- 137 source. Doses were determined based on a pre-calculated dose per time rate per irradiator. The dose per time rates were calculated based on the degradation of the irradiation source. The dose rate at this time was 0.638 Gy per second. Once exposed all potatoes were then transported to College Station for the storage treatment. The selected tubers with the dosimeters were taken to the Electron Beam Food Research Facility at the Institute of Food Science & Engineering at Texas A&M University so doses could be verified using a PC interfaced bench top EPR (electron paramagnetic resonance) spectrometer (Bruker, Billerica, MA).

IRRADIATION. The USDA/APHIS Moore Air Field Base in Mission facility determined dose based on dose per time rates that are calculated periodically based on the degradation of the irradiation source. The dose rate at this time was 0.638 Gy per second. The 0 Gy tuber was surrounded by alanine tablets, while the 75 and 200 Gy

tubers were surrounded by both alanine tablets and films. Films and pellets were placed on two locations of the tuber to determine variability of dose. The selected tubers with the dosimeters were taken to the Electron Beam Food Research Facility at the Institute of Food Science & Engineering, at Texas A&M University after exposure to the gamma- irradiation, therefore doses could be verified, by using a PC interfaced bench top EPR spectrometer. Although, doses were verified, the measured dose should not be taken as the exact dose exposed. Dose based on alanine films and tablets are known to degraded at 20 min after exposure. Doses were measured at almost two days after exposure to irradiation, due to long traveling times from Mission to College Station (Table 6.1). The EPR spectrometer was also not normally used to measure such low doses, so doses were measured based on difference of previous exposed dosage. The alanine films were determined to be too variable for such low doses. Alanine tablets were determined to have less variability; therefore, they will be used in future experiments with electron beam irradiation (Chapter VII). Control, 0 Gy alanine tablets measured an average dose of 40 Gy, and this may be due to spectrometer error or degradation error.

Table 6.1 Measured gamma- irradiation dose via alanine dosimeter films and alanine dosimeter tablets after two days.

Projected Irradiation dose (Gy)	Average Top Films	Average Bottom Films	Average Films	Average Top Pellets	Average Bottom Pellets	Average Pellets
0	N/A	N/A	N/A	35	45	40
75	60	100	80	95	85	90
200	90	170	130	180	220	200

STORAGE TREATMENTS. All samples were stored at 20 ^{o}C for 0, 10, 20, 75 or 110 days. At this time, the samples were analyzed for carotenoid content, antioxidant activity, and phenolic content. Samples stored 0 days were analyzed fresh, or within 24 h after irradiation.

SAMPLE PREPARATION. Three to five potato samples were removed from storage at previously designated times. Each tuber was analyzed separately, and three samples were taken from each of the tubers; samples were then diced with a manual vegetable dicer (The Redco Insta Cut 3.5, Lincoln Foodservice, Fort Wayne, IN). The size of the diced samples was about 0.64 cm cubes. The diced potatoes were mixed, so a randomized sample was obtained. A 5 g sample was taken. Once diced, samples were frozen at -20 ^{o}C (0 ^{o}F) until extraction.

EXTRACTION OF CAROTENOIDS. Due to the lack of carotenes found in preliminary studies, only the xanthophylls were analyzed. The xanthophylls were extracted with methanol (plus 1 g/ L of BHT for stabilization). This extraction procedure was used to quantify the total carotenoid content based on the content of xanthophylls, and the individual carotenoid content via HPLC. Twenty-five mL of methanol plus BHT was added to a 5 g sample of diced potato. This mixture was then homogenized with an ultra turrax tissumizer from Tekmar (Cincinnati, OH). Samples and solvent were stored at -20 ^{o}C for at least 12 h to ensure that the solvent extracted all carotenoids. Samples were then placed in a J-17 rotor at 17,000 rpm for 20 min in a refrigerated centrifuge manufactured by Beckman (model J2-21, Fullerton, CA). Individual carotenoids were analyzed with the combined 2 mL samples from each replication from each tuber, producing a 6 mL which was sample saved in a glass vial (Fig. 6.1). The extracted samples were stored at -29 ^{o}C (-20 ^{o}F).

HPLC ANALYSIS FOR INDIVIDUAL CAROTENOID COMPOUNDS. The extracted samples were concentrated under nitrogen gas and filtered through a 0.45 μm syringe filter (Hale, 2003). The samples were resuspended in 0.5 mL ethanol and 0.5 mL nanopure water. A PC-operated Waters high performance liquid chromatograph was used to analyze individual carotenoid compounds by spectra and retention time.

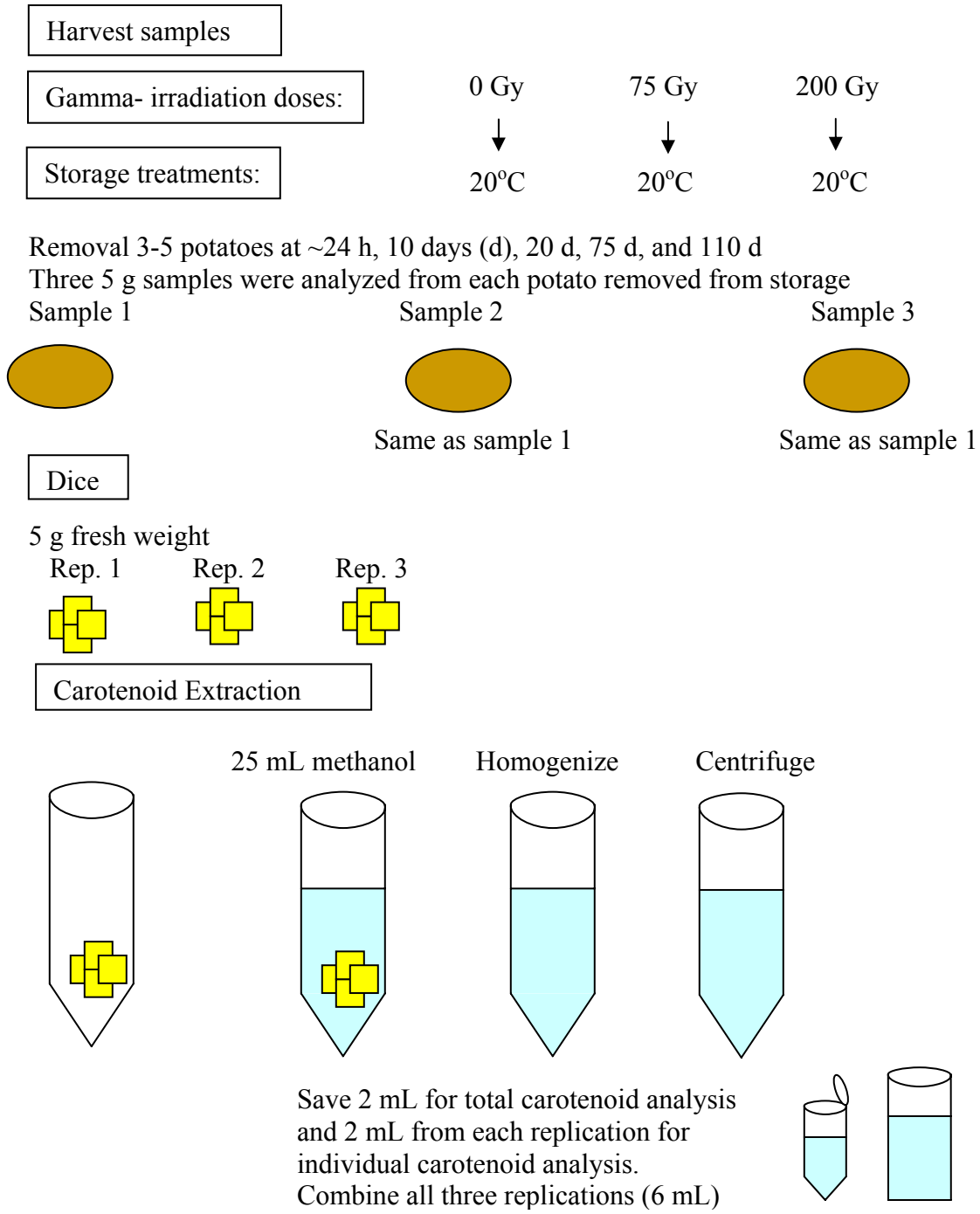

Fig. 6.1 Carotenoid extraction procedure for the factors gamma – irradiation and storage time.

The samples were analyzed using Waters Millennium 3.2 software, Waters 515 binary pump system (Waters 515), an autoinjector (Waters 717 plus), and photodiode detector (Waters 996), along with a column heater (SpectraPhysics SP8792) maintained at 35 ^{o}C. A 4.6 x 250 mm, 5 µm, YMC Carotenoid Column (C-30 reverse phase) (Waters, Milford, MA) was used to separate the carotenoid compounds. The compounds analyzed and used to create a library included: 1) violaxanthin (CaroteNature, Lupsingen, Switzerland), 2) neoxanthin, 3) antheraxanthin (CaroteNature, Lupsingen, Switzerland), 4) β-cryptoxanthin (Hoffman La Roche, Basel, Switzerland), 5) canthaxanthin (Hoffman La Roche, Basel, Switzerland), 6) zeaxanthin (Hoffman La Roche, Basel, Switzerland), and 7) lutein (Hoffman La Roche, Basel, Switzerland). Two filtered and de-gassed solution solvents were used for carotenoid extraction: "solvent A" consisted of methanol, water, and triethylamine (90:10:0.1), while "solvent B" consisted of methanol, MTBE, and triethylamine (6:90:0.1). The following was the gradient for the analysis: (min/ %A) 0/99, 8/99, 8/99, 45/0, 50/0, and 53/99 (Breithaupt and Barmedi, 2002; Hale, 2003).

EXTRACTION OF PHENOLICS AND TOTAL ANTIOXIDANT ACTIVITY. The same extraction procedure was conducted for total phenolic content, individual phenolic content, and total antioxidant activity. Fifteen mL of methanol was added to a 5 g sample of diced potato. This mixture was then homogenized with an ultra turrax tissumizer from Tekmar (Cincinnati, OH). After homogenizing, samples were placed in a J-17 rotor at 17,000 rpm for 20 min in a refrigerated centrifuge (Beckman model J2-21). Two mL of the methanol extract were saved in snap-cap tubes for analysis of total antioxidant activity and total phenolic content. Individual phenolics were analyzed with the combined 2 mL samples from each replication from each tuber; producing a 6 mL sample saved in a glass vial (Fig. 6.2). The extracted samples were stored at -29 ^{o}C (-20 ^{o}F).

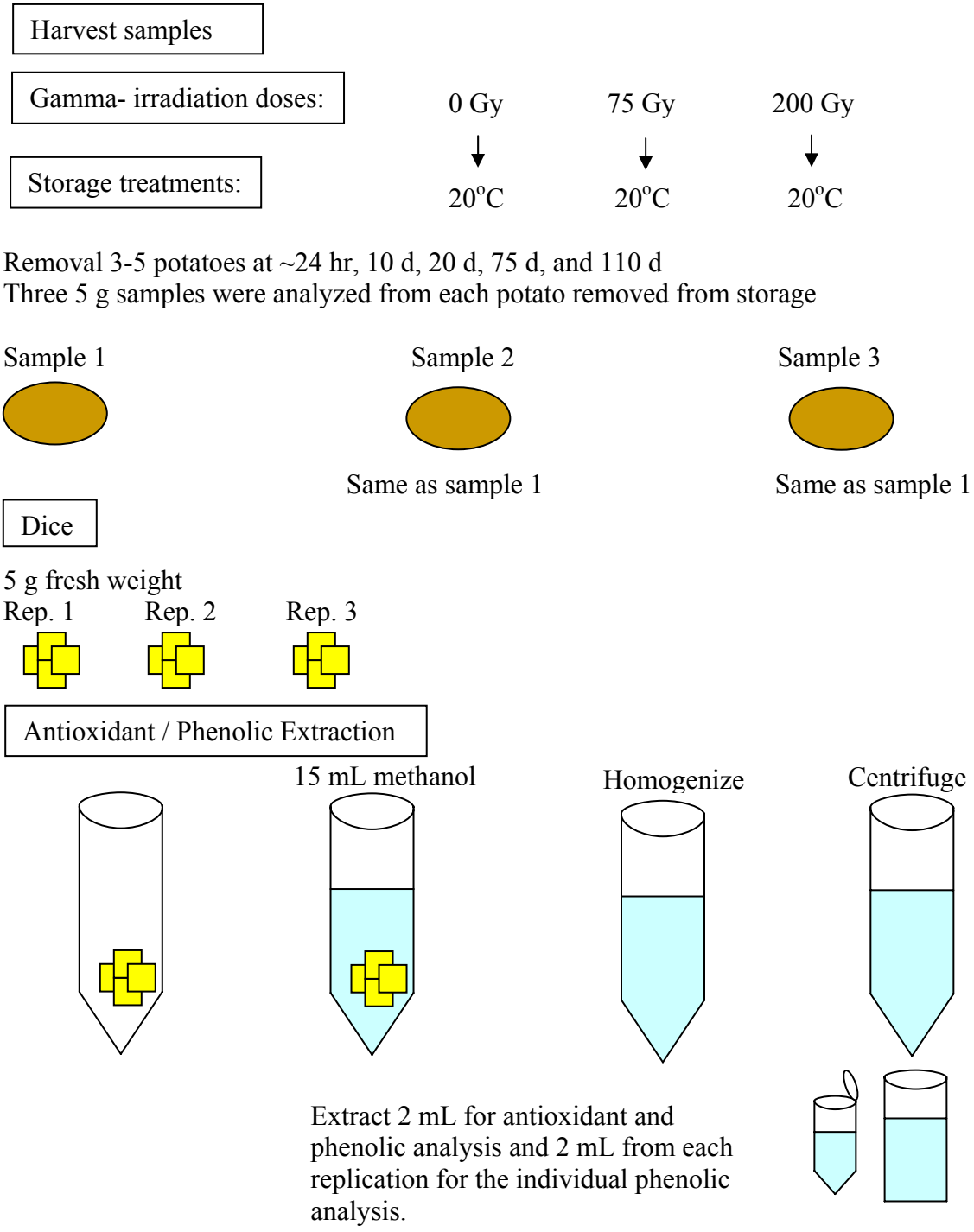

Fig. 6.2 Antioxidant/ phenolic extraction procedure for the factors gamma – irradiation and storage time.

DPPH ASSAY FOR TOTAL ANTIOXIDANT ACTIVITY. Total antioxidant activity was analyzed by using DPPH (1,1 Diphenyl-2 picrylhydrazyl), which is a colorimetric assay first described by Brand-Williams et al. (1995). DPPH is a stable radical which causes oxidation and can be reduced by natural antioxidants, which reduce the oxidizing power of DPPH. Non-reduced DPPH is dark purple, while reduction shifts the color from dark purple to lighter purple to light yellow. This decrease in color and reduction power can be measured at 515 nm. Lower absorbance correlates with a greater amount of antioxidant activity in the sample.

The DPPH solution was diluted by dissolving 24 mg DPPH in 100 mL methanol, which created a 607 μM DPPH stock solution. The solution was then diluted to ~10:55 with methanol until the spectrophotometer read 1.1 at 515 nm. The extracted methanol sample of 150 μL was combined with 2.85 mL of diluted DPPH in a scintillation vial, along with a blank which contained 150 μL of pure methanol (instead of methanol extract) with the diluted 2.85 mL DPPH. The samples were allowed to react for 15 min. The level of reduction was then determined by the absorption at 515 nm in a plastic UV-spectrophotometeric cuvette. This reading is based on the activity of the sample after 15 min (initial antioxidant activity, AOAI), but the total reaction is a kinetic one, which continues for about 24 h until stabilization (stabilized antioxidant activity, AOAS). Each antioxidant compound reacts with the oxidizing substance at a given time; therefore, two readings were taken, with second after 24 h, when the samples and the DPPH had stabilized. The first reading represents an initial response, whereas the second represents a final response. It is currently unknown how long consumed antioxidants are functional. Therefore, these readings may represent two responses. Absorptions was subtracted from the blank, a standard curve using a known antioxidant, trolox, (6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid) was prepared, and a regression curve was calculated to convert the antioxidant activity into equivalents of trolox.

TOTAL PHENOLIC CONTENT. The Folin-Ciocalteau phenol method to determine phenolic content was first described by Swain and Hillis (1959) and modified by

Singleton and Rossi (1965). This method, like the total antioxidant activity method, is a colorimetric reaction that is determined by absorbance. A 0.25 N Folin - Ciocalteau phenol reagent solution with nanopure water and a 1.0 N Na_2Co_3 solution with nanopure water were prepared. The extracted methanol sample of 150 μL was combined with 2.4 mL of nanopure water in a scintillation vial, along with a blank which contained 150 μL of pure methanol (instead of methanol extract) with 2.4 mL of nanopure water. The samples and blank reacted with 150 μL of the 0.25 N Folin - Ciocalteau phenol reagent solution for 3 min. Afterwards, 300 μL of the 1.0 N Na_2Co_3 solution were added to both the samples and blank. The reaction again was kinetic, and stabilization occurred after 1 h and 55 min. Data was recorded at stabilization. Absorption was determined at 725 nm in plastic UV-spectrophotometeric cuvettes. The blank was read first, and the sample absorption based on the cleared response of the blank. The phenolic content was determined by a prepared regression curve to equivalents of chlorogenic acid.

HPLC ANALYSIS FOR INDIVIDUAL PHENOLC COMPOUNDS. The extracted samples were concentrated under nitrogen gas or by using a heated speed vacuum centrifuge and filtered through a 0.45 μm syringe filter (Hale, 2003). A PC-operated Waters high performance liquid chromatograph was used to analyze individual phenolic compounds by spectra and retention time. Samples were analyzed using Waters Millennium 3.2 software, Waters 515 binary pump system (Waters 515), an autoinjector (Waters 717 plus), and a photodiode detector (Waters 996), along with a column heater (SpectraPhysics SP8792) maintained at 40 ^{o}C (104 ^{o}F). A 4.6 x 150 mm, 5 μm, Atlantis C-18 reverse-phase column (Milford, MA) was used to separate phenolic compounds. The compounds analyzed included: 1) 5,7-Trihydroxyflavanone, 2) sinapic acid, 3) kampherol, 4) (-) epicatechin, 5) catechin, 6) quercetin dehydrate, 7) rutin hydrate, 8) protocatechuic acid, 9) salicylic acid, 10) myricetin, 11) syringic acid, 12) gallic acid, 13) vanillic acid, 14) t-cinnamic acid, 15) p-coumaric acid, 16) ferulic acid, 17) caffeic acid, and 18) chlorogenic acid; the standard compounds were obtained from Agros Organics (Pittsburgh, PA).

Two filtered and de-gassed solution solvents were used for the phenolic extraction, "solvent A" consisted of acetonitrile, and "solvent B" consisted of nanopure water, and HCL (pH 2.3). The following gradient was used, (min/%A) 0:85, 5:85, 30:0, 35:0 (Hale, 2003).

STATISTICAL ANALYSIS. A multiple analysis of variance (MANOVA) general linear model was used to determine significant factors. The statistical model of the experiment was a full factorial design. The dependent variables included total carotenoid content, total phenolic content, total antioxidant activity initially (AOAI) (measurements taken after 15 min), and total antioxidant activity stabilization (AOAS) (measurements taken after 24 h). The fixed factors included irradiation dose and storage time. Factor comparison and mean separation was conducted using the post hoc multiple comparison methods of S-N-K tests. A test to measure the estimate of magnitude of effect or strength of association was also conducted. This test determines how strongly two or more variables are related, or how large differences are between the groups. The effect size is reported as eta squared values and is defined as the sums of squares of the effect of interest divided by the total sums of squares (Levine and Hullett, 2002). The analysis was conducted using the statistical package (SPSS) version 11.5.

Results

The objective of this study was to determine the effects of storage and gamma-irradiation on antioxidant activity, phenolics, and carotenoids, with the notion of finding the time when higher irradiation doses began to produce lower antioxidant activity values, and lower irradiation doses produced higher antioxidant activity values. The original schedule for this experiment was 20 days; however, after the 20 days of storage it was determined that this inflection point was not obtained and further storage must occur. Therefore, only the 10 days and 20 days of storage loss values were recorded, because original weight before storage was not recorded (Table 6.2). Past studies (Chapter IV) reported a weight loss with the average of 8.5 % for tubers stored 110 days at 20 ^{o}C.

Table 6.2 Percent weight loss after 10 and 20 days of storage at 20 °C with three irradiation doses.

Irradiation dose (Gy)	10 days storage	20 days storage	Average
0	0.48	0.97	0.73
75	0.63	0.91	0.77
200	0.53	1.10	0.82
Average	0.55	0.99	0.77

STANDARD CURVE FOR LUTEIN. The linear regression equation to equate the spectrophotometric absorbance readings of the methanol extract at 445 nm into lutein equivalents was the following: 3028.6x + 8.1063, where x is the absorbance at 445 nm and y is the µg lutein equivalents per hundred g fresh weight. The R^2 value of this equation was 0.9991.

The average amount of xanthophylls or lutein equivalents was 210 µg/100gfw. Analysis of variance (Table 6.3) indicates that there were significant differences in irradiation dose ($p < 0.000$) and storage time ($p < 0.000$), but the two-factor interaction of irradiation dose and storage time was not significant ($p = 0.180$). The factors' magnitude of strength, or the eta squared values, were irradiation dose 1 %; storage time 90 %; the interaction of irradiation dose and storage 1 %; and error 9 %.

Table 6.3 Analysis of variance results for carotenoid (xanthophyll) content for the factors gamma-irradiation and storage time, McCook 2004.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	920881.895[z]	14	65777.278	131.813	.000
Intercept	7846436.767	1	7846436.767	15723.754	.000
Dose	8707.145	2	4353.573	8.724	.000
Store	905459.912	4	226364.978	453.621	.000
Dose * Store	5775.941	8	721.993	1.447	.180
Error	86829.136	174	499.018		
Total	10795938.752	189			
Corrected Total	1007711.031	188			

[z] $R^2 = 0.914$ (Adjusted $R^2 = 0.907$)

The analysis of variance determined that there were significant differences among storage times. Carotenoid content decreased over time, with the highest carotenoid content at 0 days (282 µg/100gfw) and the lowest at 75 days (115 µg/100gfw) (Table 6.4). This was slightly different than the ranking of the past experiments (Chapters IV and V), where carotenoid content increased with storage time. Although the earlier storage experiments were conducted with tubers harvested near Dalhart, these potatoes were harvested near McCook. These two locations are about 900 miles apart, and some discrepancy may be related to harvest location. Although not significant, the carotenoid content increased after 75 days. This increase was slightly earlier in past studies, and may account for the increased carotenoid content in past studies.

Table 6.4 Storage time ranking for carotenoid (xanthophyll) content, McCook 2004.

Storage time	Eq. Lutein (µg/100gfw)	
0 days	282	a[z]
10 days	270	b
20 days	261	b
110 days	123	c
75 days	115	c

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The analysis of variance determined that there were significant difference among irradiation doses. The dose with the highest carotenoid content was the 0 Gy dose (234 µg/100gfw), while the lowest was the 75 Gy dose at 218 µg/100gfw (Table 6.5). Past studies with irradiation doses ranked both the 75 Gy and 150 Gy doses higher than the 0 Gy dose. There was a significant difference with irradiation dosage. However, the range was rather small, 16 µg/100gfw. Also, irradiation dose only accounted for 1% of the variability via the eta square value.

Table 6.5 Irradiation dose ranking for carotenoid (xanthophyll) content, McCook 2004.

Irradiation dose	Eq. Lutein (µg/100gfw)	
0 Gy	234	a[z]
200 Gy	231	a
75 Gy	218	b

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The interaction of irradiation dose and storage time was not significant, but may help explain some discrepancies. In the first two storage periods (0 and the 10 days) the irradiation dose with the highest amount of carotenoids was 200 Gy (Table 6.6). At 20, 75 and 110 days, the dosage with the highest carotenoid content was 0 Gy. In past studies (Chapter V) the carotenoid content at 75 and 150 Gy ranked higher than the 0 Gy control. However, this may largely be due to the time of analysis (0 and 110 days), since in this study there was a noticeable drop in carotenoid content in higher doses after 20 days of storage. The highest interaction was 0 days, 200 Gy dose (291 µg/100gfw) and the lowest interaction was 75 days, 75 Gy dose (110 µg/100gfw). After 75 days, the carotenoid content began to increase, and this may also help explain why past experiments reported carotenoid content was greater at storage of 20 ^{o}C rather than no storage.

Table 6.6 Carotenoid (xanthophyll) content as influenced by storage time and irradiation dose, McCook 2004.

Storage time and irradiation dose	Eq. Lutein (µg/100gfw)
0 days	
0 Gy	285
75 Gy	271
200 Gy	291
10 days	
0 Gy	270
75 Gy	268
200 Gy	271
20 days	
0 Gy	275
75 Gy	241
200 Gy	267
75 days	
0 Gy	123

Table 6.6 (continued).

Storage time and irradiation dose	Eq. Lutein (µg/100gfw)
75 Gy	110
200 Gy	112
110 days	
0 Gy	134
75 Gy	113
200 Gy	121

DPPH ASSAY FOR TOTAL ANTIOXIDANT ACTIVITY - STANDARD CURVE FOR TROLOX. The linear regression equation to convert the spectrophotometric absorbance readings of the methanol extract and reduced DPPH at 515 nm into trolox equivalents was the following: $y = 891.69x$, where x is the absorbance at 515 nm and y is the µg trolox equivalents per gram fresh weight. The R^2 value of this equation was 0.997.

The average antioxidant activity reported as trolox equivalents was 279 µg/gfw for AOAI and 507 µg/gfw for AOAS. The analysis of variance for AOAI indicates that there were significant differences in storage time ($p < 0.000$) and the interaction of irradiation dose and storage time ($p = 0.005$); however there was no significant difference in irradiation dose ($p = 0.775$) (Table 6.8). The magnitude of strength, or the eta squared values, for AOAI were irradiation dose 0 %; storage time 18 %; the interaction of irradiation dose and storage 10 %; and error 71 %. The analysis of variance for AOAS determined that there were significant differences in storage time ($p < 0.000$) and the interaction of irradiation dose and storage time ($p = 0.003$). There were no significant differences in irradiation dose ($p = 0.541$) (Table 6.7). The magnitude of strength, or the eta squared values, for AOAS were irradiation dose 1 %; storage time 15 %; the interaction of irradiation dose and storage 10 %; and error 73 %.

Table 6.7 Analysis of variance results for antioxidant activity for the factors gamma- irradiation and storage time, McCook 2004.

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	AOAI	387204.753[z]	14	27657.482	5.012	.000
	AOAS	916116.394[y]	14	65436.885	4.644	.000
Intercept	AOAI	13715495.161	1	13715495.161	2485.666	.000
	AOAS	45918758.032	1	45918758.032	3258.843	.000
Dose	AOAI	2822.139	2	1411.069	.256	.775
	AOAS	17383.852	2	8691.926	.617	.541
Store	AOAI	243046.754	4	60761.689	11.012	.000
	AOAS	529668.339	4	132417.085	9.398	.000
Dose * Store	AOAI	128023.728	8	16002.966	2.900	.005
	AOAS	345297.364	8	43162.170	3.063	.003
Error	AOAI	960103.413	174	5517.836		
	AOAS	2451748.861	174	14090.511		
Total	AOAI	16070894.211	189			
	AOAS	51862239.044	189			
Corrected Total	AOAI	1347308.165	188			
	AOAS	3367865.255	188			

[z] $R^2 = 0.287$ (Adjusted $R^2 = 0.230$)
[y] $R^2 = 0.272$ (Adjusted $R^2 = 0.213$)

There were significant differences among storage times. Antioxidant activity for both AOAI and AOAS decreased, then increased with time at 110 days (Table 6.8). The range of AOAI was 94 µg/gfw, with a high at 0 days (334 µg/gfw) and a low at 75 days (240 µg/gfw). The range of AOAS was 118 µg/gfw, with a high at 110 days (577 µg/gfw) and a low at 75 days (459 µg/gfw).

Table 6.8 Storage time ranking for antioxidant activity, McCook 2004.

Storage time	AOAI[z] (µg/gfw)		Storage time	AOAS[y] (µg/gfw)	
0 days	334	a[x]	110 days	577	a
110 days	303	a	0 days	572	a
20 days	264	b	20 days	467	b
10 days	249	b	10 days	466	b
75 days	240	b	75 days	459	b

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Although there were no significant differences between irradiation doses, it is still useful to note the ranking. In both, tests antioxidant activity was highest at 200 Gy and lowest at 0 Gy (Table 6.9). In Chapter V, a trend was noted that early in storage higher doses resulted in greater antioxidant activity, while later in storage, lower doses resulted in greater antioxidant activity. Antioxidant activity was measured five times during this study. Three of those times, 0, 10, and 20 days, are much earlier than 75 and 110 days. If the inflection point, the time where higher doses switched from having greater antioxidant activity to lower antioxidant activity, occurred between 20 and 75 days then the overall ranking of irradiation dose, independent of storage time, would have ranked higher doses greater in antioxidant activity. Therefore, the ranking in Table 9 was greatly dependent on the times chosen to measure antioxidant activity, due to the significant interaction, and if more measurements occurred after the inflection point then the lower dosages would have ranked higher.

Table 6.9 Irradiation dose ranking for antioxidant activity, McCook 2004.

Irradiation dose	AOAI[z] (µg/gfw)	Irradiation dose	AOAS[y] (µg/gfw)
200 Gy	292 a[x]	200 Gy	522 a
75 Gy	276 a	75 Gy	511 a
0 Gy	269 a	0 Gy	487 a

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[x] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

There were significant differences in the interaction of storage time and irradiation dose. The highest AOAI was at 0 days, 200 Gy (363 µg/gfw), with the lowest at 10 days, 0 Gy (215 µg/gfw). The highest AOAS was at 110 days, 75 Gy (635 µg/gfw), while the lowest was at 20 days, 0 Gy (422 µg/gfw) (Table 6.10). This interaction is similar to past interactions of irradiation and storage. During early storage (0-20 days) higher irradiation doses resulted in greater antioxidant activity, while at 75 days the lower doses or 0 Gy resulted in higher antioxidant activity. At 110

days, the 200 Gy irradiation dose was also lower in antioxidant activity than the lower irradiation doses. Irradiation may have caused an induction of antioxidants initially after exposure, while continued storage may have caused an induction of antioxidants in the 0 Gy due to the added stress of dehydration.

Table 6.10 Antioxidant activity as influenced by storage time and irradiation dose, McCook 2004.

Storage time and irradiation dose	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
0 days		
0 Gy	302	527
75 Gy	336	549
200 Gy	363	620
10 days		
0 Gy	215	431
75 Gy	258	528
200 Gy	272	440
20 days		
0 Gy	258	422
75 Gy	232	426
200 Gy	302	553
75 days		
0 Gy	260	510
75 Gy	225	441
200 Gy	235	428
110 days		
0 Gy	332	562
75 Gy	332	635
200 Gy	244	535

TOTAL PHENOLIC CONTENT. The linear regression equation to equate the spectrophotometric absorbance readings at 727 nm using the Folin method into chlorogenic acid equivalents was as follows: $y = 0.5775x - 0.0279$, where x was the absorbance at 727 nm after zeroing the spectrophotometer with a blank lacking antioxidant extract, but containing all other solutions, and y was the µg chlorogenic acid equivalents per g fresh weight. The R^2 value of this equation was 0.970.

The average phenolic content reported as equivalents of chlorogenic acid was 553 µg/gfw. There were significant differences in storage time ($p < 0.000$) and the interaction of irradiation dose and storage time ($p = 0.014$), while irradiation dose alone

was not significant (p = 0.832) (Table 6.11). The magnitude of strength, or the eta squared values, were irradiation dose 0 %; storage time 17 %; the interaction of irradiation dose and storage 8 %; and error 74 %.

Table 6.11 Analysis of variance results for phenolic content for the factors gamma- irradiation and storage time, McCook 2004.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	1768614.154[z]	14	126329.582	4.369	.000
Intercept	57898725.751	1	57898725.751	2002.424	.000
Dose	10668.692	2	5334.346	.184	.832
Store	1158638.187	4	289659.547	10.018	.000
Dose * Store	575257.722	8	71907.215	2.487	.014
Error	5031092.017	174	28914.322		
Total	64569492.034	189			
Corrected Total	6799706.171	188			

[z] $R^2 = 0.260$ (Adjusted $R^2 = 0.201$)

There were significant differences among storage times. Phenolic content increased with storage (Table 6.12). The range of phenolic content was 224 µg/gfw. The highest phenolic content was 714 µg/gfw at 110 days, while the lowest was 490 µg/gfw at 0 days. In Chapter IV, the 20^{o}C for 110 day storage treatment ranked slightly below the no storage treatmentp; however, not significantly lower. Also in Chapter V, the 4 ^{o}C for 110 day storage treatment ranked significantly lower than the no storage treatment. The individual phenolics as reported in Chapter IV also indicated the storage treatments were significantly lower than the no storage treatments. However, in Chapter V the storage treatments ranked significantly greater than the no storage treatments. The individual phenolics via HPLC for this study may help explain past discrepancies.

Table 6.12 Storage time ranking for phenolic content, McCook 2004.

Storage time	Eq. chlorogenic acid (µg/gfw)	
110 days	714	a[z]
75 days	618	b
20 days	540	bc
10 days	493	c
0 days	490	c

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

There were no significant differences among irradiation treatments (Table 6.13). The range for phenolic content was 32 µg/gfw, with the highest phenolic content of 572 µg/gfw at 200 Gy, and the lowest 540 µg/gfw at 0 Gy. This is similar to past experiments (Chapter V), where higher doses ranked higher in phenolic content than lower doses.

Table 6.13 Irradiation dose ranking for phenolic content, McCook 2004.

Irradiation dose	Eq. chlorogenic acid (µg/gfw)	
200 Gy	572	a[z]
75 Gy	547	a
0 Gy	540	a

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The interaction of storage time and irradiation dose was significant. The greatest phenolic content (787 µg/gfw) was at 110 days at 75 Gy, and the lowest (430 µg/gfw) was at 0 days, 75 Gy (Table 6.14). Although the interaction was significant, no discernable trend was revealed by with this interaction.

Table 6.14 Storage by irradiation dose interaction for phenolic content, McCook 2004.

Storage time and irradiation dose	Eq. chlorogenic acid (μg/gfw)
0 days	
0 Gy	548
75 Gy	430
200 Gy	492
10 days	
0 Gy	386
75 Gy	549
200 Gy	545
20 days	
0 Gy	531
75 Gy	474
200 Gy	616
75 days	
0 Gy	633
75 Gy	619
200 Gy	601
110 days	
0 Gy	707
75 Gy	787
200 Gy	647

HPLC ANALYSIS FOR CAROTENOID COMPOUNDS. Sixty-three samples were analyzed for individual carotenoid content via HPLC. Although there were seven compounds analyzed, only three (lutein, zeaxanthin, and canthaxanthin) were identified through retention time. Storage time significantly ($p < 0.000$) influenced the presence or amounts of all three compounds based on retention time (Table 6.15). Lutein increased with storage, zeaxanthin and canthaxanthin decreased, while total carotenoid content decreased then increased at 75 days. Individual carotenoid content was determined at the five storage times based on spectra and the combination of spectra and retention time (Table 6.15) and the only compound detected was lutein. The amount of lutein increased with storage, with an insignificant dip at 10 days. This finding differs with those in past studies (Chapter V); however, lutein content also increased with storage in Chapter IV. In Chapter IV, where multiple temperatures were included in the analysis, both total carotenoid content and lutein content increased with storage at 20 ^{o}C, which corresponds to the results of the present analysis.

Table 6.15 Storage ranking for individual carotenoid compounds[z] (µg/100 gfw), McCook 2004.

Storage time	LUT	ZEA	CAN	Total-R	LUT-SP	LUT-RSP
0 days	11 b[y]	12 a	9 a	31 a	7 b	0 b
10 days	11 b	0 b	5 ab	16 b	2 b	2 b
20 days	11 b	2 b	1 b	14 b	9 b	7 b
75 days	37 a	0 b	0 b	37 a	22 a	18 a
110 days	33 a	0 b	0 b	33 a	26 a	26 a

[z]LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The ranking of irradiation doses based on retention time, spectra, and retention time and spectra is presented in Table 6.16. Irradiation dose did not significantly affect any of the individual carotenoids analyzed based on retention time. Therefore, no discernable trend based on irradiation dose could be determined. While again not significant, lutein content based on spectra and retention time and spectra tended to increase with higher irradiation dose.

Table 6.16 Irradiation dose ranking for individual carotenoid compounds[z] (µg/100 gfw), McCook 2004.

Irradiation dose	LUT	ZEA	CAN	Total-R	LUT-SP	LUT-RSP
0 Gy	19 a[y]	3 a	2 a	24 a	11 a	6 a
75 Gy	19 a	2 a	3 a	26 a	14 a	11 a
200 Gy	24 a	2 a	5 a	29 a	15 a	15 a

[z]LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The interaction of storage time and irradiation dose based on retention time, spectra, and retention time and spectra is shown in Table 6.17. The only significant differences, were with lutein content and total carotenoid content (p =0.010 and 0.031 respectively) (not shown). Over all, levels of lutein and total carotenoids based on retention time were greater at higher irradiation doses, except at 10 days, where the 0 Gy dose resulted in more lutein and higher total carotenoids. The low lutein levels during early storage might indicate that lutein was overlooked by the spectra.

Table 6.17 Storage by irradiation dose interaction for individual carotenoid compounds[z] (µg/100 gfw), McCook 2004.

Storage	Irradiation dose	LUT	ZEA	CAN	Total-R	LUT-SP	LUT-RSP
0 days							
	0	7	11	10	28	10	0
	75	13	12	9	34	9	0
	200	13	12	8	33	3	0
10 days							
	0	27	0	0	27	7	7
	75	0	0	10	10	0	0
	200	7	0	5	12	0	0
20 days							
	0	0	5	0	5	0	0
	75	7	0	4	11	7	0
	200	27	0	0	27	21	21
75 days							
	0	34	0	0	34	12	0
	75	40	0	0	40	40	40
	200	35	0	0	35	13	13
110 days							
	0	25	0	0	25	25	25
	75	34	0	0	34	13	13
	200	40	0	0	40	40	40

[z]LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

HPLC ANALYSIS FOR PHENOLIC COMPOUNDS. Sixty-one HPLC phenolic samples were analyzed. Although there were eighteen compounds analyzed for individual phenolics, only thirteen (chlorogenic acid, caffeic acid, t-cinnamic acid, gallic acid, rutin hydrate, sinapic acid, epicatechin, quercetin dihydrate, protocatechuic acid, myricetin, p-coumaric acid, catechin, and vanillic acid) were identified based on retention time. Storage time resulted in significant differences in caffeic acid ($p = 0.003$), t-cinnamic acid ($p < 0.000$), epicatechin ($p = 0.037$), quercetin dihydrate ($p < 0.000$), catechin ($p = 0.019$), and vanillic acid ($p < 0.000$) (Table 6.18). The ranges for the compounds that were significantly affected by storage were very small. The averages of gallic acid, rutein hydrate, and protocatechuic acid tended to increase with storage; however, storage was an insignificant factor for these compounds. This increase may be related to the increase in phenolic content based on the Folin method during storage. Past studies (Chapter IV and V) did not report such a large increase in these compounds.

Table 6.18 Storage ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, McCook 2004.

Storage	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
0 days	231	41	10	145	90	53	8	20	59	24	16	197	10	905
	a[y]	a	a	a	a	a	b	a	a	a	a	ab	a	a
10 days	208	41	9	383	81	41	8	14	88	21	17	198	10	1118
	a	a	b	a	a	a	b	b	a	a	a	ab	a	a
20 days	177	40	9	522	266	46	8	14	85	21	14	196	9	1408
	a	b	b	a	a	a	b	b	a	a	a	b	b	a
75 days	163	41	10	225	535	44	8	18	96	22	16	198	10	1385
	a	ab	a	a	a	a	b	a	a	a	a	ab	a	a
110 days	185	41	9	546	443	47	8	15	126	24	19	199	10	1675
	a	a	b	a	a	a	a	b	a	a	a	a	a	a

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The effect of storage time on individual phenolic content based on spectra and the combination of spectra and retention time is presented in Table 6.19. The only compounds to match spectra were chlorogenic acid, caffeic acid, rutin hydrate, and epicatechin. The only compounds to match both spectra and retention time were chlorogenic acid, caffeic acid, and rutin hydrate. The greatest phenolic contents were during the early stages of storage, as compared to later stages, as seen with total phenolic content via the Folin method and individual phenolics based on retention time.

Table 6.19 Storage ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both retention time and spectra, McCook 2004.

Storage	CH-SP	CA-SP	RU-SP	EP-SP	Total-SP	CH-RSP	CA-RSP	RU-RSP	Total-RSP
0 days	254 a[y]	246 a	80 a	4 a	585 a	214 a	41 a	66 a	321 a
10 days	264 a	237 a	56 a	1 a	558 ab	163 ab	41 a	47 a	251 b
20 days	243 a	186 ab	65 a	2 a	495 bc	140 ab	37 a	55 a	232 ab
75 days	256 a	186 ab	50 a	0 a	491 bc	146 ab	36 a	45 a	227 ab
110 days	244 a	143 b	35 a	0 a	423 c	105 b	27 b	32 a	164 c

[z]CH-SP : Chlorogenic acid based on spectra
CA-SP : Caffeic acid based on spectra
RU-SP : Rutin hydrate based on spectra
EP-SP : Epicatechin based on spectra
Total-SP : the addition of all measured phenolics based on spectra
CH-RSP : Chlorogenic acid based on retention time and spectra
CA-RSP : Caffeic acid based on retention time and spectra
EP-RSP : Epicatechin based on retention time and spectra
Total-RSP : the addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

There were few significant differences in the effect of irradiation on individual phenolics based on retention time (Table 6.20). The effects on t-cinnamic acid ($p = 0.011$) and quercetin dihydrate ($p = 0.001$) (ANOVA table not shown) were significantly different, but the range between doses for these two compounds was very

small. Over all, these results are similar to those for total phenolic content based on both the Folin method and past experiments (Chapter V).

Table 6.20 Irradiation dose ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, McCook 2004.

Irradiation dose	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
0 Gy	197 a[y]	41 a	10 b	233 a	369 a	44 a	8 a	14 b	87 a	22 a	17 a	198 a	10 a	1249 a
75 Gy	187 a	41 a	10 b	317 a	277 a	47 a	8 a	15 b	96 a	22 a	17 a	198 a	10 a	1244 a
200 Gy	195 a	41 a	10 a	543 a	204 a	48 a	8 a	19 a	90 a	23 a	16 a	197 a	10 a	1402 a

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 6.21 ranks the effect of the irradiation doses on individual phenolics, based on spectra and the combination of spectra and retention time. Although not significant caffeic acid, rutin hydrate, and total individual phenolic content tended to increase with increasing irradiation dose.

Table 6.21 Irradiation dose ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both retention time and spectra, McCook 2004.

Irradiation dose	CH-SP	CA-SP	RU-SP	EP-SP	Total-SP	CH-RSP	CA-RSP	RU-RSP	Total-RSP
0 Gy	248 a[y]	172 a	54 a	3 a	476 a	152 a	35 a	46 a	233 a
75 Gy	260 a	197 a	57 a	2 a	515 a	154 a	35 a	47 a	236 a
200 Gy	249 a	231 a	61 a	0 a	541 a	155 a	39 a	54 a	248 a

[z]CH-SP : Chlorogenic acid based on spectra
CA-SP : Caffeic acid based on spectra
RU-SP : Rutin hydrate based on spectra
EP-SP : Epicatechin based on spectra
Total-SP : the addition of all measured phenolics based on spectra

CH-RSP : Chlorogenic acid based on retention time and spectra
CA-RSP : Caffeic acid based on retention time and spectra
EP-RSP : Epicatechin based on retention time and spectra
Total-RSP : the addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Due to limited significant differences in the interaction between storage time and irradiation dose, discussion of the interaction will be omitted. Over all, storage had a greater influenced the amounts of the compounds than irradiation dose, and trends mimicked those found in Tables 6.18 and 6.19.

Discussion and Conclusion

This study further analyzed the effects of storage and irradiation dose on carotenoids, phenolics and antioxidant activity. Not all trends were similar to earlier studies, however, the experimental design was not the same as in past experiments. The potatoes used in this study were harvested near McCook, Texas, but were compared to those harvested near Dalhart, Texas (Chapters IV, and V), almost 900 miles away. This study also included only one storage temperature, but numerous storage times. In spite of those design differences certain trends could be seen across experiments.

Total carotenoid content decreased with storage but, although not significant, tended to increase after 75 days. Individual carotenoids also decreased with storage, but later increased, based on retention time. A similar trend was also noted with individual carotenoid content based on spectra. These results supported the findings of

Craft and Wise (1993) and Thomas and Joshi (1977) who also found decreases in carotenoid content during storage. In the present study, the carotenoids identified by both spectra and retention time increased with storage. Storage increased carotenoid content in earlier experiments, and carotenoid content was also reported to increase with storage at 25-30 ^{o}C in a study conducted by Bhushan and Thomas (1990) and Janave and Thomas (1979). Over all, irradiation dose had little effect on carotenoid content.

Antioxidant activity decreased, then increased with storage. Higher irradiation doses tended to increase antioxidant activity, but not significantly more than the 0 Gy dose. This trend differed from past experiments (Chapter V), but may be related to the times selected for measurement of antioxidant activity. As in past studies (Chapter V), antioxidant activity was higher with higher dosages early in storage, but decreased as storage progressed. Past studies conducted by Bergers (1981), Patil et al. (1999), and Penner and Fromm (1972) also noted interactions with storage and irradiation in antioxidant phenolics. In the present study, a decrease in antioxidant activity with higher dosages was also noted, but after 75 days.

Total phenolic content based on the Folin method increased with storage; however, past experiments (Chapters IV and V) reported decreases with storage. Individual phenolics identified based on HPLC retention time also indicated an increase in phenolic content, most notably due to some large values for gallic acid, rutein hydrate, and protocatechuic acid found in the later storage times. Zafrilla et al. (2001) also reported an increase in ellagic acid in strawberry with storage. In the present study, phenolics, based on identification of spectra and the combination of spectra and retention time did not include the compounds with large increases; therefore, storage caused a decrease in phenolics based on these methods. A decrease in phenolic compounds with prolonged storage was also reported in the studies conducted by Häkkinen et al. (2000) and Percival and Baird (2000). Irradiation dose did not significantly affect phenolic content; however, the ranking of total phenolic content based on the Folin method, and individual phenolics identified based on

retention time, spectra, or the combination of spectra and retention time all ranked higher at doses higher than 0 Gy. This trend was also identified for total phenolic content based on the Folin method in earlier experiments (Chapter V). This may be due to the increase of certain compounds, such as gallic acid, in the individual phenolics based on retention time, and increases of caffeic acid and rutein hydrate in the individual phenolic analysis based on spectra and the combination of spectra and retention time. The mechanism of increase due to irradiation should be investigated in the future. One possible cause of phenolic increases is the induction of the PAL enzyme. Pendharker and Nair (1975, 1987) have reported two separate inductions of PAL with exposure to irradiation.

CHAPTER VII
THE EFFECTS OF LOW-DOSE ELECTRON BEAM IRRADIATION, AND STORAGE TIME AND TEMPERATURE ON CAROTENOIDS, ANTIOXIDANT ACTIVITY, AND PHENOLICS IN THE POTATO CULTIVAR ATLANTIC

Synopsis

Potatoes are stored to ensure a continuous supply; however, losses due to shrinkage and sprouting can be large. It is believed that ionizing irradiation will become more prominent for sprout inhibition due to the increasingly higher operating costs of low-temperature storage and possible phase-out of chemical sprout inhibitors. Moreover, electron beam irradiation may become more popular than gamma-irradiation due to no radioactive waste, more precise dosing, and security reasons. The effects of storage and low-dose electron beam irradiation on carotenoid content, antioxidant activity, and phenolic content were analyzed using the potato cultivar Atlantic. Tubers were subjected to a surface dose of 0 or 200 Gy e-beam irradiation, stored at either 4 ^{o}C or 20 ^{o}C, and then whole tubers were analyzed after 0, 10, 20, 75, and 110 days. A separate analysis was conducted on the exterior and interior surfaces of tubers for each treatment. Carotenoid content (xanthophyll content) was determined via absorbance at 445 nm. Individual carotenoid compounds were quantified via HPLC identification based on retention time, spectra, and the combination of retention time and spectra corresponding to standards. Antioxidant activity was determined by the DPPH method and the kinetic reaction was quantified at two times, initially and at stabilization. Phenolic content was determined by the Folin-Ciocalteau method, and individual phenolic compounds were quantified via HPLC identification based on retention time, spectra, and the combination of retention time and spectra corresponding to standards. Carotenoid content decreased with storage and then later increased. The 110 day storage stage may have ranked significantly higher using both spectrophotometric methods and HPLC, perhaps due to dehydration and concentration. This trend was more prominent when both the exterior and interior surfaces were

evaluated. A similar trend where some of the later stages of storage ranked higher was observed for both antioxidant activity and phenolic content. Storage temperature appeared to influence dehydration. The exterior and interior surfaces were higher in antioxidants and phenolics at 20 ^{o}C than at 4 ^{o}C, while whole tubers were higher at 4 ^{o}C. Irradiation dose appeared to have little to no significant affect on carotenoid content and antioxidant activity, and only resulted in a slight increase in phenolic content. This effect was less prominent in the HPLC identification for total phenolics of both exterior and interior samples. There were numerous significant interactions, most notably storage time and irradiation dose; storage temperature and irradiation dose for carotenoid content, and the storage time and irradiation dose for antioxidant activity.

Introduction

Many national and international organizations, including the United States Food and Drug Administration, the United States Department of Agriculture (USDA), the American Medical Association, the National Center for Food Safety and Technology, the United States Army, the World Health Organization, the Food and Agricultural Organization of the United Nations (FAO), and the Codex Alimentarius Commission, have approved the use of irradiated foods and declared the food safe when Good Manufacturing Practices (GMPs) and Good Irradiation Practices are conducted. The current reasons for food irradiation include: reduction of losses due to spoilage, contamination, or infestation; control of food borne diseases; reduction of disease transmittal, pests, and insects by international trade; lessen the use of food chemical treatments that are either restrictive or prohibitive; and extend shelf life (Gerst, 2003). Potatoes, a food commodity that is stored for extended periods, could benefit from the use of low-dose irradiation by inhibiting sprouting. Spouting renders potatoes unusable for processing, and the glycoalkaloids in spouts are toxic if consumed in high amounts. In 1999, the International Consultative Group on Food Irradiation, recommended a dose of 0.05-0.15 kGy for the purpose of sprout inhibition in potatoes. Low temperature storage and chemical sprout inhibitors are currently used

in most modern industrial nations, but this option is not always available in less modernized nations, due to the high operating costs of storage facilities. Furthermore, chemical sprout inhibitors are subject to increasingly stringent regulations and possible of phase-out. In the United States, with the combination of low-temperature storage and chemical sprout inhibitors, the percentage of potato crop loss to sprouting is about 5 % (USDA, 1965).

Potatoes, although considered to be mostly a western commodity, are a growing food in Asia, with per capita consumption rose from 12 kg/capita in 1991-92 to 14 kg/capita in 1994-96 (International Potato Center, 2004). In India, there are two harvest seasons which result in a production over six months. The market supply must be continuous after this period; therefore, radiation processing could help to ensure a steady supply (Thomas et al., 1978). Other countries, such as Nigeria, experience high losses of their tuber and root crops; the losses of yams range from 15-60 % from farm to distribution (Ogundana, 1971). These losses are due to desiccation, loss of carbohydrate due to respiration, breakage of dormancy and sprouting, microbial decay, and nematodes. Although irradiation could be used to prevent losses in many nations, only one country, Japan, is currently using irradiation for the purpose of inhibiting sprouts commercially. About 15,000 tons of potatoes are irradiated per year in Hokkaido, Japan, with a crop value of 16 million dollars (Kume et al. 2002).

Many consumers have reservations about food irradiation, mostly due to the misconception that the food is radioactive, as well as the danger of storage, disposal, and control of radioactive sources. An alternative to producing radiation from a radioactive source is the use of electron beam irradiation. This type of irradiation uses electricity as the energy source; therefore, it can simply be switched on and off. Electrons are emitted by heating a tungsten filament. A large potential (voltage) difference is built between the cathode and the anode in an electron accelerator. The negatively charged electrons are accelerated via microwaves, through a vacuum, towards the positive anode almost at the speed of light (~190,000 miles/second) and are steered through a hole in the anode to form an intense electron beam. Products to be

irradiated are loaded on a conveyor belt, and passed under the electron beam (Panel on Gamma and Electron Irradiation, 2002). Another major difference between electron beam irradiation and gamma- irradiation, is that electrons have mass so they are also greatly affected by density. Also, doses are not as consistent, as with gamma-irradiation, because they are attenuated throughout the mass (Figs. 7.1, 7.2).

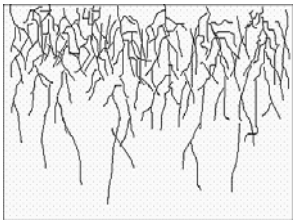

Fig 7.1 Dose penetration of a single beam through a mass. (Vestal, 2004).

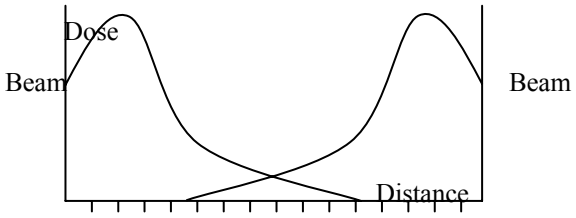

Fig 7.2 Dual beam measured dose through a mass.

Therefore, electron beam irradiation is currently limited to relatively thin packages (< 2 inches) because of its low penetrating power. In 2000, the Institute of Food Science and Engineering at Texas A&M University formed a partnership with the SureBeam Corporation, a corporation that conducts electron beam food irradiation. Recently, the Institute has assumed control of the Sure Beam research facility from parent company, Titan and it now houses the new National Center for Electron Beam

Food Research, which investigates uses of electron beam irradiation, and is a federally inspected USDA inspection center.

Past research has reported significant levels of antioxidants, phenolics and carotenoids within cultivars and advanced breeding lines in the Texas Potato Variety Development Program (Hale, 2003; Al-Saikhan, 1994, 2000). Earlier chapters have reported the effects of cooking, storage and low-dose gamma- irradiation on these phytochemicals. The effects of electron beam irradiation on phytochemical levels were analyzed and reported in the present chapter. It is unknown how these photochemical levels are affected by low-dose electron beam irradiation and storage over a period of time. The objectives of the present experiment were to study the effects of low-dose electron beam irradiation, storage time, and storage temperature on carotenoid content, antioxidant activity, and phenolic content in the cultivar, Atlantic. The long term objective was to provide the Texas Potato Variety Development Program, and the potato industry with information about low-dose electron beam irradiation effects and storage effects.

Materials and Methods

HARVEST LOCATION. Planting and harvesting were conducted near Springlake, Texas, located in north central Lamb County on the High Plains, 59 miles northwest of Lubbock.

PLANT MATERIAL. One processing cultivar Atlantic, was used in this study. Atlantic is a standard for potato chip quality in the United States and also is a good cultivar for other cooking processes such as boiling, baking, and frying.

Three different processing methods were involved, electron beam irradiation, storage temperature, and storage time. Tubers of similar size and weight were used in each treatment. Each potato sample was subjected to one of two surface-doses (0, or 200 Gy) of irradiation, then to one of two storage temperatures (4 ^{o}C, or 20 ^{o}C), and one of five storage times (0, 10, 20, 75 and 110 days).

DOSIMETRY AND ELECTRON BEAM IRRADIATION TREATMENT. The dosing process for the electron beam irradiation treatment was slightly more

complicated than that for gamma- irradiation, due to the mechanics of the system and the lack of protocols for similar products. All electron beam irradiation was conducted at the National Center for Electron Beam Food Research of the Institute of Food Science & Engineering, at Texas A&M University. The facility houses two electron accelerators which can be used separately or in combination, as a dual over and under beam. A series of preliminary dosimetry experiments were conducted before irradiating the samples. Dosimetry experiments were necessary because the desired irradiation dose was much lower than that commonly used at that facility. The shape of the tuber presented some dilemmas because commonly used products are much thinner and more compact. Also, the low penetration power of electron irradiation presented a unique problem, as well. Doses were measured via alanine dosimeter pellets (Bruker, Billerica, MA) and a PC-interfaced bench top EPR (electron paramagnetic resonance) spectrometer (Bruker, Billerica, MA). A series of experiments were conducted by surrounding potato tubers with high density polyethylene plastics to achieve a desired dose. A selected tuber was surrounded with four dosimetric alanine tablets, wrapped in a plastic party balloon, and used for dosimetry experiments. A group of 10 tubers (including the one with the alanine tablets which was surrounded on all four sides by other potatoes) were placed on a series of high density polyethylene (HDPE) sheets used as attenuation (attenuation scheme). Attenuator sheets were placed on the sides and top of the stack of potatoes. The speed of the conveyor and the width of the attenuation were used to control the dose. Experiments were conducted until an even desirable dose was obtained on all four sides (top, bottom, left and right sides) of the dosimetric potato. An example of this dosimetry process was the following: if the top dose was too high during the experiments, one could increase width of the top HDPE sheets, or increase the speed of the conveyor. If the dose was uneven, one could slow down the conveyor, add high density polyethylene pellets to the spaces between the tubers, or change the attenuation scheme. Preliminary experiments were conducted until an appropriate attenuation scheme and conveyor speed was achieved. A desired average surface dose of 200 Gy

was achieved with the following attenuation scheme: 4.8 cm HDPE plastic sheets on the top, 4.3 cm HDPE plastic sheets on all four sides, 4.6 cm HDPE plastic sheets on the bottom; both top and bottom electron accelerators were used; and the conveyor speed was 18.2 meters per min (Fig. 7.3).

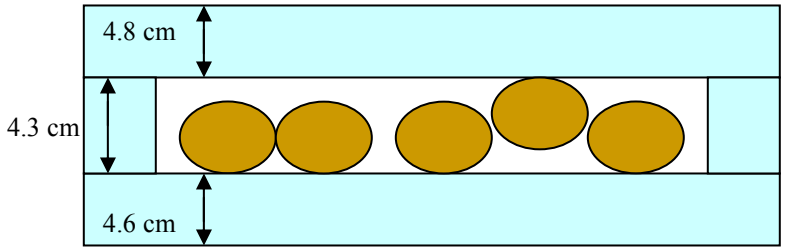

Fig 7.3 Attenuation scheme.

All tubers were then subjected to the same attenuation scheme and conveyor speed determined in the dosimetry tests. Following exposure to irradiation, tubers were transported to the Horticultural Sciences building and subjected to a various storage treatments.

STORAGE TREATMENTS. All samples were stored at either 4 ^{o}C or 20 ^{o}C for 0, 10, 20, 75 or 110 days. Following storage, samples were analyzed for carotenoid content, antioxidant activity, and phenolic content. Samples stored 0 days were analyzed fresh, or within 24 hours after irradiation.

SAMPLE PREPARATION. Three tubers from each treatment were removed from storage at the designated times. Each tuber was analyzed separately, and three samples were obtained from each of the three tubers, resulting in three replications per tuber. They were diced with a manual vegetable dicer (The Redco Insta Cut 3.5, Lincoln Foodservice, Fort Wayne, IN). The size of the diced samples was roughly 0.64 cm cubes. The diced potatoes were mixed, so a randomized sample was obtained, and a 5 g sample was taken. Once diced, samples were frozen at -20 ^{o}C (0 ^{o}F) until extraction.

EXTRACTION OF CAROTENOIDS. Due to the lack of carotenes found in preliminary studies, only the xanthophylls were analyzed. The xanthophylls were extracted with methanol (plus 1 g/ L of BHT for stabilization) and this extraction procedure quantified the total carotenoid content based on xanthophyll content and individual carotenoid content via HPLC. Twenty-five mL of methanol plus BHT was added to a 5 g sample of diced potato. This mixture was then homogenized with an ultra turrax tissumizer from Tekmar (Cincinnati, OH). Samples and solvent were stored at -20 ^{o}C for at least 12 h to ensure that the solvent extracted all carotenoids. Samples were then placed in a J-17 rotor at 17,000 rpm for 20 min in a refrigerated centrifuge manufactured by Beckman (model J2-21, Fullerton, CA). Individual carotenoids were analyzed with the combined 2 mL samples from each replication from each tuber; producing a 6 mL sample saved in a glass vial (Fig. 7.4). The extracted samples were stored at -29 ^{o}C (-20 ^{o}F).

HPLC ANALYSIS FOR INDIVIDUAL CAROTENOID COMPOUNDS. The extracted samples were concentrated under nitrogen gas and filtered through a 0.45 μm syringe filter (Hale, 2003). The samples were resuspended in 0.5 mL ethanol and 0.5 mL nanopure water. A PC-operated Waters high performance liquid chromatograph was used to analyze individual carotenoid compounds through spectra and retention time. Samples were analyzed using Waters Millennium 3.2 software, Waters 515 binary pump system (Waters 515), an autoinjector (Waters 717 plus), and photodiode detector (Waters 996), along with a column heater (SpectraPhysics SP8792) maintained at 35 ^{o}C. A 4.6 x 250 mm, 5 μm, YMC Carotenoid Column (C-30 reverse phase) (Waters, Milford, MA) was used to separate the carotenoid compounds.

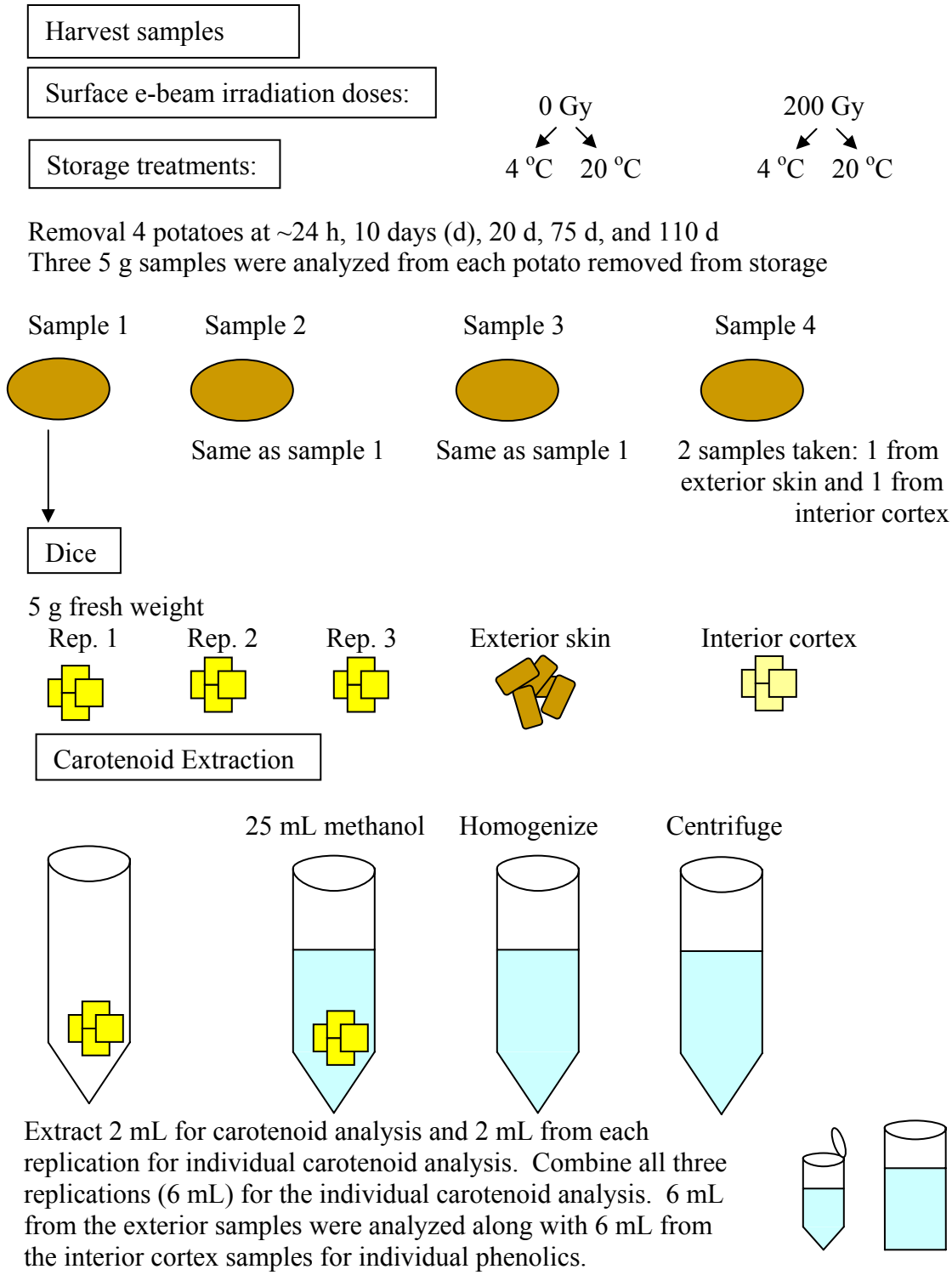

Fig. 7.4 Carotenoid extraction procedure for the factors electron beam- irradiation, storage time, and storage temperature.

A library was created including 1) violaxanthin (CaroteNature, Lupsingen, Switzerland), 2) neoxanthin (CaroteNature, Lupsingen, Switzerland), 3) antheraxanthin (CaroteNature, Lupsingen, Switzerland), 4) β-cryptoxanthin (Hoffman La Roche, Basel, Switzerland), 5) canthaxanthin (Hoffman La Roche, Basel, Switzerland), 6) zeaxanthin (Hoffman La Roche, Basel, Switzerland), and 7) lutein (Hoffman La Roche, Basel, Switzerland). Two filtered and de-gassed solution solvents were used for carotenoid extraction. "Solvent A" consisted of methanol, water, and triethylamine (90:10:0.1), while "solvent B" consisted of methanol, MTBE, and triethylamine (6:90:0.1). The following was the gradient for the analysis: (min/ %A) 0/99, 8/99, 8/99, 45/0, 50/0, and 53/99 (Breithaupt and Barmedi, 2002; Hale, 2003).

EXTRACTION OF PHENOLICS AND ANTIOXIDANT ACTIVITY. The same extraction procedure was conducted for total phenolics content, individual phenolic content, and total antioxidant content. Fifteen mL of methanol was added to a 5 g sample of diced potato. This mixture was then homogenized with an ultra turrax tissumizer from Tekmar (Cincinnati, Ohio). After homogenizing, samples were placed in a J-17 rotor at 17,000 rpm for 20 min in a refrigerated centrifuge (Beckman model J2-21). Two mL of the methanol extract were saved in snap-cap tubes for analysis of total antioxidant activity and total phenolic content. Individual phenolics were analyzed with the combined 2 mL samples from each replication from each tuber; producing a 6 mL sample saved in glass vials (Figure 7.5). The extracted samples were stored at -29 ^{o}C (-20 ^{o}F).

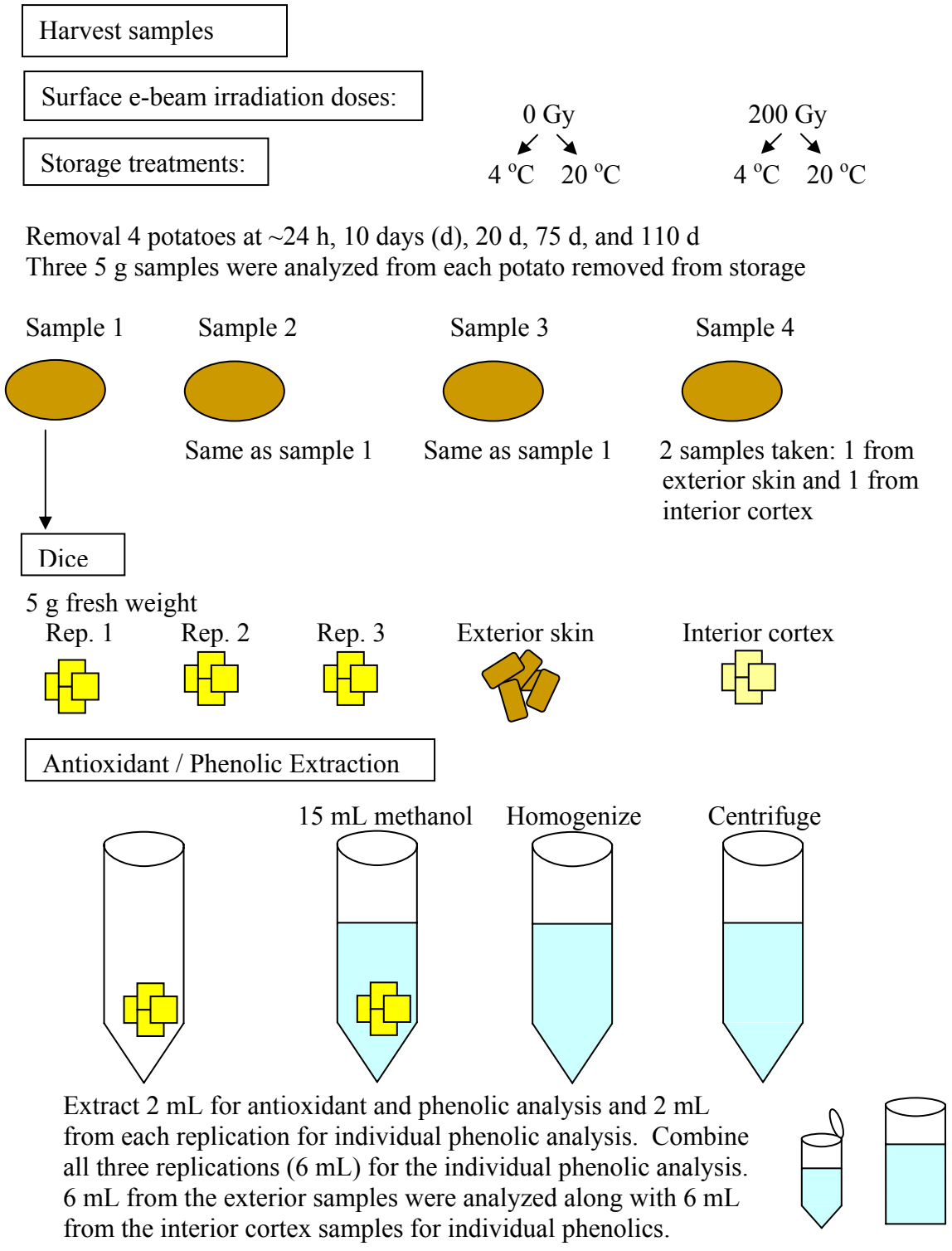

Fig. 7.5 Antioxidant/ phenolic extraction procedure for the factors electron beam-irradiation, storage time, and storage temperature.

DPPH ASSAY FOR TOTAL ANTIOXIDANT ACTIVITY. Total antioxidant activity was analyzed by using DPPH (1,1 Diphenyl-2 picrylhydrazyl), a colorimetric assay first described by Brand-Williams et al. (1995). DPPH is a stable radical which causes oxidation and can be reduced by natural antioxidants, which reduce the oxidizing power of DPPH. Non-reduced DPPH is dark purple, while reduction shifts the color from dark purple to lighter purple to light yellow. This decrease in color and reduction power can be measured at 515 nm; the lower the absorbance, the greater the antioxidant activity in the sample.

The DPPH solution was diluted by dissolving 24 mg DPPH in 100 mL methanol, which created a 607 μM DPPH stock solution. The solution was then diluted to ~10:55 with methanol until the spectrophotometer read 1.1 at 515 nm. The extracted methanol sample of 150 μL was combined with 2.85 mL of diluted DPPH in a scintillation vial, along with a blank which contained 150 μL of pure methanol (instead of methanol extract) with the diluted 2.85 mL DPPH. The samples reacted with each other for 15 min. After this time, the level of reduction was determined by the absorption at 515 nm in a plastic UV-spectrophotometeric cuvette. This reading was based on the activity of the sample after 15 min (initial antioxidant activity, AOAI), but the total reaction is a kinetic one, which continues for about 24 h until stabilization (stabilized antioxidant activity, AOAS). Each antioxidant compound reacts with the oxidizing substance at a given time; therefore, two readings were recorded. The second reading was taken after 24 h, when the samples and the DPPH had stabilized. The first reading (after 15 min) represents an initial response and the second represents a final response. It is currently unknown how long consumed antioxidants are functional; therefore, these readings may represent two responses. Absorptions were subtracted from the blank. A standard curve using a known antioxidant, trolox (6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid), was prepared, and a regression curve was calculated to convert the antioxidant activity into trolox equivalents.

TOTAL PHENOLIC CONTENT. The Folin-Ciocalteau phenol method to determine phenolic content was first described by Swain and Hillis (1959) and later modified by Singleton and Rossi (1965). This method, like the total antioxidant activity method, is a colorimetric reaction that is determined by absorbance. A 0.25 N Folin - Ciocalteau phenol reagent solution with nanopure water and a 1.0 N Na_2Co_3 solution with nanopure water was prepared. The extracted methanol sample of 150 μL was combined with 2.4 mL of nanopure water in a scintillation vial, along with a blank which contained 150 μL of pure methanol (instead of methanol extract) with 2.4 mL of nanopure water. The samples and blank reacted with 150 μL of the 0.25 N Folin - Ciocalteau phenol reagent solution for 3 min. Afterwards, 300 μL of the 1.0 N Na_2Co_3 solution was added to both the samples and blank. The reaction again was kinetic, and stabilization occurred after 1 hour and 55 min. Data was recorded at stabilization. Absorption was determined at 725 nm in plastic UV-spectrophotometeric cuvettes. The blank was read first, and the sample absorption was based on the cleared response of the blank. The phenolic content was determined by a prepared regression curve to equivalents of chlorogenic acid.

HPLC ANALYIS FOR INDIVIDUAL PHENOLIC COMPOUNDS. The extracted samples were concentrated under nitrogen gas or by using a heated speed vacuum centrifuge and filtered through a 0.45 μm syringe filter (Hale, 2003). A PC-operated Waters high performance liquid chromatograph was used to analyze individual phenolic compounds through spectra and retention time. The samples were analyzed using Waters Millennium 3.2 software, Waters 515 binary pump system (Waters 515), an autoinjector (Waters 717 plus), and photodiode detector (Waters 996), along with a column heater (SpectraPhysics SP8792) maintained at 40 ^{o}C (104 ^{o}F). A 4.6 x 150 mm, 5 μm, Atlantis C-18 reverse-phase column (Milford, MA) was used to separate phenolic compounds. The compounds analyzed included: 1) 5,7-trihydroxyflavanone, 2) sinapic acid, 3) kampherol, 4) (-) epicatechin, 5) catechin, 6) quercetin dehydrate, 7) rutin hydrate, 8) protocatechuic acid, 9) salicylic acid, 10) myricetin, 11) syringic acid, 12) gallic acid, 13) vanillic acid, 14) t-cinnamic acid, 15) p-

coumaric acid, 16) ferulic acid, 17) caffeic acid, and 18) chlorogenic acid; the standard compounds were obtained from Agros Organics (Pittsburgh, PA). Two filtered and de-gassed solution solvents were used for the phenolic extraction. "Solvent A" consisted of acetonitrile, and solvent "B" consisted of nanopure water and HCL adjusted to a pH of 2.3. The following gradient was used, (min/%A) 0:85, 5:85, 30:0, 35:0 (Hale, 2003).

STATISTICAL ANALYIS. Multiple analysis of variance (MANOVA) and analysis of variance (ANOVA) general linear models were used. The statistical model of the experiment was a full factorial design. The dependent variables included total carotenoid content, total phenolic content, total antioxidant activity initially (AOAI) (measurements taken after 15 min), total antioxidant activity at stabilization (AOAS) (measurements taken after 24 h), individual carotenoid compounds, and individual phenolic compounds. The fixed factors included storage time, storage temperature, irradiation dose, and area of tuber analyzed. Factor comparison was conducted using the post hoc multiple comparison methods of S-N-K tests. Also, a test to measure the estimate of magnitude of effect or strength of association was conducted. This test determines how strongly two or more variables are related, or the magnitude of the difference between the groups. The effects are reported as eta squared values and are defined as the sums of squares of the effect of interest divided by the total sums of squares (Levine and Hullett, 2002). The analysis was conducted using the statistical package (SPSS) version 11.5.

Results

The dosimetry scheme experiments via the alinine dosimetry tablets and a PC-interfaced bench top EPR spectrometer produced the following doses (Table 7.1).

Table 7.1 Measured irradiation dose via alanine dosimeter tablets, Springlake, 2004.

Projected Irradiation dose (Gy)	Average top dose	Average bottom dose	Average right dose	Average left dose	Average overall dose
200	285	255	185	210	235

Percent weight loss due to storage time and temperature was recorded after the storage treatment (Table 7.2). Weight loss was more severe at higher storage temperatures and also with longer storage time. Irradiation dose did not appear to affect the weight loss.

Table 7.2 Percent weight loss at selected storage times and irradiation doses.

Irradiation dose and storage	10 days storage	20 days storage	75 days storage	110 days storage	Average
0 Gy 4°C	0.17	0.60	1.81	3.06	1.41
0 Gy 20°C	0.69	1.73	6.21	8.60	4.31
200 Gy 4°C	0.14	1.34	1.69	3.37	1.64
200 Gy 20°C	0.77	1.55	4.51	8.12	3.74
Average	0.44	1.31	3.56	5.79	2.77

STANDARD CURVE FOR LUTEIN. The linear regression equation to equate the spectrophotometric absorbance readings of the methanol extract at 445 nm into lutein equivalents was the following: 3028.6x + 8.1063, where x is the absorbance at 445 nm and y is the µg lutein equivalents per hundred mL. The R^2 value of this equation was 0.9991.

The average amount of xanthophylls in lutein equivalents was 172 µg/100gfw. Analysis of variance (Table 7.3) indicated that there were significant differences in storage time (Store) ($p < 0.000$), storage temperature (Temp) ($p < 0.000$), and the interactions of storage time and storage temperature ($p = 0.016$), and storage time and irradiation dose ($p = 0.021$). There were no significant differences in irradiation dose (Dose) ($p = 0.654$), or the interactions of irradiation dose and storage temperature ($p = 0.292$), and storage time, storage temperature and irradiation dose ($p = 0.083$). The factor's magnitude of strength (eta squared values) were irradiation dose 0 %, storage time 12 %, storage temperature 11 %, the interactions of irradiation dose and storage time 5 %, irradiation dose and storage temperature 0 %, storage time and storage temperature 5 %, irradiation dose, storage time, and storage temperature 3 %, and error 63 %.

Table 7.3 Analysis of variance results for carotenoid (xanthophyll) content for the factors e-beam irradiation, storage time, and temperature, Springlake 2004.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	49017.837[z]	19	2579.886	4.902	.000
Intercept	5275063.163	1	5275063.163	10023.618	.000
Dose	1.762	1	1.762	.003	.954
Store	16472.334	4	4118.084	7.825	.000
Temp	14672.789	1	14672.789	27.881	.000
Dose * Store	6234.750	4	1558.687	2.962	.021
Dose * Temp	588.225	1	588.225	1.118	.292
Store * Temp	6615.183	4	1653.796	3.143	.016
Dose * Store * Temp	4432.795	4	1108.199	2.106	.083
Error	84202.142	160	526.263		
Total	5408283.143	180			
Corrected Total	133219.979	179			

[z] $R^2 = 0.368$ (Adjusted $R^2 = 0.293$)

Storage time was a significant factor as indicated by analysis of variance and eta square values. Carotenoid content peaked at 10 days (187 µg/100gfw), and the lowest carotenoid level was at 75 days (158 µg/100gfw) (Table 7.4). The general trend was that carotenoid content decreased over time, then increased slightly at 110 days. In Chapter VI, a general decrease in carotenoids was found, while Chapters IV and V there was an increase in carotenoids. In the present study, the first and last days of analysis were not significantly different. In Chapters IV and V, the only times studied were 0 and 110 days of storage; therefore, it may have been more difficult to observe a general trend.

Table 7.4 Storage time ranking for carotenoid (xanthophyll) content, Springlake 2004.

Storage time	Eq. Lutein (µg/100gfw)	
10 days	187	a[z]
0 days	175	b
110 days	170	bc
20 days	166	bc
75 days	158	c

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The 4 ^{o}C storage treatment (180 µg/100gfw) ranked significantly higher than the 20 ^{o}C treatment (162 µg/100gfw) (Table 7.5). This ranking is similar to the results found in Chapter IV, where the colder temperature resulted in higher carotenoid content then the warmer temperatures.

Table 7.5 Storage temperature ranking for carotenoid (xanthophyll) content, Springlake 2004.

Storage temperature	Eq. Lutein (µg/100gfw)
4 ^{o}C	180 a[z]
20 ^{o}C	162 b

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

There were no significant differences in the effect of irradiation dose on carotenoid content (Table 7.6). In Chapter VI, it was reported that gamma- irradiation doses of 0 and 200 Gy were not significantly different, while in Chapter V higher irradiation doses ranked significantly higher than the control. The discrepancy may be due to the difference in experimental design between the two experiments.

Table 7.6 Irradiation dosage ranking for carotenoid (xanthophyll) content, Springlake 2004.

Irradiation dose	Eq. Lutein (µg/100gfw)
0 Gy	171 a[z]
200 Gy	171 a

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Although the interaction of storage time and temperature was significant, temperature appeared to have a greater influence. Carotenoid levels were higher at the colder storage temperatures throughout the study.

Table 7.7 Storage time by storage temperature interaction for carotenoid (xanthophyll) content, Springlake 2004.

Storage time and storage temperature	Eq. Lutein (µg/100gfw)
0 days	
4C	175
20 C	175
10 days	
4C	203
20 C	170
20 days	
4C	170
20 C	162
75 days	
4C	171
20 C	145
110 days	
4C	181
20 C	158

Carotenoid content appeared to have a similar trend as shown in past studies with the interaction of storage time and irradiation dose on antioxidant activity. As seen in Chapters V and VI, antioxidant activity was greater at high irradiation doses at earlier storage stages, while high irradiation doses at later stages of storage had lower antioxidant activity. This similar trend is shown in Table 7.8 with carotenoid content.

Table 7.8 Storage time by irradiation dose interaction for carotenoid (xanthophyll) content, Springlake 2004.

Storage time and irradiation dose	Eq. Lutein (µg/100gfw)
0 days	
0 Gy	169
200 Gy	182
10 days	
0 Gy	184
200 Gy	189
20 days	
0 Gy	163
200 Gy	169
75 days	
0 Gy	158
200 Gy	158

Table 7.8 (continued).

Storage time and irradiation dose	Eq. Lutein (µg/100gfw)
110 days	
0 Gy	181
200 Gy	159

Due to the low penetrating power of electron beam irradiation, significant differences between treatments may have been spurious because of the area of the tuber sampled and analyzed. Different areas (exterior or surface; and interior or cortex) of the tuber were also analyzed throughout this study and were a significant factor ($p = 0.000$) in carotenoid content (Table 7.9).

Table 7.9 Analysis of variance results for carotenoid (xanthophyll) content based on area analyzed, Springlake 2004.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	1394292.647[z]	1	1394292.647	141.732	.000
Intercept	6604467.340	1	6604467.340	671.353	.000
Area	1394292.647	1	1394292.647	141.732	.000
Error	1042780.084	106	9837.548		
Total	9041540.071	108			
Corrected Total	2437072.731	107			

[z] $R^2 = 0.572$ (Adjusted $R^2 = 0.568$)

Since area was significant, results for both the exterior and interior surface results will be discussed separately. Table 7.10 presents the analysis of variance results for the exterior surfaces of the tuber. All factors for the exterior surfaces, including storage time, storage temperature, and irradiation dose were significant ($p < 0.000$), along with all two-factor interactions ($p < 0.000$) and three-factor interactions ($p < 0.000$). The magnitude of strength, or eta squared values for the exterior analysis were irradiation dose 6 %, storage time 1 %, storage temperature 4 %, the interactions of irradiation dose and storage time 24 %, irradiation dose and storage temperature 3 %, storage time and storage temperature 14 %, irradiation dose, storage time, and storage

temperature 4 %, and error 3 %. The average surface carotenoid content was 346 µg/100gfw.

Table 7.10 Analysis of variance results for exterior surface carotenoid (xanthophyll) content for the factors e-beam irradiation, storage time, and temperature, Springlake 2004.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	1074299.265[z]	19	56542.067	60.390	.000
Intercept	7188164.121	1	7188164.121	7677.359	.000
Dose	65558.576	1	65558.576	70.020	.000
Store	462980.411	4	115745.103	123.622	.000
Temp	42187.176	1	42187.176	45.058	.000
Dose * Store	264037.528	4	66009.382	70.502	.000
Dose * Temp	32879.042	1	32879.042	35.117	.000
Store * Temp	157217.716	4	39304.429	41.979	.000
Dose * Store * Temp	49438.816	4	12359.704	13.201	.000
Error	37451.231	40	936.281		
Total	8299914.617	60			
Corrected Total	1111750.497	59			

[z] $R^2 = 0.966$ (Adjusted $R^2 = 0.950$)

Table 7.11 presents the analysis of variance results for the interior surfaces of the tuber. All factors in the analysis of interior surfaces were significant ($p < 0.000$), except storage temperature ($p = 0.245$) and irradiation dose ($p = 0.103$). All interactions were also significant ($p < 0.000$). The magnitude of strength, or eta squared values, for the interior analysis were irradiation dose 1 %, storage time 23 %, storage temperature 0 %, the interactions of irradiation dose and storage time 31 %, irradiation dose and storage temperature 5 %, storage time and storage temperature 16 %, irradiation dose, storage time, and storage temperature 12 %, and error 12 %. The average interior area carotenoid content was 131 µg/100gfw.

Table 7.11 Analysis of variance results for interior surface carotenoid (xanthophyll) content for the factors e-beam irradiation, storage time, and temperature, Springlake 2004.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	62226.887[z]	19	3275.099	14.858	.000
Intercept	1030434.959	1	1030434.959	4674.700	.000
Dose	613.322	1	613.322	2.782	.103
Store	15990.824	4	3997.706	18.136	.000
Temp	307.098	1	307.098	1.393	.245
Dose * Store	22010.886	4	5502.721	24.964	.000
Dose * Temp	3428.659	1	3428.659	15.555	.000
Store * Temp	11369.842	4	2842.461	12.895	.000
Dose * Store * Temp	8506.257	4	2126.564	9.647	.000
Error	8817.123	40	220.428		
Total	1101478.969	60			
Corrected Total	71044.010	59			

[z] $R^2 = 0.876$ (Adjusted $R^2 = 0.817$)

The exterior surfaces contained more than twice the carotenoid content than the interior surfaces. Both surfaces showed a trend where the carotenoid content increased after 0 days, then decreased, followed by a second increase (Table 7.12). This trend was also seen in the total surfaces of this study.

Table 7.12 Storage time ranking for carotenoid (xanthophyll) content for exterior and interior samples, Springlake 2004.

Storage time	Eq. Lutein (Surface area) (µg/100gfw)	Storage time	Eq. Lutein (Interior area) (µg/100gfw)
110 days	479 a[z]	110 days	154 a
10 days	383 b	10 days	143 ab
75 days	352 c	75 days	131 bc
20 days	302 d	20 days	120 c
0 days	213 e	0 days	107 d

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Temperature was a significant factor for surface area, but not for interior areas (Table 7.13). Carotenoid levels were higher at the lower storage temperatures for total surfaces area (Table 7.5), but carotenoid content was significantly greater at 20 ^{o}C for

the exterior surfaces. The discrepancy may be due to the greater dehydration at 20 ^{o}C, causing concentration in the exterior surfaces.

Table 7.13 Storage temperature ranking for carotenoid (xanthophyll) content for exterior and interior samples, Springlake 2004.

Storage temperature	Eq. Lutein (Surface area) (µg/100gfw)	Storage temperature	Eq. Lutein (Interior area) (µg/100gfw)
20 C	373 a[z]	20 C	133 a
4 C	319 b	4 C	129 a

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.14 ranks the irradiation dose based on area of sample. Over all, carotenoid content was significantly higher at 0 Gy in the exterior area, while carotenoid content was also higher in the interior surfaces at the same dosage, but not significantly so. These results may also be related to dehydration. The surfaces of the potato are dehydrated in the 0 Gy samples due to sprouting, while the 200 Gy samples are not dehydrated and therefore ranked lower in carotenoid content.

Table 7.14 Irradiation dose ranking for carotenoid (xanthophyll) content for exterior and interior samples, Springlake 2004.

Irradiation dose	Eq. Lutein (Surface area) (µg/100gfw)	Irradiation dose	Eq. Lutein (Interior area) (µg/100gfw)
0 Gy	379 a[z]	0 Gy	134 a
200 Gy	313 b	200 Gy	128 a

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

All interactions for both surface area analysis and interior area analysis were significant, but no trends could be identified, so the interactions will be omitted from further analysis.

DPPH ASSAY FOR TOTAL ANTIOXIDANT ACTIVITY - STANDARD CURVE FOR TROLOX. The linear regression equation to convert the spectrophotometric absorbance readings of the methanol extract and reduced DPPH at 515 nm into trolox equivalents was the following: $y = 891.69x$, where x is the absorbance at 515 nm and y is the µg trolox equivalents per g fresh weight. The R^2 value of this equation was 0.997.

The average antioxidant activity as reported in trolox equivalents was 264 µg/gfw for AOAI and 486 µg/gfw for AOAS. The analysis of variance for AOAI indicated that there were significant differences in storage time ($p < 0.000$), the interaction of irradiation dose and storage time ($p < 0.000$), and the interaction of storage time and storage temperature ($p = 0.003$). There were no significant differences in irradiation dose ($p = 0.068$), storage temperature ($p = 0.155$), the interaction of irradiation dose, and storage temperature ($p = 0.285$), and the interaction of storage time, storage temperature, and irradiation dose ($p = 0.195$) (Table 7.15). The magnitude of strength, or eta squared values, for AOAI were irradiation dose 1 %, storage time 51 %, storage temperature 0 %, the interactions of irradiation dose and storage time 8 %, irradiation dose and temperature 0 %, storage time and storage temperature 4 %, irradiation dose, temperature, storage time 1 %, and error 34 %.

The analysis of variance for AOAS indicates that there were significant differences in storage time ($p < 0.000$) and in the interaction of irradiation dose and storage time ($p = 0.006$). There were no other significant differences in all other factors (Table 7.15). The magnitude of strength, or eta squared values, for AOAS were irradiation dose 0 %, storage time 47 %, storage temperature 0 %, the interactions of irradiation dose and storage time 4 %, irradiation dose and storage temperature 0 %, storage temperature and storage time 0 %, irradiation dose, storage temperature, and storage time 1 %, and error 46 %.

Table 7.15 Analysis of variance results for antioxidant activity for the factors e-beam irradiation, storage time, and temperature, Springlake 2004.

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	AOAI	1998025.718 [z]	19	105159.248	16.196	.000
	AOAS	2191475.597 [y]	19	115340.821	9.811	.000
Intercept	AOAI	13681460.080	1	13681460.080	2107.180	.000
	AOAS	44604660.177	1	44604660.177	3794.230	.000
Dose	AOAI	21876.178	1	21876.178	3.369	.068
	AOAS	10582.419	1	10582.419	.900	.344
Store	AOAI	1573863.683	4	393465.921	60.601	.000
	AOAS	1914871.355	4	478717.839	40.721	.000
Temp	AOAI	13238.835	1	13238.835	2.039	.155
	AOAS	18328.940	1	18328.940	1.559	.214
Dose * Store	AOAI	230970.402	4	57742.601	8.893	.000
	AOAS	178538.715	4	44634.679	3.797	.006
Dose * Temp	AOAI	7469.804	1	7469.804	1.150	.285
	AOAS	1081.470	1	1081.470	.092	.762
Store * Temp	AOAI	110772.194	4	27693.048	4.265	.003
	AOAS	17501.580	4	4375.395	.372	.828
Dose * Store * Temp	AOAI	39834.621	4	9958.655	1.534	.195
	AOAS	50571.117	4	12642.779	1.075	.371
Error	AOAI	1038845.168	160	6492.782		
	AOAS	1880947.088	160	11755.919		
Total	AOAI	16718330.966	180			
	AOAS	48677082.861	180			
Corrected Total	AOAI	3036870.886	179			
	AOAS	4072422.684	179			

[z] $R^2 = 0.658$ (Adjusted $R^2 = 0.617$)
[y] $R^2 = 0.538$ (Adjusted $R^2 = 0.483$)

Both initial and stabilized antioxidant activity followed similar trends in storage (Table 7.16). In the gamma- irradiation study (Chapter VI) antioxidant activity decreased over time, then increased after 75 days, reaching similar values as before storage. In the present study there was a significant decrease in antioxidant activity, and at later stages of storage antioxidant activity did increase, but not significantly. For both AOAI and AOAS the antioxidant activity was greatest at 10 days, at 400 and 637 µg/gfw, respectively. AOAI was lowest at 20 days with 192 µg/gfw, while AOAS was lowest at 75 days with 399 µg/gfw.

Table 7.16 Storage time ranking for antioxidant activity, Springlake 2004.

Storage time		AOAI[z] (µg/gfw)		Storage time		AOAS[y] (µg/gfw)	
10	days	400	a[x]	10	days	637	a
0	days	380	a	0	days	609	a
110	days	210	b	110	days	424	b
75	days	197	b	20	days	420	b
20	days	192	b	75	days	399	b

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Both AOAI and AOAS were higher at 4 ^{o}C than at 20 ^{o}C, but there was no significant difference between temperatures (Table 7.17). In past experiments (Chapter IV) antioxidant activity was higher at the lower temperatures.

Table 7.17 Storage temperature ranking for antioxidant activity samples, Springlake 2004.

Storage temperature	AOAI[z] (µg/gfw)		Storage temperature	AOAS[y] (µg/gfw)	
4 C	284	a[x]	4 C	508	a
20 C	267	a	20 C	488	a

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Irradiation dose did not affect AOAI and AOAS (Table 7.18). In past studies irradiation dose was also a non-significant factor (Chapter VI). In Chapter VI, antioxidant activity also ranked higher with higher irradiation doses. In Chapter V, the 0 Gy dose resulted in higher antioxidant activity; however, this may be related to the cold storage temperature used in Chapter V or the addition of cooking.

Table 7.18 Irradiation dosage ranking for antioxidant activity samples, Springlake 2004.

Irradiation dose	AOAI[z] (µg/gfw)	Irradiation dose	AOAS[y] (µg/gfw)
200 Gy	287 a[x]	200 Gy	505 a
0 Gy	265 a	0 Gy	490 a

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The interaction between storage temperature and irradiation dose was not significant. The interaction describes the main factor effects of irradiation dose and the main factor effects of storage temperature. Each 200 Gy dose ranked higher in antioxidant activity than the 0 Gy dose, and each 4 ^{o}C storage ranked higher in antioxidant activity than the 20 ^{o}C storage treatment. The interaction of storage time and storage temperature also followed past trends. Over all, the 4 ^{o}C storage resulted in higher antioxidant activity, but antioxidant activity decreased after 10 days. Therefore these two interactions are not shown.

Trends in the interaction of storage time and irradiation dose are presented in Table 7.19. Trends were noted in Chapter V and VI, where antioxidant activity was greater at the higher irradiation dose during the earlier stages of storage, and higher at lower irradiation doses during later stages of storage. At 0, 10 and 20 days, the 200 Gy irradiation dose resulted in antioxidant activity greater or equal to the 0 Gy dose. However, at 75 and 110 days 0 Gy resulted in greater antioxidant activity than the 200 Gy dose.

Table 7.19 Storage time by irradiation dose interaction for antioxidant activity, Springlake 2004.

Storage time and storage temperature	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
0 days		
0 Gy	314	546
200 Gy	445	673
10 days		
0 Gy	363	619
200 Gy	437	655
20 days		
0 Gy	187	420
200 Gy	196	420

Table 7.19 (continued).

Storage time and storage temperature	AOAI[z] (µg/gfw)	AOAS[y] (µg/gfw)
75 days		
0 Gy	219	417
200 Gy	175	381
110 days		
0 Gy	240	448
200 Gy	181	399

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox

Due to the low penetrating power of electron beam irradiation, significant differences between treatments may have only occurred based on area of the tuber sampled. Different areas (exterior or surface, and interior or cortex) of the tuber were also analyzed throughout this study, and area analyzed was a significant factor ($p = 0.000$) for antioxidant activity (Table 7.20).

Table 7.20 Analysis of variance results for antioxidant activity based on area analyzed, Springlake 2004.

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	AOAI	3350738.225 [z]	2	1675369.113	88.867	.000
	AOAS	2857968.781 [y]	2	1428984.390	114.280	.000
Intercept	AOAI	4436428.503	1	4436428.503	235.323	.000
	AOAS	5738507.816	1	5738507.816	458.927	.000
Area	AOAI	3350738.225	2	1675369.113	88.867	.000
	AOAS	2857968.781	2	1428984.390	114.280	.000
Error	AOAI	2017215.516	107	18852.481		
	AOAS	1337948.234	107	12504.189		
Total	AOAI	22466240.353	110			
	AOAS	34036204.387	110			
Corrected Total	AOAI	5367953.741	109			
	AOAS	4195917.015	109			

[z] $R^2 = 0.624$ (Adjusted $R^2 = 0.617$)
[y] $R^2 = 0.681$ (Adjusted $R^2 = 0.675$)

The exterior tuber surface AOAI analysis indicated significance for irradiation dose ($p < 0.000$), storage time ($p < 0.000$), storage temperature ($p = 0.010$), the

interaction of irradiation dose and storage time ($p < 0.000$), the interaction of irradiation dose and storage temperature ($p = 0.007$), the interaction of storage time and storage temperature ($p < 0.000$), and the interaction of irradiation dose, storage time, and storage temperature ($p = 0.002$) (Table 7.21). The magnitude of strength, or eta squared values, for exterior area AOAI were irradiation dose 5 %, storage time 51 %, storage temperature 1 %, the interactions of irradiation dose and storage time 28 %, irradiation dose and storage temperature 1 %, storage time and storage temperature 7 %, irradiation dose, storage time, and storage temperature 3 %, and error 5 %. The average exterior surface AOAI was 551 µg/gfw.

The exterior surface AOAS analysis indicated the significance of irradiation dose ($p = 0.006$), storage time ($p < 0.000$), the interaction of irradiation dose and storage time ($p < 0.000$), the interaction of storage time and storage temperature ($p = 0.006$), and the three factor interaction ($p < 0.000$). Storage temperature ($p = 0.394$), and the interaction of irradiation dose and storage temperature ($p = 0.368$) were not significant. The magnitude of strength, or eta squared values, for exterior area AOAS were irradiation dose 1 %, storage time 52 %, storage temperature 0 %, the interactions of irradiation dose and storage time 31 %, irradiation dose and storage temperature 0 %, storage time and storage temperature 3 %, irradiation dose, storage time, and storage temperature 8 %, and error 5 %. The average exterior surface AOAS was 683 µg/gfw.

Table 7.21 Analysis of variance results for exterior surface antioxidant activity for the factors e-beam irradiation, storage time, and temperature, Springlake 2004.

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	AOAI	1300538.032 [z]	19	68449.370	41.656	.000
	AOAS	94655.814 [y]	19	4981.885	36.175	.000
Intercept	AOAI	18177496.674	1	18177496.674	11062.315	.000
	AOAS	27974389.387	1	27974389.387	203131.564	.000
Dose	AOAI	61656.211	1	61656.211	37.522	.000
	AOAS	1235.989	1	1235.989	8.975	.005

Table 7.21 (continued).

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Store	AOAI	697229.961	4	174307.490	106.079	.000
	AOAS	51973.207	4	12993.302	94.349	.000
Temp	AOAI	12381.411	1	12381.411	7.535	.009
	AOAS	107.818	1	107.818	.783	.382
Dose * Store	AOAI	380559.061	4	95139.765	57.899	.000
	AOAS	31077.554	4	7769.388	56.416	.000
Dose * Temp	AOAI	14212.178	1	14212.178	8.649	.005
	AOAS	119.598	1	119.598	.868	.357
Store * Temp	AOAI	98814.790	4	24703.697	15.034	.000
	AOAS	2591.270	4	647.818	4.704	.003
Dose * Store * Temp	AOAI	35684.422	4	8921.105	5.429	.001
	AOAS	7550.379	4	1887.595	13.706	.000
Error	AOAI	65727.643	40	1643.191		
	AOAS	5508.625	40	137.716		
Total	AOAI	19543762.349	60			
	AOAS	28074553.826	60			
Corrected Total	AOAI	1366265.676	59			
	AOAS	100164.439	59			

[z] $R^2 = 0.952$ (Adjusted $R^2 = 0.929$)
[y] $R^2 = 0.945$ (Adjusted $R^2 = 0.919$)

The interior area AOAI analysis indicated the significance of irradiation dose ($p < 0.000$), storage time ($p < 0.000$), the interaction of irradiation dose and storage time ($p < 0.000$), the interaction of storage time and storage temperature ($p < 0.000$), and the three factor interaction ($p < 0.000$). Storage temperature ($p = 0.143$) and the interaction of irradiation dose and storage temperature ($p = 0.063$) were not significant (Table 7.22). The magnitude of strength, or eta squared values, for exterior area AOAI were irradiation dose 9 %, storage time 81 %, storage temperature 0 %, the interactions of irradiation dose and storage time 4 %, irradiation dose and storage temperature 0 %, storage time and storage temperature 3 %, irradiation dose, storage time, and storage temperature 2 %, and error 2 %. The average interior area AOAI was 235 µg/gfw.

The interior AOAS analysis indicated the significance of irradiation dose ($p < 0.000$), storage time ($p < 0.000$), the interaction of irradiation dose and storage time ($p < 0.000$), the interaction of storage time and storage temperature ($p < 0.000$), and the

three factor interaction (p = 0.004). Storage temperature (p = 0.134) and the interaction of irradiation dose and storage temperature (p = 0.556) were not significant. The magnitude of strength, or eta squared values, were irradiation dose 6 %, storage time 73 %, storage temperature 0 %, the interactions of irradiation dose and storage time 7 %, irradiation dose and storage temperature 0 %, storage time and storage temperature 4 %, irradiation dose, storage time, and storage temperature 3 %, and error 6 %. The average interior area AOAS was 372 µg/gfw.

Table 7.22 Analysis of variance results for interior surface antioxidant activity for the factors e-beam irradiation, storage irradiation, storage time, and temperature, Springlake 2004.

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	AOAI	975563.933[z]	19	51345.470	88.448	.000
	AOAS	1302608.114[y]	19	68558.322	30.371	.000
Intercept	AOAI	3292047.734	1	3292047.734	5670.916	.000
	AOAS	8288371.241	1	8288371.241	3671.742	.000
Dose	AOAI	87431.816	1	87431.816	150.611	.000
	AOAS	86833.761	1	86833.761	38.467	.000
Store	AOAI	804769.434	4	201192.358	346.576	.000
	AOAS	1019117.392	4	254779.348	112.867	.000
Temp	AOAI	1298.271	1	1298.271	2.236	.143
	AOAS	5279.716	1	5279.716	2.339	.134
Dose * Store	AOAI	35325.546	4	8831.386	15.213	.000
	AOAS	92221.631	4	23055.408	10.214	.000
Dose * Temp	AOAI	2118.176	1	2118.176	3.649	.063
	AOAS	796.742	1	796.742	.353	.556
Store * Temp	AOAI	27766.199	4	6941.550	11.958	.000
	AOAS	57787.245	4	14446.811	6.400	.000
Dose * Store * Temp	AOAI	16854.492	4	4213.623	7.258	.000
	AOAS	40571.628	4	10142.907	4.493	.004
Error	AOAI	23220.572	40	580.514		
	AOAS	90293.612	40	2257.340		
Total	AOAI	4290832.239	60			
	AOAS	9681272.966	60			
Corrected Total	AOAI	998784.505	59			
	AOAS	1392901.726	59			

[z] $R^2 = 0.977$ (Adjusted $R^2 = 0.966$)
[y] $R^2 = 0.935$ (Adjusted $R^2 = 0.904$)

The effects of storage time on antioxidant activity in the exterior and interior surfaces of the tubers are presented in Table 7.23. Over all, antioxidant activity of the tuber interior was similar to that of the total tuber (Table 7.16). Surface antioxidant activity was similar, but lower in AOAS at 110 days. Antioxidant activity was high at the start of storage, decreased with storage, then increased again in the later stages of storage.

Table 7.23 Storage time ranking for antioxidant activity for exterior and interior samples, Springlake 2004.

Storage time	AOAI[z] (Surface Area) (µg/gfw)	Storage time	AOAI (Interior Area) (µg/gfw)	Storage time	AOAS[y] (Surface Area) (µg/gfw)	Storage time	AOAS (Interior Area) (µg/gfw)
10 days	736 a[x]	10 days	378 a	0 days	731 a	10 days	545 a
110 days	590 b	0 days	373 a	10 days	692 b	0 days	505 a
0 days	502 c	110 days	161 c	20 days	684 b	110 days	309 b
20 days	497 c	75 days	133 d	110 days	655 c	20 days	290 b
75 days	416 d	20 days	127 d	75 days	649 c	75 days	209 c

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

When antioxidant activity of both surface and interior areas was measured at different storage temperatures, only the surface area AOAI was significantly affected (Table 7.24). The total tuber area results described earlier indicated that antioxidant activity at 4 ^{o}C storage was higher than at 20 ^{o}C. Both AOAI and AOAS surface area antioxidant activity was greater at 20 ^{o}C than at 4 ^{o}C, perhaps due to the greater amount of dehydration experienced by the tubers stored at 20 ^{o}C. The dehydration process may result in concentration of the compounds responsible for antioxidant activity.

Table 7.24 Storage temperature ranking for antioxidant activity for exterior and interior samples, Springlake 2004.

Storage temp.	AOAI[z] (Surface Area) (µg/gfw)	Storage temp.	AOAI (Interior Area) (µg/gfw)	Storage temp.	AOAS[y] (Surface Area) (µg/gfw)	Storage temp.	AOAS (Interior Area) (µg/gfw)
20 C	565 a[x]	4 C	239 a	20 C	684 a	20 C	381 a
4 C	536 b	20 C	230 a	4 C	681 a	4 C	362 a

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The 200 Gy irradiation dose resulted in greater, but not significantly different, antioxidant activity for total tuber area (Table 7.18). The 200 Gy dose resulted in greater AOAI at the tuber exterior surface than the 0 Gy dose (Table 7.25). The 0 Gy irradiation dose resulted in higher AOAI in the tuber interior and higher AOAS at both tuber surfaces. This may indicate that there was an increase in antioxidant activity with e-beam irradiation; however, it was only at the surface of the tuber.

Table 7.25 Irradiation dosage ranking for antioxidant activity for exterior and interior samples, Springlake 2004.

Irradiation Dose	AOAI[z] (Surface Area) (µg/gfw)	Irradiation Dose	AOAI (Interior Area) (µg/gfw)	Irradiation Dose	AOAS[y] (Surface Area) (µg/gfw)	Irradiation Dose	AOAS (Interior Area) (µg/gfw)
200 Gy	582 a[x]	0 Gy	272 a	0 Gy	687 a	0 Gy	410 a
0 Gy	519 b	200 Gy	196 b	200 Gy	678 b	200 Gy	334 b

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox
[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Analysis of variance indicated that all interactions significantly affected surface area AOAI, and surface area AOAS was similarly affected, with the exception of the interaction of irradiation dose, and storage temperature (Table 7.21). Analysis of variance for AOAI and AOAS interior area indicated significant differences for all

interactions, irradiation dose, and storage temperature (Table 7.22). The only interaction discussed here is the interaction of storage time and irradiation dose (Table 7.26). The trends noted in the other interactions were less prominent. Both interior area antioxidant activity readings were higher at the 0 Gy dosage throughout the storage process. However, both surface area antioxidant activity readings were higher at the 200 Gy dose at 0 days of storage, after which surface area AOAS readings were higher at the 0 Gy dose, while surface area AOAI was higher at the 200 Gy dose at later stages of storage.

Table 7.26 Storage time by irradiation dose interaction for antioxidant activity for exterior and interior samples, Springlake 2004.

Storage time and irradiation dose	AOAI[z] (Surface Area) (µg/gfw)	AOAI (Interior Area) (µg/gfw)	AOAS[y] (Surface Area) (µg/gfw)	AOAS (Interior Area) (µg/gfw)
0 days				
0 Gy	338	422	693	536
200 Gy	667	324	769	475
10 days				
0 Gy	739	453	699	633
200 Gy	732	303	685	457
20 days				
0 Gy	589	128	717	275
200 Gy	429	125	656	305
75 days				
0 Gy	361	162	659	219
200 Gy	471	103	639	199
110 days				
0 Gy	567	197	669	387
200 Gy	613	125	642	232

[z] AOAI = Initial antioxidant activity eq. trolox
[y] AOAS = Stabilized antioxidant activity eq. trolox

TOTAL PHENOLIC CONTENT. The linear regression equation to equate the spectrophotometric absorbance readings of the Folin method at 727 nm into chlorogenic acid equivalents was the following: $y = 0.5775x - 0.0279$, where x was the absorbance at 727 nm after zeroing the spectrophotometer with a blank lacking

antioxidant extract but containing all other solutions and y was the milligrams chlorogenic acid per 100 grams. The R^2 value for this equation was 0.970.

The average phenolic content reported as equivalents of chlorogenic acid was 571 µg/gfw. Analysis of variance for phenolic acids indicated that there were significant differences in irradiation dose ($p < 0.000$); storage time ($p < 0.000$); and storage temperature ($p = 0.029$). All two-factor and three-factor interactions were not significant (Table 7.27). Magnitude of strength, or eta squared values, were irradiation dose 8 %, storage time 51 %, storage temperature 1 %, the interactions of irradiation dose and storage time 0 %, irradiation dose and storage temperature 1 %, storage time and storage temperature 1 %, irradiation dose, storage time, and storage temperature 1 %, and error 38 %.

Table 7.27 Analysis of variance results for phenolic content for the factors e-beam irradiation, storage time, and temperature, Springlake 2004.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	1853507.752 [z]	19	97553.040	13.829	.000
Intercept	58628476.153	1	58628476.153	8311.304	.000
Dose	245426.665	1	245426.665	34.792	.000
Store	1516681.657	4	379170.414	53.752	.000
Temp	34328.649	1	34328.649	4.867	.029
Dose * Store	11449.607	4	2862.402	.406	.804
Dose * Temp	394.445	1	394.445	.056	.813
Store * Temp	24420.791	4	6105.198	.865	.486
Dose * Store * Temp	20805.937	4	5201.484	.737	.568
Error	1128650.344	160	7054.065		
Total	61610634.248	180			
Corrected Total	2982158.096	179			

[z] $R^2 = 0.622$ (Adjusted $R^2 = 0.577$)

Analysis of variance indicated that there were significant differences among storage times, and storage time had the highest eta squared value. Generally, phenolic

content increased with storage up to 10 days, then decreased (Table 7.28). Phenolic content was significantly greater at day 10 than day 0, while it was significantly lower at day 75 than day 110, which indicates that phenolic content did not increase/decrease linearly. Phenolics decreased with storage in previous studies, (Chapter IV and V), but increased in Chapter VI.

Table 7.28 Storage time ranking for phenolic content, Springlake 2004.

Storage time	Eq. Chlorogenic acid (µg/gfw)
10 days	716 a[z]
0 days	632 b
20 days	535 c
110 days	511 c
75 days	460 d

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The storage temperature significantly affected phenolic content, with 4 ^{o}C storage resulting in more phenolics than the 20 ^{o}C temperature (Table 7.29). In past studies (Chapter IV) these two temperatures were not significantly different; however, only two storage times were used.

Table 7.29 Storage temperature ranking for phenolic content, Springlake 2004.

Storage temperature	Eq. Chlorogenic acid (µg/gfw)
4 C	585 a[z]
20 C	557 b

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The 200 Gy irradiation treatment resulted in significantly higher phenolics than the 0 Gy dose (Table 7.30). This agrees with earlier studies (Chapters V and VI) where phenolic levels were greater at the higher irradiation dose.

Table 7.30 Irradiation dosage ranking for phenolic samples, Springlake 2004.

Irradiation dose	Eq. Chlorogenic acid (µg/gfw)	
200 Gy	608	a[z]
0 Gy	534	b

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

All interactions as indicated by the analysis of variance, were non-significant (Table 7.27). Therefore these interactions will not be displayed.

Due to the low penetrating power of electron beam irradiation, significant differences between treatments may have occurred due only to area of the tuber analyzed. Different areas (exterior or surface, and interior or cortex) of the tuber were also analyzed throughout this study, and area analyzed was a significant factor ($p = 0.000$) for phenolic content (Table 7.31).

Table 7.31 Analysis of variance results for phenolic content based on areas analyzed, Springlake 2004.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	5720563.286 [z]	2	2860281.643	116.024	.000
Intercept	13370113.912	1	13370113.912	542.343	.000
Area	5720563.286	2	2860281.643	116.024	.000
Error	2613167.627	106	24652.525		
Total	62349197.327	109			
Corrected Total	8333730.913	108			

[z] $R^2 = 0.686$ (Adjusted $R^2 = 0.681$)

Exterior surface area (Table 7.32) and interior surface area (Table 7.33) of the tubers were analyzed separately. The analysis of variance for exterior surface area phenolic content indicated that all factors were significantly different, as were all possible interactions (Table 7.32). Magnitude of strength, or eta squared values, for surface area phenolic content were irradiation dose 3 %, storage time 76 %, storage temperature 1 %, the interactions of irradiation dose and storage time 13 %, irradiation dose and storage temperature 0 %, storage time and storage temperature 5 %,

irradiation dose, storage time, and storage temperature 1 %, and error 2 %. The average exterior surface phenolic content was 913 µg/gfw.

Table 7.32 Analysis of variance results for exterior surface phenolic content for the factors e-beam irradiation, storage time, and temperature, Springlake 2004.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	1914588.100 [z]	19	100767.795	92.809	.000
Intercept	48346404.021	1	48346404.021	44527.945	.000
Dose	51888.718	1	51888.718	47.790	.000
Store	1482959.653	4	370739.913	341.458	.000
Temp	22680.779	1	22680.779	20.889	.000
Dose * Store	252261.024	4	63065.256	58.084	.000
Dose * Temp	6356.896	1	6356.896	5.855	.020
Store * Temp	88662.332	4	22165.583	20.415	.000
Dose * Store * Temp	18242.793	4	4560.698	4.200	.006
Error	42344.415	39	1085.754		
Total	50403934.641	59			
Corrected Total	1956932.515	58			

[z] $R^2 = 0.978$ (Adjusted $R^2 = 0.968$)

Analysis of variance for interior area phenolic content indicated that there were significant differences among all factors and interactions, except irradiation dose ($p = 0.422$), storage temperature ($p = 0.581$), and the interaction of irradiation dose and storage temperature ($p = 0.227$) (Table 7.33). Magnitude of strength, or eta squared values, for interior area phenolic content were irradiation dose 0 %, storage time 79 %, storage temperature 0 %, the interactions of irradiation dose and storage time 9 %, irradiation dose and storage temperature 0 %, storage time and storage temperature 4 %, irradiation dose, storage time, and storage temperature 3 %, and error 6 %. The average interior surface phenolic content was 486 µg/gfw.

Table 7.33 Analysis of variance results for interior surface phenolic content for the factors e-beam irradiation, storage time, and temperature, Springlake 2004.

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	766816.478 [z]	19	40358.762	33.647	.000
Intercept	14144710.981	1	14144710.981	11792.326	.000
Dose	789.177	1	789.177	.658	.422
Store	639719.005	4	159929.751	133.332	.000
Temp	371.715	1	371.715	.310	.581
Dose * Store	69524.444	4	17381.111	14.490	.000
Dose * Temp	1805.936	1	1805.936	1.506	.227
Store * Temp	29507.472	4	7376.868	6.150	.001
Dose * Store * Temp	25098.728	4	6274.682	5.231	.002
Error	47979.376	40	1199.484		
Total	14959506.835	60			
Corrected Total	814795.854	59			

[z] $R^2 = 0.941$ (Adjusted $R^2 = 0.913$)

The trends in phenolic content for both exterior and interior surfaces as affected by storage time are presented in Table 7.34. These trends are similar to that reported for total area. Phenolic content initially increased with time, then decreased. Phenolic content decreased, when comparing day 0 to day 110, however, the difference was quite small especially for the surface area (14 µg/gfw).

Table 7.34 Storage time ranking for phenolic content for exterior and interior samples, Springlake 2004.

Storage time	Eq. Chlorogenic acid (Surface Area) (µg/gfw)	Storage time	Eq. Chlorogenic acid (Interior Area) (µg/gfw)
10 days	1181 a[z]	10 days	633 a
20 days	944 b	0 days	550 b
0 days	858 c	20 days	492 c
110 days	844 c	110 days	417 d
75 days	707 d	75 days	335 e

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Storage temperature significantly affected phenolic content in the exterior surface area of the tuber (Table 7.36). This increase in phenolics might be due to a concentration / dehydration effect caused by the warmer temperatures. The total area

analysis ranked the lower temperature significantly greater, while past studies (Chapter IV) indicated there was no significant difference between the two storage temperatures.

Table 7.35 Storage temperature ranking for phenolic content for exterior and interior samples, Springlake 2004.

Storage temperature	Eq. Chlorogenic acid (Surface Area) (µg/gfw)	Storage temperature	Eq. Chlorogenic acid (Interior Area) (µg/gfw)
20 C	928 a[z]	20 C	488 a
4 C	897 b	4 C	483 a

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Over all, higher irradiation doses resulted in higher phenolic content than the lower dose; this difference was significant for the surface area, but not significant for the interior area (Table 7.36).

Table 7.36 Irradiation dosage ranking for phenolic content for exterior and interior samples, Springlake 2004.

Irradiation dose	Eq. chlorogenic acid (Surface Area) (µg/gfw)	Irradiation dose	Eq. chlorogenic acid (Interior Area) (µg/gfw)
200 Gy	939 a[z]	200 Gy	486 a
0 Gy	879 b	0 Gy	482 a

[z] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The interaction between storage time and storage temperature and the interaction between storage time and irradiation dose are presented in Tables 7.38 and 7.39, respectively. These two interactions were chosen because both were significant in both areas of the tuber, and these interactions clarify some discrepancies. The three-factor interaction, although significant in both areas, will not be described due to lack of a determined trend, as well as the complicated nature of the interaction.

The interaction between storage time and storage temperature for both exterior and interior areas of the tuber can be seen in Table 7.37. The 4 ^{o}C storage treatment

resulted in significantly higher phenolics in whole tubers than the 20 ^{o}C, while both exterior and interior had higher phenolic content at 4 ^{o}C. This interaction does not fully explain this discrepancy, but it does portray some variability in ranking based on storage time. It is believed that with longer storage at 20 ^{o}C, dehydration and concentration occurred. Therefore, as storage increased, phenolic content increased at a greater rate in tubers stored at warmer temperatures. A similar trend can be noted for interior-area samples.

Table 7.37 Storage time by storage temperature interaction for phenolic content for exterior and interior samples, Springlake 2004.

Storage time and storage temperature	Eq. Chlorogenic acid (Surface Area) (µg/gfw)	Eq. Chlorogenic acid (Interior Area) (µg/gfw)
0 days		
4C	858	550
20 C	858	550
10 days		
4C	1201	666
20 C	1161	601
20 days		
4C	857	476
20 C	1051	509
75 days		
4C	697	340
20 C	717	330
110 days		
4C	834	384
20 C	855	451

The interaction between storage time and irradiation dose indicates that irradiation dose ranking was dependent on storage time (Table 7.39). It was noted that during later stages of storage the higher irradiation dose resulted in greater phenolic content than the lower irradiation dose.

Table 7.38 Storage time by irradiation dose interaction for phenolic content for exterior and interior samples, Springlake 2004.

Storage time and irradiation dose	Eq. chlorogenic acid (Surface Area) (µg/gfw)	Eq. chlorogenic acid (Interior Area) (µg/gfw)
0 days		
0 Gy	732	578
200 Gy	984	521
10 days		
0 Gy	1244	666
200 Gy	1118	601
20 days		
0 Gy	972	430
200 Gy	936	554
75 days		
0 Gy	648	325
200 Gy	765	345
110 days		
0 Gy	799	410
200 Gy	890	424

HPLC ANALYSIS FOR CAROTENOID COMPOUNDS. Ninety samples were analyzed for individual carotenoid content. Although there were seven compounds via HPLC, only four compounds, neoxanthin, lutein, zeaxanthin, and canthaxanthin, were identified through retention time. Only lutein was identified via spectra; therefore, only lutein was reported in spectra results and in the combined spectra and retention time results.

Total carotenoid content differed significantly in exterior and interior areas of the tubers based on retention time ($p = 0.021$), but was not significant for any other dependent value. Individual carotenoids and total carotenoids via HPLC were similarly affected by storage, as was total carotenoid content via spectrophotometric methods. Carotenoid content based on total area was greatest at 10 days, decreased at 20 days, followed by an increase (Table 7.39). Carotenoid content (total area) based on spectra and both retention time and spectra decreased with time. Exterior total carotenoid content via retention time was highest at 110 days of storage, while exterior total carotenoid content based on spectra and both retention time and spectra decreased over time (Table 7.39). Interior total carotenoid content via retention time, for 10 and 110 days were similar, and the 110 day storage ranked higher than the 0 day (Table

7.41). Interior total carotenoid content via spectra and both spectra and retention time indicated a greater decrease in carotenoids with storage than the results based on retention time.

Table 7.39 Total area storage ranking for individual carotenoid compounds[z] (µg/100gfw) based on retention time, spectra, and both retention time and spectra, Springlake 2004.

Storage time	NEO	LUT	ZEA	CAN	Total	LUT-SP	LUT-RSP
0 days	0 a[y]	37 ab	0 b	0 a	37 a	37 a	37 a
10 days	0 a	41 a	0 b	0 a	41 a	38 a	38 a
20 days	0 a	23 b	0 b	1 a	24 a	6 b	4 b
75 days	0 a	29 ab	0 b	0 a	29 a	6 b	6 b
110 days	2 a	0 c	32 a	0 a	34 a	4 b	0 b

[z]NEO : Neoxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.40 Exterior surface area storage ranking for individual carotenoid compounds[z] (µg/100gfw) based on retention time, spectra, and both retention time and spectra, Springlake 2004.

Storage time	NEO	LUT	ZEA	CAN	Total	LUT-SP	LUT-RSP
0 days	0 a[y]	44 a	0 b	0 b	44 a	28 a	28 abc
10 days	6 a	65 a	0 b	0 b	80 a	65 a	65 a
20 days	0 a	34 a	0 b	0 b	34 a	19 a	19 bc
75 days	0 a	55 a	0 b	0 b	59 a	55 a	55 ab
110 days	13 a	0 b	80 a	10 a	107 a	15 a	0 c

[z]NEO : Neoxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.41 Interior area storage ranking for individual carotenoid compounds[z] (µg/100gfw) based on retention time, spectra, and both retention time and spectra, Springlake 2004.

Storage time	NEO	LUT	ZEA	CAN	Total	LUT-SP	LUT-RSP
0 days	0 a[y]	35 a	0 b	0 a	35 a	35 a	35 a
10 days	0 a	46 a	0 b	0 a	46 a	38 a	38 a
20 days	0 a	21 a	0 b	0 a	21 a	21 a	21 a
75 days	0 a	33 a	0 b	0 a	33 a	16 a	16 a
110 days	0 a	0 a	46 a	0 a	46 a	26 a	0 a

[z]NEO : Neoxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Storage temperature was not a significant factor in any of the results for carotenoid content based on HPLC (Tables 7.42, 7.43, and 7.44), but storage temperature was significant for total area and exterior area based on spectrophotometric methods. The total area HPLC retention time results match the ranking of the spectrophotometric results. Both ranked the 4 ^{o}C storage higher, while the spectra and the combination ranked the 20 ^{o}C higher. The ranking for both exterior and interior areas for total carotenoids based on retention time both matched the ranking based on the spectrophotometric results, while those based on spectra did not.

Table 7.42 Total area temperature ranking for individual carotenoid compounds[z] (µg/100gfw) based on retention time, spectra, and both retention time and spectra, Springlake 2004.

Storage temp.	NEO	LUT	ZEA	CAN	Total	LUT-SP	LUT-RSP
4 ^{o}C	1 a[y]	25 a	9 a	0 a	35 a	14 a	14 a
20 ^{o}C	0 a	25 a	6 a	0 a	31 a	18 a	16 a

Table 7.42 (continued).

[z]NEO : Neoxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.43 Exterior surface area temperature ranking for individual carotenoid compounds[z] (µg/100gfw) based on retention time, spectra, and both retention time and spectra, Springlake 2004.

Storage temp.	NEO	LUT	ZEA	CAN	Total	LUT-SP	LUT-RSP
4 °C	6 a[y]	38 a	11 b	0 b	57 a	46 a	38 a
20 °C	3 a	40 a	23 a	4 a	75 a	31 a	31 a

[z]NEO : Neoxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.44 Interior area temperature ranking for individual carotenoid compounds[z] (µg/100gfw) based on retention time, spectra, and both retention time and spectra, Springlake 2004.

Storage temp.	NEO	LUT	ZEA	CAN	Total	LUT-SP	LUT-RSP
4 °C	0 a[y]	23 a	10 a	0 a	32 a	28 a	19 a
20 °C	0 a	28 a	12 a	0 a	40 a	24 a	21 a

[z]NEO : Neoxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Results of the HPLC analysis on the effect of irradiation dose on carotenoids are presented in Tables 7.45, 7.46, and 7.47. Irradiation dose was not significant in the

HPLC samples. Total carotenoid content based on retention time ranking matched the ranking based on the spectrophotometric results for all areas sampled, with the 0 Gy dose higher than the 200 Gy dose. Over all, the spectra matched the ranking based on spectrophotometric results, except for interior samples.

Table 7.45 Total area irradiation dose ranking for individual carotenoid compounds[z] (µg/100gfw) based on retention time, spectra, and both retention time and spectra, Springlake 2004.

Irradiation dose	NEO	LUT	ZEA	CAN	Total	LUT-SP	LUT-RSP
0 Gy	1 a[y]	27 a	8 a	0 a	36 a	18 a	16 a
200 Gy	0 a	23 a	6 a	0 a	29 a	14 a	14 a

[z]NEO : Neoxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

[x] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.46 Exterior surface area irradiation dose ranking for individual carotenoid compounds[z] (µg/100gfw) based on retention time, spectra, and both retention time and spectra, Springlake 2004.

Irradiation dose	NEO	LUT	ZEA	CAN	Total	LUT-SP	LUT-RSP
0 Gy	6 a[y]	42 a	24 a	3 a	79 a	45 a	38 a
200 Gy	3 a	37 a	12 a	2 a	55 a	30 a	30 a

[z]NEO : Neoxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.47 Interior area irradiation dose ranking for individual carotenoid compounds[z] (µg/100gfw) based on retention time, spectra, and both retention time and spectra, Springlake 2004.

Irradiation dose	NEO	LUT	ZEA	CAN	Total	LUT-SP	LUT-RSP
0 Gy	0 a[y]	27 a	14 a	0 a	41 a	25 a	19 a
200 Gy	0 a	24 a	7 b	0 a	31 a	27 a	20 a

[z]NEO : Neoxanthin content based on retention time
LUT : Lutein content based on retention time
ZEA : Zeaxanthin content based on retention time
CAN : Canthaxanthin content based on retention time
Total-R : the addition of all measured carotenoids based on retention time
LUT-SP : Lutein based on spectra
LUT-RSP : Lutein based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Analysis of variance results indicated that the interactions involving the carotenoid HPLC data were not significant for most dependent variables; therefore, none of these interactions are described.

HPLC ANALYSIS FOR PHENOLIC COMPOUNDS. Ninety samples were analyzed for phenolics via HPLC. Although there were eighteen compounds analyzed, only thirteen, chlorogenic acid, caffeic acid, t-cinnamic acid, gallic acid, rutin hydrate, sinapic acid, epicatechin, quercetin dihydrate, protocatechuic acid, myricetin, p-coumaric acid, catechin, and vanillic acid were identified though retention time. Area of the tuber analyzed was a significant factor for all measured dependent values, with the exception of rutin hydrate content based retention time, rutin hydrate content based on spectra, and caffeic acid content based on the combination of retention time and spectra (Tables 7.48 and 7.49). The largest differences between areas analyzed appeared to be in chlorogenic acid content.

Table 7.48 Area ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, Springlake 2004.

Area	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
Surface	413	80	11	304	126	122	12	29	52	34	13	242	60	1516
	a[y]	a	a	a	a	a	a	a	a	a	a	a	a	a
Interior	98	43	10	218	132	38	8	13	36	18	10	196	21	842
	b	b	b	b	a	b	b	b	b	b	b	b	b	b

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.49 Area ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both spectra and retention time, Springlake 2004.

Area	CH-SP	CA-SP	RU- SP	EP-SP	Total-SP	CH-RSP	CA-RSP	Total-RSP
Surface	614 a[y]	119 a	41 a	7 a	781 a	321 a	15 a	336 a
Interior	241 b	20 b	23 a	1 b	285 b	37 b	2 a	39 b

[z]CH-SP : Chlorogenic acid based on spectra
CA-SP : Caffeic acid based on spectra
RU-SP : Rutin hydrate based on spectra
EP-SP : Epicatechin based on spectra
Total-SP : the addition of all measured phenolics based on spectra
CH-RSP : Chlorogenic acid based on retention time and spectra
CA-RSP : Caffeic acid based on retention time and spectra
Total-RSP : The addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The effects of storage time on phenolic content as identified by HPLC retention time are shown in Tables 7.50, 7.51, and 7.52. A similar trend was seen with the spectrophotometric results, where an increase in phenolics occurred at days 10 and 110. An increase in the individual phenolics (gallic acid, catechin, and chlorogenic acid) also took place at 10 and 110 days of storage.

Table 7.50 Total area storage ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, Springlake 2004.

Storage time	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
0 days	202	41	10	219	34	33	8	14	54	19	11	196	9	851
	b[y]	b	a	a	b	a	a	b	b	a	a	a	a	a
10	291	38	11	246	775	49	10	25	69	22	11	202	11	1782
days	a	b	a	a	a	a	a	a	a	a	a	a	a	a
20	57	49	10	241	114	48	10	14	39	21	9	213	24	853
days	b	b	a	a	b	a	a	b	c	a	a	a	b	a
75	80	65	10	217	97	43	11	16	38	21	10	224	30	862
days	b	a	a	a	b	a	a	b	c	a	a	a	b	a
110	78	61	10	810	157	45	11	18	36	21	10	412	68	1755
days	b	a	a	a	b	a	a	b	c	a	a	a	a	a

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics
[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.51 Exterior surface area storage ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, Springlake 2004.

Storage time	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
0 days	468	45	10	250	47	38	8	17	55	23	15	204	18	1198
	b[y]	e	a	b	a	a	d	a	a	a	a	d	e	d
10	1354	59	11	302	138	99	8	37	47	33	20	219	32	2360
days	a	d	a	b	a	a	c	a	a	a	a	c	d	a
20	98	85	10	263	81	145	12	25	42	36	10	249	69	1129
days	c	c	a	b	a	a	b	a	a	a	a	b	b	e
75	182	87	11	305	169	159	12	34	46	43	12	249	56	1365
days	c	b	a	b	a	a	c	a	a	a	a	b	c	c
110	161	112	11	379	170	140	17	31	68	31	12	276	109	1585
days	c	a	a	a	a	a	a	a	a	a	a	a	a	b

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics
[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.52 Interior area storage ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, Springlake 2004.

Storage time	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
0 days	79	20	10	175	52	36	8	10	39	17	8	196	10	658
	a[y]	a	a	a	a	a	a	a	a	a	b	a	a	a
10 days	203	39	10	235	164	53	8	19	45	15	12	149	8	961
	a	a	a	a	a	a	a	a	a	a	a	b	a	a
20 days	66	47	10	221	115	35	8	12	35	20	9	207	19	803
	a	a	a	a	a	a	a	a	a	a	ab	a	a	a
75 days	59	46	10	222	133	38	8	11	38	18	9	203	14	808
	a	a	a	a	a	a	a	a	a	a	ab	a	a	a
110 days	66	53	10	216	151	28	9	12	26	21	9	227	47	877
	a	a	a	a	a	a	a	a	a	a	ab	a	a	a

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The effects of storage time via spectra and the combination of spectra and retention time are shown in Tables 7.53, 7.54, and 7.55. Increases in phenolics during the late stages of storage were noted for spectra results, but this increase was less apparent in the results based on the combination of spectra and retention time, which indicated a general trend of decreasing phenolics with storage, and ranked day 10 higher than the other days of storage.

Table 7.53 Total area storage ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both spectra and retention time, Springlake 2004.

Storage time	CH-SP		CA-SP		RU- SP		EP-SP		Total-SP		CH-RSP		CA-RSP		Total-RSP	
0 days	222	a[y]	200	a	4	a	0	b	425	a	164	b	27	a	191	b
10 days	332	a	116	b	5	a	12	a	465	a	260	a	28	a	288	a
20 days	231	a	61	bc	12	a	2	ab	305	a	3	c	0	b	3	c
75 days	343	a	17	c	19	a	7	ab	386	a	6	c	0	b	6	c
110 days	526	a	22	c	78	a	2	ab	629	a	0	c	4	b	4	c

Table 7.53 (continued).

[z]CH-SP	: Chlorogenic acid based on spectra	CH-RSP	: Chlorogenic acid based on retention time and spectra
CA-SP	: Caffeic acid based on spectra		
RU-SP	: Rutin hydrate based on spectra	CA-RSP	: Caffeic acid based on retention time and spectra
EP-SP	: Epicatechin based on spectra		
Total-SP	: the addition of all measured phenolics based on spectra	Total-RSP	: The addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.54 Exterior surface area storage ranking for individual phenolic compounds (µg/gfw) based on spectra and both spectra and retention time, Springlake 2004.

Storage time	CH-SP		CA-SP		RU- SP		EP-SP		Total-SP		CH-RSP		CA-RSP		Total-RSP	
0 days	466	a[y]	123	a	21	a	7	a	616	b	436	b	12	a	447	b
10 days	1331	a	310	a	6	a	21	a	1667	a	1314	a	59	a	1372	a
20 days	402	a	65	a	0	a	0	a	467	b	0	c	0	a	0	c
75 days	455	a	48	a	83	a	11	a	597	b	81	c	13	a	94	c
110 days	504	a	65	a	77	a	0	a	645	b	0	c	0	a	0	c

[z]CH-SP	: Chlorogenic acid based on spectra	CH-RSP	: Chlorogenic acid based on retention time and spectra
CA-SP	: Caffeic acid based on spectra		
RU-SP	: Rutin hydrate based on spectra	CA-RSP	: Caffeic acid based on retention time and spectra
EP-SP	: Epicatechin based on spectra		
Total-SP	: the addition of all measured phenolics based on spectra	Total-RSP	: The addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.55 Interior area storage ranking for individual phenolic compounds (µg/gfw) based on spectra and both spectra and retention time, Springlake 2004.

Storage time	CH-SP		CA-SP		RU- SP		EP-SP		Total-SP		CH-RSP		CA-RSP		Total-RSP	
0 days	142	a[y]	6	a	0	a	0	a	148	a	0	a	0	a	0	a
10 days	177	a	61	a	2	a	5	a	245	a	158	a	10	a	168	a
20 days	273	a	3	a	3	a	0	a	279	a	0	a	0	a	0	a
75 days	263	a	0	a	52	a	0	a	315	a	0	a	0	a	0	a
110 days	309	a	18	a	40	a	0	a	368	a	0	a	0	a	0	a

[z]CH-SP	: Chlorogenic acid based on spectra	CH-RSP	: Chlorogenic acid based on retention time and spectra
CA-SP	: Caffeic acid based on spectra		
RU-SP	: Rutin hydrate based on spectra	CA-RSP	: Caffeic acid based on retention time and spectra
EP-SP	: Epicatechin based on spectra		
Total-SP	: the addition of all measured phenolics based on spectra	Total-RSP	: The addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.56 presents results for the effects of storage temperature on phenolic content in the three areas of the tuber, total area (Table 7.56), exterior surface area (Table 7.57), and interior area (Table 7.58). The spectrophotometric results for the total area indicated that phenolics were significantly higher at 4 ^{o}C, while both the exterior and interior areas had significantly higher phenolics at 20 ^{o}C. The results based on retention time identification of total phenolics indicate a similar trend, except that the interior total phenolics were higher at 4 ^{o}C, but not significantly higher than at 20 ^{o}C. The compounds that appeared to be most affected by storage temperature were chlorogenic acid, caffeic acid, gallic acid, rutin hydrate, and catechin.

Table 7.56 Total area temperature ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, Springlake 2004.

Storage temp.	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
4 ^{o}C	145 a[y]	56 a	10 a	246 a	362 a	49 a	10 a	17 a	45 a	21 a	10 a	305 a	32 a	1308 a
20 ^{o}C	128 b	48 b	10 a	453 a	174 a	41 a	10 a	18 a	47 a	21 a	10 a	216 a	30 a	1218 a

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.57 Exterior surface area temperature ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, Springlake 2004.

Storage temp.	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
4 ^{o}C	416 a[y]	86 a	11 a	278 b	117 a	121 a	14 a	32 a	49 a	34 a	12 a	245 a	59 b	1498 b
20 ^{o}C	382 a	79 a	11 a	330 a	140 a	132 a	11 b	27 a	54 a	34 a	14 a	244 b	66 a	1534 a

Table 7.57 (continued).

[z]CH : Chlorogenic acid	QU : Quercetin dihydrate
CA : Caffeic acid	PR : Protocatechuic acid
CI : t-Cinnamic acid	MY : Myricetin
GA : Gallic acid	PC : p-Coumaric acid
RU : Rutin hydrate	CT : Catechin
SI : Sinapic acid	VA : Vanillic acid
EP : Epicatechin	Total : the addition of all measured phenolics

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.58 Interior area temperature ranking for individual phenolic compounds[z] (μg/gfw) based on retention time, Springlake 2004.

Storage temp.	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
4 °C	108	45	10	237	170	40	8	13	37	19	9	208	22	927
	a[y]	a	a	a	a	a	a	a	a	a	b	a	a	a
20 °C	92	45	10	208	111	38	8	13	36	18	10	187	21	796
	a	a	a	a	a	a	a	a	a	a	a	b	a	a

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics
[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The effects of storage temperature on phenolics based on spectra and the combination of spectra and retention time can be seen in Tables 7.59, 7.60, and 7.61. The only significant results were with chlorogenic acid content based on the combination of spectra and retention time, and total phenolics based on the combination of spectra and retention time. Over all, the spectra and combination results were similar to those based on spectrophotometric methods.

Table 7.59 Total area temperature ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both spectra and retention time, Springlake 2004.

Storage temp.	CH-SP	CA-SP	RU- SP	EP-SP	Total-SP	CH-RSP	CA-RSP	Total-RSP
4 °C	432 a[y]	47 a	35 a	5 a	519 a	88 a	7 a	95 a
20 °C	271 a	89 a	19 a	5 a	384 a	70 b	13 a	83 b

[z]CH-SP : Chlorogenic acid based on spectra
CA-SP : Caffeic acid based on spectra
RU-SP : Rutin hydrate based on spectra
EP-SP : Epicatechin based on spectra
Total-SP : the addition of all measured phenolics based on spectra
CH-RSP : Chlorogenic acid based on retention time and spectra
CA-RSP : Caffeic acid based on retention time and spectra
Total-RSP : The addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.60 Exterior surface area temperature ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both spectra and retention time, Springlake 2004.

Storage temp.	CH-SP	CA-SP	RU- SP	EP-SP	Total-SP	CH-RSP	CA-RSP	Total-RSP
4 °C	652 a[y]	111 a	2 a	13 a	779 a	328 a	14 a	342 a
20 °C	572 a	111 a	76 a	2 a	760 a	279 a	16 a	295 a

[z]CH-SP : Chlorogenic acid based on spectra
CA-SP : Caffeic acid based on spectra
RU-SP : Rutin hydrate based on spectra
EP-SP : Epicatechin based on spectra
Total-SP : the addition of all measured phenolics based on spectra
CH-RSP : Chlorogenic acid based on retention time and spectra
CA-RSP : Caffeic acid based on retention time and spectra
Total-RSP : The addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.61 Interior area temperature ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both spectra and retention time, Springlake 2004.

Storage temp.	CH-SP	CA-SP	RU- SP	EP-SP	Total-SP	CH-RSP	CA-RSP	Total-RSP
4 °C	248 a[y]	31 a	3 a	3 a	284 a	52 a	0 a	52 a
20 °C	247 a	13 a	41 a	0 a	301 a	30 a	4 a	34 a

[z]CH-SP : Chlorogenic acid based on spectra
CA-SP : Caffeic acid based on spectra
RU-SP : Rutin hydrate based on spectra
EP-SP : Epicatechin based on spectra
Total-SP : the addition of all measured phenolics based on spectra
CH-RSP : Chlorogenic acid based on retention time and spectra
CA-RSP : Caffeic acid based on retention time and spectra
Total-RSP : The addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

All spectrophotometric methods reported an increase in phenolics with higher doses of irradiation. The total tuber area showed an little difference in individual phenolic content with higher doses of irradiation (Table 7.62), while the exterior and interior tuber surfaces indicated a decrease in phenolics (Tables 7.63 and 7.64 respectively). The discrepancy may be due to the difference between measurement mechanisms of the two methods. The Folin method may be measuring different phenolics than those measured by HPLC. Total phenolic content in the exterior surface based on retention time was significantly different based on irradiation dose, but there were no significant differences for the total or interior area samples.

Table 7.62 Total area irradiation dose ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, Springlake 2004.

Irradiation dose	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
0 Gy	132 a[y]	52 a	10 a	461 a	178 a	44 a	9 a	18 a	43 b	21 a	10 a	289 a	26 b	1298 a
200 Gy	139 a	52 a	10 a	261 a	337 a	46 a	10 a	18 a	49 a	21 a	10 a	221 a	35 a	1200 a

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.63 Exterior surface area irradiation dose ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, Springlake 2004.

Irradiation dose	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
0 Gy	469 a[y]	85 a	11 a	275 b	110 a	121 a	11 b	30 a	53 a	36 a	14 a	244 a	55 b	1525 a
200 Gy	318 a	80 b	11 a	340 a	150 a	133 a	13 a	30 a	50 a	32 a	13 a	245 a	72 a	1509 b

Table 7.63 (continued).

[z]CH : Chlorogenic acid	QU : Quercetin dihydrate
CA : Caffeic acid	PR : Protocatechuic acid
CI : t-Cinnamic acid	MY : Myricetin
GA : Gallic acid	PC : p-Coumaric acid
RU : Rutin hydrate	CT : Catechin
SI : Sinapic acid	VA : Vanillic acid
EP : Epicatechin	Total : the addition of all measured phenolics

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.64 Interior area irradiation dose ranking for individual phenolic compounds[z] (µg/gfw) based on retention time, Springlake 2004.

Irradiation dose	CH	CA	CI	GA	RU	SI	EP	QU	PR	MY	PC	CT	VA	Total
0 Gy	108	48	10	217	154	36	8	12	35	18	10	183	19	858
	a[y]	a	a	a	a	a	a	a	a	a	a	b	a	a
200 Gy	90	42	10	225	120	41	8	14	38	19	9	208	24	849
	a	a	a	a	a	a	a	a	a	a	b	a	a	a

[z]CH : Chlorogenic acid
CA : Caffeic acid
CI : t-Cinnamic acid
GA : Gallic acid
RU : Rutin hydrate
SI : Sinapic acid
EP : Epicatechin
QU : Quercetin dihydrate
PR : Protocatechuic acid
MY : Myricetin
PC : p-Coumaric acid
CT : Catechin
VA : Vanillic acid
Total : the addition of all measured phenolics
[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

In Tables 7.65, 7.66, and 7.67 there were no significant differences between irradiation doses based on spectra and the combination of spectra and retention time; however, certain compounds appeared to be increased by higher irradiation doses, e.g. as rutin hydrate, while chlorogenic acid and caffeic acid appeared to decrease at higher doses of irradiation.

Table 7.65 Total area irradiation dose ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both spectra and retention time, Springlake 2004.

Irradiation dose	CH-SP	CA-SP	RU- SP	EP-SP	Total-SP	CH-RSP	CA-RSP	Total-RSP
0 Gy	361 a[y]	79 a	18 a	5 a	463 a	74 a	11 a	85 a
200 Gy	325 a	61 a	34 a	5 a	425 a	82 a	10 a	91 a

Table 7.65 (continued).

[z]CH-SP	: Chlorogenic acid based on spectra	CH-RSP	: Chlorogenic acid based on retention time and spectra
CA-SP	: Caffeic acid based on spectra		
RU-SP	: Rutin hydrate based on spectra	CA-RSP	: Caffeic acid based on retention time and spectra
EP-SP	: Epicatechin based on spectra		
Total-SP	: the addition of all measured phenolics based on spectra	Total-RSP	: The addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.66 Exterior surface area irradiation dose ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both spectra and retention time, Springlake 2004.

Irradiation dose	CH-SP		CA-SP		RU- SP		EP-SP		Total-SP		CH-RSP		CA-RSP		Total-RSP	
0 Gy	661	a[y]	127	a	0	a	4	a	792	a	383	a	19	a	402	a
200 Gy	551	a	93	a	87	a	11	a	792	a	211	a	10	a	221	a

[z]CH-SP	: Chlorogenic acid based on spectra	CH-RSP	: Chlorogenic acid based on retention time and spectra
CA-SP	: Caffeic acid based on spectra		
RU-SP	: Rutin hydrate based on spectra	CA-RSP	: Caffeic acid based on retention time and spectra
EP-SP	: Epicatechin based on spectra		
Total-SP	: the addition of all measured phenolics based on spectra	Total-RSP	: The addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

Table 7.67 Interior area irradiation dose ranking for individual phenolic compounds[z] (µg/gfw) based on spectra and both spectra and retention time, Springlake 2004.

Irradiation dose	CH-SP		CA-SP		RU- SP		EP-SP		Total-SP		CH-RSP		CA-RSP		Total-RSP	
0 Gy	256	a[y]	39	a	1	a	2	a	298	a	45	a	5	a	50	a
200 Gy	239	a	3	a	47	a	0	a	289	a	34	a	0	a	34	a

[z]CH-SP	: Chlorogenic acid based on spectra	CH-RSP	: Chlorogenic acid based on retention time and spectra
CA-SP	: Caffeic acid based on spectra		
RU-SP	: Rutin hydrate based on spectra	CA-RSP	: Caffeic acid based on retention time and spectra
EP-SP	: Epicatechin based on spectra		
Total-SP	: the addition of all measured phenolics based on spectra	Total-RSP	: The addition of all measured phenolics based on spectra and retention time

[y] Mean separation within columns by Student-Newman-Keuls (SNK) multiple range test at $P \leq 0.05$.

The analysis of variance results indicated that the interactions involving the phenolic HPLC data were not significant for most dependent variables; therefore, none of these interactions are described further.

Discussion and Conclusion

The present chapter analyzed postharvest treatments such as storage time and storage temperature that were reported earlier (Chapters IV, V, and VI); however, this study also involved electron beam irradiation. While electron beam irradiation is also a type of ionizing irradiation, its source, mechanism, and penetrating power are starkly different than those of gamma- irradiation. Similar interactions were observed in this study which confirmed earlier trends. There are still some unanswered questions which may relate to other factors not investigated in this study, such as harvest location, harvest time, cultural practices, maturity, and humidity during storage.

No previous studies have been found in the literature on the effect of electron beam irradiation on phytochemical levels. However, the effect of gamma- irradiation on phytochemical levels has been studied to a limited extent.

Over all, carotenoids were most affected by storage time, storage temperature, the interaction of storage time and temperature, and the interaction of storage time and irradiation dose. Carotenoid levels decreased with time, although in later stages of storage an increase did occur. Carotenoid levels also decreased in the previous studies conducted by Craft and Wise (1993); and Thomas and Joshi (1977). This increase was seen most dramatically in the exterior surfaces, where concentration due to dehydration may have occurred. Total carotenoid levels measured spectrophotometrically were significantly higher at 4 ^{o}C than at 20 ^{o}C storage. This trend was less prominent in the HPLC results. Carotenoids were higher in both exterior and interior surfaces at the higher temperature than the lower, which may again relate to dehydration. Irradiation dose effects on carotenoid levels appear to be related to both storage time and storage temperature. During the earlier stages of storage, the higher dose resulted in higher levels of carotenoids than the lower dose, while at later storage times, the lower dose resulted in higher carotenoid levels. Dehydration from

storage may be the reason for this trend. Bhushan and Thomas (1990); Janave and Thomas (1979); and Thomas and Janave (1975) reported interactions between storage and gamma- irradiation dose. While important results were obtained in the present study regarding carotenoids, "Atlantic" may not have been the best cultivar choice, as it is relatively low in carotenoids (Chapters III, IV, and V).

Antioxidant activity was most affected by storage time and the interaction of storage time and irradiation dose. Storage time caused decreases in antioxidant activity, but antioxidant activity increased again during the later stages of storage. Temperature was statistically significant, however it did not have a large influence on antioxidant activity based on eta squared values. Tubers stored at the lower temperature ranked significantly higher in antioxidant activity than those stored at higher temperatures. This was not as consistent in the exterior and interior areas of the tuber, which may have also experienced a dehydration effect. In the total tuber higher irradiation doses resulted in higher antioxidant activity than lower doses; however, the irradiation results were varied for the surface and interior samples. During the early stages of storage, the higher irradiation dose resulted in higher antioxidant activity, while the lower dose resulted in higher antioxidant activity in later stages. This trend was also noted in Chapters V and VI.

Phenolic content was most affected by storage time, peaking at 10 days. Phenolic content followed a similar trend to antioxidant activity where it decreased after 10 days, then increased again at 110 days. Phenolic content was only minimally affected by storage temperature. Phenolic content in the total tuber area was significantly greater at 4 ^{o}C than at 20 ^{o}C. Analysis of surface and interior results were varied, where storage at 20 ^{o}C caused phenolic content to be significantly higher via spectrophotometric methods, while the results via HPLC were more varied. Higher irradiation doses resulted in significantly greater phenolic content via spectrophotometric methods, while HPLC analysis indicated that there were no differences. Bergers (1981), Patil et al. (1999), and Penner and Fromm (1972) reported increases in phenolic content with exposure to gamma- irradiation; however, Patil et al.

(1999) and Penner and Fromm (1972) both reported decreases following the initial increase in phenolics.

In conclusion, storage time and temperature influenced carotenoid content, antioxidant activity, and phenolic content to a much greater extent than irradiation dosage. However, the levels of carotenoids, antioxidants, and phenolics were not linearly affected by storage time. The warmer storage temperature appeared to create dehydration and concentration effects especially on exterior surfaces. Irradiation dose caused only minor changes, most notable was a slight increase in total phenolic content via the Folin method. The interaction between irradiation dose and storage time influenced both carotenoid content and antioxidant activity. Future studies are needed to clarify the interaction between irradiation dose and storage time. The use of electron beam irradiation to extend shelf life of fruits and vegetables and its effect on quality, nutritional, and phytochemical levels should also be pursued further.

CHAPTER VIII
SUMMARY AND CONCLUSIONS

Numerous epidemiological studies have reported that the consumption of fruits and vegetables is correlated to disease prevention. This disease prevention is believed to be related to the content of certain chemicals found in fruits and vegetables, most notably phytochemicals with antioxidant potential. Numerous families and species of fruits and vegetables have been screened for antioxidant activity, and wide variability has been reported among families, species and genotypes within species. Many berry species, which have high antioxidant activity and phenolics, have been promoted and marketed as healthy foods. Misconceptions have led to the belief that potatoes are not nutritious, since they don't contain as high levels of certain phytochemicals. Past studies have determined that there are significant levels of phytochemicals and antioxidants in potato tubers, and that there is wide variability among genotypes (Al-Saikhan, 1994, 2000; Hale, 2003). The compounds analyzed in this study were compared to the amount of the same specific compounds found in blueberry (Table 8.1). Blueberry contains higher levels of some compounds; however, potato contains comparable or higher levels of other compounds. When considering the current average per capita consumption of potatoes, 136 lbs, compared to 0.75 lbs for blueberry (Givan, 2002), one may receive more phytochemicals from potato.

Table 8.1 Average amount of antioxidant compounds found in potatoes (average throughout all experiments) and blueberries (average of three fresh samples obtained from H-E-B grocery store in June).

Antioxidant Compound	Potatoes	Blueberries
Total Xanthophylls [z]	148	N/A
Total Carotenes [z]	24	N/A
Neoxanthin (R) [z v]	1	0
Antheraxanthin (R) [z]	1	0
Lutein (R) [z]	14	0
Zeaxanthin (R) [z]	4	0
Canthaxanthin (R) [z]	2	0
β-cryptoxanthin (R) [z]	< 0	0
Total Carotenoids (R) [z]	22	0
Lutein (SP) [z u]	9	0
Lutein (RSP) [z v]	7	0
AOAI [y]	167	684
AOAS [y]	363	836
Total Phenolics [x]	413	1519
Chlorogenic acid (R) [w]	113	105
Caffeic acid (R) [w]	52	208
t-Cinnamic acid (R) [w]	10	9
Gallic acid (R) [w]	287	1608
Rutin hydrate (R) [w]	115	1577
Sinapic acid (R) [w]	54	371
Epicatechin (R) [w]	212	59
Quercetin dihydrate (R) [w]	10	4
Protocatechuic acid (R) [w]	75	113
Myricetin (R) [w]	22	71
p-Coumaric acid (R) [w]	13	21
Catechin (R) [w]	253	291
Vanillic acid (R) [w]	20	185
Total Phenolics (R) [w]	1238	4622
Chlorogenic acid (SP) [w]	229	684
Caffeic acid (SP) [w]	158	836
Rutin hydrate (SP) [w]	19	0
Epicatechin (SP) [w]	2	0
Total Phenolics (SP) [w]	408	1519
Chlorogenic acid (RSP) [w]	77	0
Caffeic acid (RSP) [w]	25	0
Rutin hydrate (RSP) [w]	9	0
Total Phenolics (RSP) [w]	112	0

[z] expressed as µg/100gfw
[y] expressed as equivalents of trolox µg/gfw
[x] expressed as equivalents of chlorogenic acid µg/gfw
[w] expressed as µg/gfw
[v] (R) : based on HPLC retention time
[u] (SP) : based on HPLC spectra
[t] (RSP) : based on HPLC the combination of retention time

Postharvest processing is known to affect quality and nutritional aspects of food products; however, it has not been determined if certain phytochemical compounds, such as carotenoids, phenolics, and total antioxidant activity, are stable during postharvest processing of potato. All consumed potatoes are subject to some type of processing such as cooking, or storage; therefore, it was quite important to determine the effects of postharvest processing.

This study analyzed the effects of the processing methods of cooking, storage, and ionizing irradiation on carotenoid content, antioxidant activity, and phenolic content. A series of five experiments were designed that involved combinations of cultivars, cooking methods, storage times, storage temperatures, ionizing irradiation sources and ionizing irradiation dosages (Table 8.2). The first study (Chapter III) involved a factorial experiment with 17 cultivars, 5 cooking methods, and two harvest locations. Chapter IV involved a factorial experiment with 8 cultivars, 5 cooking treatments, and 4 storage treatments. Chapter V included 8 cultivars, 5 cooking methods, 2 storage treatments, and 3 gamma- irradiation doses. Chapter VI included 5 storage times and 3 gamma- irradiation doses. Chapter VII included 5 storage times, 2 storage temperatures, 2 electron beam irradiation doses, and 3 areas of the tuber analyzed. Areas of the tuber were not treated as a factor. Therefore, three separate analyses were conducted to control bias in the experiment due to previous known differences based on area of the tuber (Burton, 1989).

Table 8.2 Experimental design of the five studies.

Factor	Exp. 1 (Chapter III)	Exp. 2 (Chapter IV)	Exp. 3 (Chapter V)	Exp. 4 (Chapter VI)	Exp. 5 (Chapter VII)
Cultivars	17	8	8	1	1
Cooking methods	5	5	5	1 (raw)	1 (raw)
Storage treatments	1 (no storage)	4	2 storage times at 4 ^{o}C	5 storage times at 20 ^{o}C	5 storage times 2 temperatures
Irradiation doses	1 (0 Gy)	1 (0 Gy)	3 (Gamma doses)	3 (Gamma doses)	2 (E-beam doses)
Other factors	2 harvest locations				3 areas of tuber analyzed

An analysis was conducted to determine level of significance of the factors, and a ranking was assigned based on mean separation. Interactions were also analyzed based on significance and visible trends. Although numerous factors had a significant effect on the compounds tested, certain factors had more influence than others. In fact, this may have been the most important contribution of the present study. Eta squared values were computed in the separate studies. This value indicated the percentage of variability that each factor contributed to the analysis of variance, so one can determine which factors have a high power or more influence in an analysis. Eta squared values should not be compared directly if experimental design is not consistant, because eta squared values are a percent of variability; however, consistency of the influence can be determined. The next three tables show eta squared values for influencial factors based on experimental design. Carotenoid content was most influenced by cultivar selection (Table 8.3). Cultivar, when included in the experimental design, was the most influential factor. Storage appears to be the second most influential factor, followed by irradiation dose and cooking method. Over all, the effects of cooking and irradiation dose were minor as compared to storage (especially storage time).

Table 8.3 Eta squared values for carotenoid content for each experimental design.

Factors	Ch. III Carot.[z]	Ch. III Xanth.[y]	Ch. IV Xanth.	Ch. V Xanth.	Ch. VI Xanth.	Ch. VII Xanth.	Ch. VII Xanth. Ext.[x]	Ch. VII Xanth. Int.[w]
Cultivar (cult)	35	42	30	17				
Cooking method (cook)	2	2	3	6				
Storage time (store)				12	90	12	42	23
Storage temperature (temp)			19			11	4	0
Irradiation dose (irr)				17	1	0	6	1
(cult)* (cook)	6	6	5	3				
(cult) * (store)				2				
(cult) * (temp)			11					
(cult) * (irr)				5				
(cook) * (store)				1				
(cook) * (temp)			3					
(cook) * (irr)				4				
(store) * (irr)				0	1	5	24	31
(store) * (temp)						5	14	16
(temp) * (irr)						0	3	5
(cult) * (cook) * (store)				1				
(cult) * (cook) * (temp)			6					
(irr) * (store) * (temp)						3	4	12
(cult) * (cook) * (irr)				3				
(cult) * (store) * (irr)				4				
(cook) * (store) * (irr)				2				
(cult) * (cook) * (store) * (irr)				3				
error	57	50	22	21	9	63	3	12
Average amount eq. β-carotene µg/100gfw[v] or eq. lutein µg/100gfw[u]	24	163	106	120	210	172	346	131

[z] Carotenes
[y] Xanthophylls
[x] Exterior surfaces
[w] Interior surfaces
[v] Total number of carotene samples: 620; overall average 24 eq. β-carotene µg/100gfw
[u] Total number of xanthophyll samples: 2309; overall average 148 eq. lutein µg/100gfw

The interaction involving storage and irradiation dose and the interaction of storage time and storage temperature were also influential. Storage temperature had more influence on carotenoid content than on antioxidant activity or phenolic content.

Storage time appeared to have a greater effect on the external surfaces of the tubers than the internal and total area, and storage temperature had a greater effect on total and external surfaces as compared to internal surfaces (Chapter VII).

The eta squared values for initial antioxidant activity (AOAI) are presented in Table 8.4. Again, cultivar was the most influential factor followed by cooking. Cooking was not very influential for carotenoid content; however, cooking appears to have a larger impact on antioxidant activity (Tables 8.4 and 8.5) and phenolic content (Table 8.6). In experiments where cultivar and cooking were omitted, the most influential factors were storage time and the interaction of storage time and irradiation dose. Storage temperature had less influence on antioxidant activity as compared to carotenoid content.

The eta squared values for the stabilized antioxidant activity (AOAS) appear to mirror the eta squared values for AOAI, with some minor deviations, e.g., the influence of cooking appears to be slightly stronger on AOAS. Again, storage time appeared to have a greater influence when the factors of cultivar and cooking method were eliminated from the experimental design. Also, the interaction of storage time and irradiation dose was quite influential. Both antioxidant activity tests indicated a wide variability within the interior surfaces of the tuber with use of electron beam irradiation. This wide variability and large influence may signify chemical changes in the internal areas of electron beam irradiated samples. This could reflect a confounding effect from uneven dept penetration by electron beam irradiation within the tuber.

Table 8.4 Eta squared values for AOAI for each experimental design.

Factors	Ch. III McCook AOAI	Ch. III Springlake AOAI	Ch. IV AOAI	Ch. V AOAI	Ch. VI AOAI	Ch. VII AOAI	Ch. VII Ext. [z] AOAI	Ch. VII Int. [y] AOAI
Cultivar (cult)	42	28	24	8				
Cooking method (cook)	14	18	16	19				
Storage time (store)				0	18	51	51	81
Storage temperature (temp)			2			0	1	0
Irradiation dose (irr)				2	0	1	5	9
(cult)* (cook)	12	15	3	3				
(cult) * (store)				2				
(cult) * (temp)			21					
(cult) * (irr)				9				
(cook) * (store)				0				
(cook) * (temp)			1					
(cook) * (irr)				1				
(store) * (irr)				3	10	8	28	4
(store) * (temp)						4	7	3
(temp) * (irr)						0	1	0
(cult) * (cook) * (store)				2				
(cult) * (cook) * (temp)			5					
(irr) * (store) * (temp)						1	3	2
(cult) * (cook) * (irr)				4				
(cult) * (store) * (irr)				9				
(cook) * (store) * (irr)				0				
(cult) * (cook) * (store) * (irr)				4				
error	32	37	28	32	71	34	5	2
Average amount eq. trolox µg/gfw [x]	120	185	128	123	279	264	551	235

[z] Exterior surfaces

[y] Interior surfaces

[x] Total count of antioxidant activity samples: 1999; overall average 167 eq. trolox µg/gfw

Table 8.5 Eta squared values for AOAS for each experimental design.

Factors	Ch. III McCook AOAS	Ch. III Springlake AOAS	Ch. IV AOAS	Ch. V AOAS	Ch. VI AOAS	Ch. VII AOAS	Ch. VII Ext. [z] AOAS	Ch. VII Int. [y] AOAS
Cultivar (cult)	42	24	18	10				
Cooking method (cook)	11	29	17	23				
Storage time (store)				0	15	47	52	73
Storage temperature (temp)			4			0	0	0
Irradiation dose (irr)				1	1	0	1	6
(cult) * (cook)	11	13	3	4				
(cult) * (store)				5				
(cult) * (temp)			24					
(cult) * (irr)				11				
(cook) * (store)				1				
(cook) * (temp)			1					
(cook) * (irr)				1				
(store) * (irr)				2	10	4	31	7
(store) * (temp)						0	3	4
(temp) * (irr)						0	0	0
(cult) * (cook) * (store)				8				
(cult) * (cook) * (temp)			5					
(irr) * (store) * (temp)						1	8	3
(cult) * (cook) * (irr)				2				
(cult) * (store) * (irr)				9				
(cook) * (store) * (irr)				0				
(cult) * (cook) * (store) * (irr)				3				
error	36	33	26	27	73	46	5	6
Average amount eq. trolox µg/gfw [x]	348	328	305	312	507	486	683	372

[z] Exterior surfaces

[y] Interior surfaces

[x] Total count of antioxidant activity samples: 1999; overall average: 363 eq. trolox µg/gfw

Eta squared values for total phenolic content appear similar to those for antioxidant activity (Table 8.6). Cultivar and cooking had the greatest influence. Storage time had the largest influence of phenolics when cultivar and cooking method were eliminated. Irradiation dose and the interaction of irradiation dose and storage time also had a modest influence.

Table 8.6 Eta squared values for phenolic content for each experimental design.

Factors	Ch. III McCook Phen.	Ch. III Springlake Phen.	Ch. IV Phen.	Ch. V Phen.	Ch. VI Phen.	Ch. VII Phen.	Ch. VII Ext. [z] Phen.	Ch. VII Int. [y] Phen.
Cultivar (cult)	49	48	39	34				
Cooking method (cook)	6	15	22	24				
Storage time (store)				1	17	51	76	79
Storage temperature (temp)			1			1	1	0
Irradiation dose (irr)				5	0	8	3	0
(cult) * (cook)	7	9	4	3				
(cult) * (store)				2				
(cult) * (temp)			12					
(cult) * (irr)				7				
(cook) * (store)				0				
(cook) * (temp)			2					
(cook) * (irr)				1				
(store) * (irr)				1	8	0	13	9
(store) * (temp)						1	5	4
(temp) * (irr)						1	0	0
(cult) * (cook) * (store)				1				
(cult) * (cook) * (temp)			3					
(irr) * (store) * (temp)						1	1	3
(cult) * (cook) * (irr)				3				
(cult) * (store) * (irr)								
(cook) * (store) * (irr)				0				
(cult) * (cook) * (store) * (irr)				2				
error	38	28	16	15	74	38	2	6
Average amount eq. chlorogenic acid µg/gfw [x]	352	384	336	371	553	571	913	486

[z] Exterior surfaces
[y] Interior surfaces
[x] Total count of phenolic samples: 1999; overall average: 413 eq. chlorogenic acid µg/gfw

The eta squared tables also included an average of the content determined in each study. There was wide variability between averages determined in the separate studies, partly due to the factors chosen in each experiment, but also to variability of site and time of year of harvest. Pendlington et al. (1965), K'osambo et al. (1998), Burton (1989), and Connor et al. (2002) determined that there were significant

differences in aspects of antioxidant activity based on cultural or environmental influences. Future research should determine the influence of this factor.

For all eta squared results, an error or unexplainable variable term was also calculated. This was the variability that was not explained by the factors determined by each separate experiment. The error term was quite large in some studies, inferring that there were other factors that controlled phytochemical levels that were not analyzed or controlled. Future experiments should identify as many factors that could influence phytochemical levels.

There are many factors that contribute to the variability of phytochemicals in potato; however, in this study the factors of cultivar, cooking method, storage treatment, and ionizing irradiation were studied. The following is a summary of some results based on each factor.

Cultivar

CAROTENOID CONTENT. Carotenes were analyzed in Chapter III, but not in subsequent studies due to their low concentration. The top three cultivars in carotene content (measured as equivalents of β-carotene µg/100gfw) were Yukon Gold, A84420-5, and Atlantic. The range of carotene content was 14 to 53 µg/100gfw. Xanthophylls were analyzed in all studies. The greatest variability for xanthophyll content was in Chapter III (McCook, 2003 harvest). The range was 96 to 276 eq. lutein µg/100gfw. The cultivars that consistently ranked high in xanthophylls in all experiments were Innovator, Russet Burbank, Santana, Krantz, and Russet Norkotah.

ANTIOXIDANT ACTIVITY. Initial antioxidant activity (AOAI) and stabilized antioxidant activity (AOAS) were determined. Stabilized antioxidant activity was about twice as large as AOAI. The greatest range of AOAI was in Chapter III (McCook,2003 harvest). The range varied from 44 to 317 eq. trolox µg/gfw. The cultivars that consistently ranked high in AOAI were Russet Burbank, Innovator, Yukon Gold, Russet Norkotah, Santana, Krantz, and Atlantic. The greatest range of AOAS was also in Chapter III (McCook, 2003 harvest). The range varied from 206 to

727 eq. trolox µg/gfw. The cultivars that consistently ranked high in AOAS were similar to those for AOAI.

PHENOLIC CONTENT. Phenolic content varied most in Chapter III (McCook, 2003 harvest). The range was 177 to 672 eq. chlorogenic acid µg/gfw. The cultivars that consistently ranked high throughout the experiments were Krantz, Russet Burbank, Santana, and Innovator. Comparing all HPLC results, the cultivar Russet Burbank contained more rutin hydrate and sinapic acid based on retention time than the other cultivars, while the cultivar Innovator contained the greatest amount of total phenolics based on the combination of spectra and retention time.

Cooking Method

CAROTENOID CONTENT. Carotenoid content was less influenced by cooking than were phenolic content and antioxidant activity. Although, there were significant differences in each study, the range was minuscule. All cooking methods, including the raw control samples, resulted in significantly greater carotenoid content than the boiled samples. Further significant separation was not definitive. HPLC results corrobrated the spectrophotometric results, and boiled samples were again lower in carotenoid levels than the other cooking methods.

ANTIOXIDANT ACTIVITY. Antioxidant activity was affected by cooking method. The treatment with the lowest levels of phenolics throughout all studies was the control (raw samples) followed by the boiled samples. The microwaved and fried samples were the highest. It is believed that the cooked samples had an altered texture, that caused antioxidants to become more available. Another possibility was that cooked samples contain Maillard reaction products which are believed to have antioxidant activity. The greatest range (198 eq. trolox µg/gfw) of antioxidant activity was in AOAS from the Springlake harvest (Chapter III); raw samples averaged 220 µg/gfw while microwaved samples had an average of 418 µg/gfw.

PHENOLIC CONTENT. Phenolic content results were very similar to those for antioxidant content. Raw and boiled samples generally ranked much lower than the microwaved, baked, and fried samples. The greatest range (166 eq. chlorogenic acid

µg/gfw) was in the Dalhart samples (Chapter V). The highest phenolic content was in microwaved samples, 444 µg/gfw, while the lowest was in boiled, 278 µg/gfw. HPLC results supported the results based on the Folin method, ranking the cooking methods of microwave, fry and baking above the methods of boil and raw. Comparing all HPLC results, microwaved samples were the highest in the following compounds, caffeic acid, epicatechin based on retention time, chlorogenic acid, and total phenolic content based on spectra and the combination of spectra and retention time. Baked samples were highest in protocatechuic acid based on retention time throughout all studies. P-coumaric acid based on retention time was higher when samples were baked, fried, or microwaved. Quercetin dihydrate and t-cinnamic acid based on retention time were higher when samples were raw or uncooked.

Storage Treatment

CAROTENOID CONTENT. Storage was the most influential factor besides cultivar, although storage effects appear riddled with interactions and other influencial factors. In Chapters IV and V, the stored samples had higher levels of carotenoids than the no storage treatment via spectrophotometric results, while the HPLC results from Chapter V reported a higher level in the no storage treatment. In Chapters VI and VII, multiple storage times were analyzed. In Chapter VI, spectrophotometric results reported a decrease in carotenoid content with storage, but HPLC results ranked later stages of storage (75 and 110 days) equal or significantly higher in carotenoid content as no storage (0 days). Chapter VII results appeared to corroborate these findings, often ranking the earlier stages of storage (0 and 10 days) equal to the later stages of storage (75 and 110 days). Storage temperature was also an influential factor. The lower temperature of 4 ^{o}C resulted in higher carotenoid content than the 20 ^{o}C; however, this trend was not consistent for the exterior and interior surfaces. The carotenoid content might decrease with time, but the separate areas may have experienced greater dehydration and concentration in the warmer temperature storage, which caused the 20 ^{o}C to rank higher. The interaction of storage time and storage temperature and the

interaction of storage time and irradiation dosage had an important role in determining the effects of storage on carotenoid content.

ANTIOXIDANT ACTIVITY. The influence of storage on antioxidant activity is believed to be influenced by a number of factors. The storage treatment of 4 ^{o}C for 110 days had significantly greater levels of antioxidant activity than the other storage treatments, including no storage in Chapter IV. However, in Chapter V, there was either no significant difference or the no storage treatment was significantly greater. In Chapters VI and VII, the effect of storage was explained. Both treatments of no storage (0 days) and 110 days of storage at 4 ^{o}C and 20 ^{o}C exhibited high antioxidant activity. This effect may have been due to a general decrease in antioxidant activity with storage; however, due to concentration and dehydration in late stages of storage, antioxidant activity increased. In both Chapters IV and VII, the colder storage temperature (4 ^{o}C) ranked significantly higher than the warmer temperature (20 ^{o}C); however, the exterior area samples from Chapter VII ranked the 20 ^{o}C treatment significantly higher than the 4 ^{o}C. This may be due to the greater dehydration of exterior surfaces that accompanied storage at higher temperatures. The interaction between storage time and irradiation dose was an influential factor throughout experiments.

PHENOLIC CONTENT. The storage treatment of 4 ^{o}C for 110 days with 10 days reconditioning was significantly greater in phenolics via the Folin method than any other treatment in Chapter IV. HPLC results indicated that the treatments of no storage and 4 ^{o}C for 110 days with 10 days reconditioning both ranked higher than the other treatments. In Chapter V, the no storage treatment again had higher phenolics via the Folin method than 4 ^{o}C for 110 days, while the storage treatment 4 ^{o}C for 110 days ranked higher via HPLC. In Chapter VI, phenolic content increased with storage time via the Folin method. This may have been due to the 20 ^{o}C storage, which caused tubers to experience dehydration and concentration. However, HPLC retention time indicated that total phenolic content ranked high at both 110 and 20 days. HPLC spectra also identified greater total phenolic content during earlier stages of storage. In

Chapter VII, phenolic content via the Folin method peaked at 10 days, decreased, then increased at later stages of storage. HPLC results for Chapter VII reported a high amount of total phenolic compounds during the storage times of 0, 10 days, and 110 days. In Chapter VII, samples obtained from whole tubers had higher levels of phenolics with the 4 ^{o}C storage treatment, while in the external and internal surfaces the higher temperatures often ranked higher or not significantly different. This may be due to dehydration and concentration in the exterior and interior surfaces when tubers were stored at higher temperatures. There were also numerous interactions involving storage treatments that played significant roles, such as the interaction of cultivar and storage temperature, storage time and irradiation dose, and storage time and storage temperature. Based on all HPLC results, chlorogenic acid was higher when samples were stored at colder temperatures, 4 ^{o}C rather than 20 ^{o}C. No other compounds appeared to be consistently affected by a certain storage treatment.

Irradiation Treatment

CAROTENOID CONTENT. Carotenoid content via spectrophotometric absorbance increased with the added treatment of irradiation in Chapter V. The HPLC results also ranked the 150 Gy dose highest in both retention time and spectra; however, there was no significant differences. In Chapter VI, the carotenoid content via spectrophotometric absorbance was highest with the 0 Gy dose, but not significantly greater than the highest dose, 200 Gy. HPLC results ranked the 200 Gy dose higher but not significantly higher than the other doses. In Chapter VII, the 0 Gy dose was ranked higher, but again not significantly higher. In both experiments, a trend was noted, where during early stages of storage the higher doses had higher carotenoid levels, while with continued storage the lower doses had higher levels. In Chapter VII, there was also a significant interaction between storage temperature and irradiation dose.

ANTIOXIDANT ACTIVITY. Significant differences in antioxidant activity were reported with irradiation dose; however, these differences were dependent on experimental design. The interaction of storage time and irradiation dose was very

influential. The higher doses had higher antioxidant activity in the early stages of storage, while the lower doses ranked higher with continued storage. Therefore, the effect of irradiation dose on antioxidant activity was dependent on when sample measurement occurred. The 0 Gy tubers may have had higher antioxidant activity at later stages of storage due to dehydration and induction of antioxidants due to the stress of sprouting.

PHENOLIC CONTENT. The phenolic content, via the Folin method, increased with added exposure to irradiation. This increase was significant in Chapter V and in the whole tuber samples in Chapter VII. In Chapter VI, the 200 Gy dose appeared higher in phenolics but not significantly so. Chapter V HPLC retention time results reported no significant differences between irradiation doses, but ranked the 75 Gy dose highest in phenolic content. However, the HPLC spectra and the combination of spectra and retention time ranked the 0 Gy dose higher than the other two doses. Chapter VII also reported higher levels of phenolics via the Folin method in the 200 Gy dose. In the total tuber area and exterior surface area, the 200 Gy dose was significantly greater. The HPLC results were less conclusive. Most results for the total surface, exterior, and interior areas ranked the 0 Gy dose higher, but not significantly higher than the 200 Gy dose. Based on HPLC results from all the chapters, no one compound appeared to be consistently affected by irradiation dose.

Interactions

The most influential interactions throughout all the studies were the interactions of cultivar and cooking method, storage time and storage temperature, storage time and irradiation dose, and storage time, storage temperature, and irradiation dose. Noticeable trends were described for many of these interactions and some theories were discussed on the causes of the interactions. Future research should further investigate the mechanism and causes of these interactions.

Conclusion

The objectives of this study were to determine the effects of the processing methods of cooking, storage and low-dose ionizing irradiation on carotenoid content, antioxidant

activity, and phenolic content in potato. Each processing factor had an effect on the phytochemicals; however, the most influential factor appeared to be cultivar selection. There may be a stimulation, induction, or release of some compounds due to processing; however, the magnitude of these effects is not as great as genetic control.

However, the effects of processing cannot be denied and should continue to be investigated. Future studies investigating the health properties of fruits and vegetables, especially potatoes, should included processing effects in the experiment.

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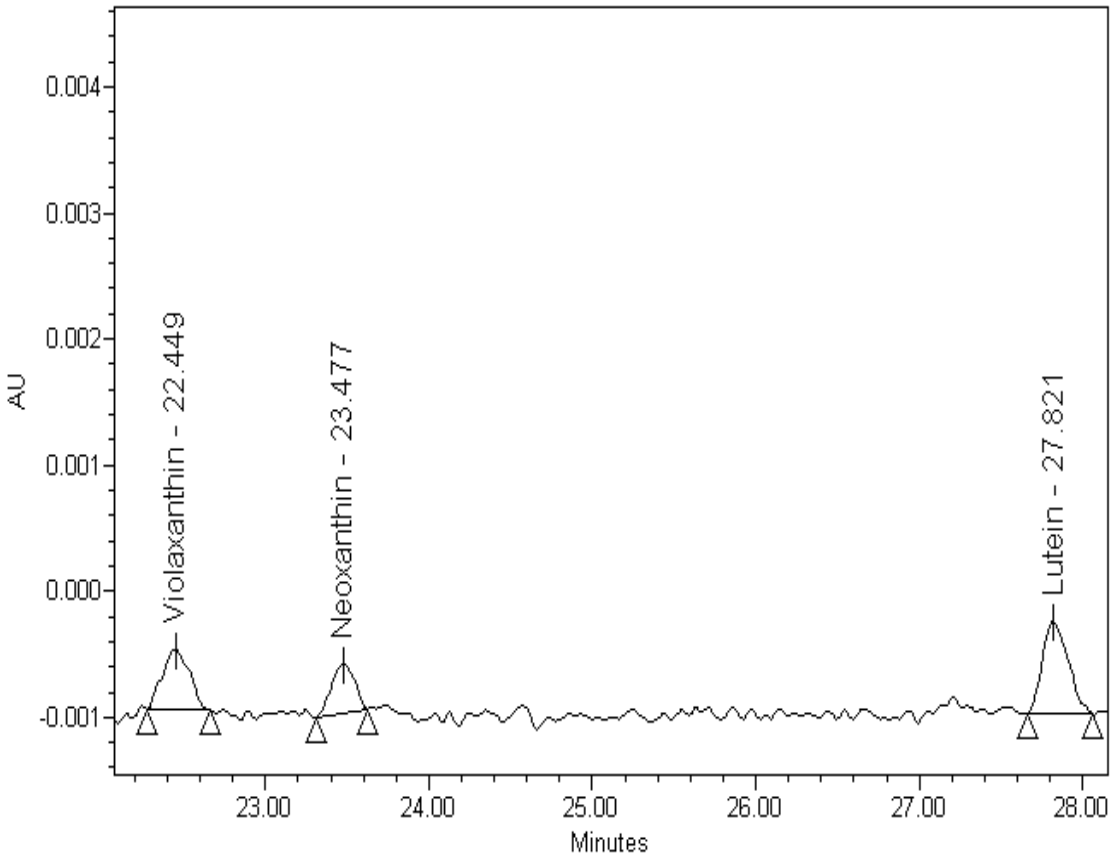
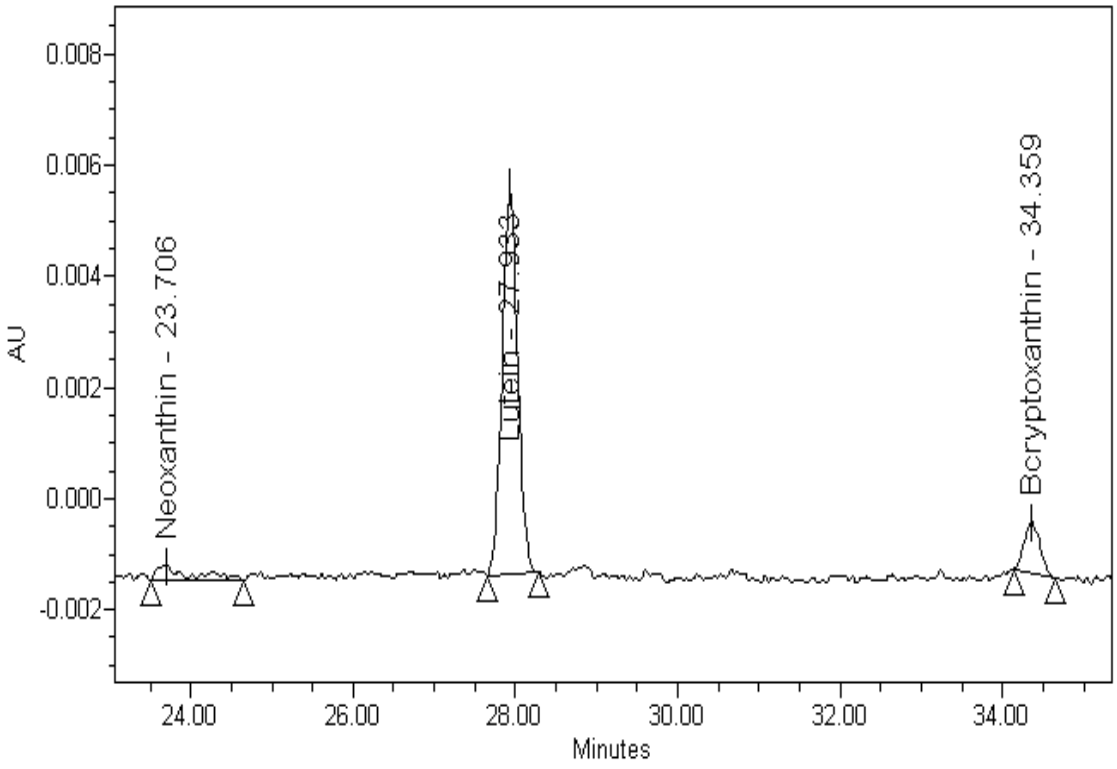
APPENDIX A



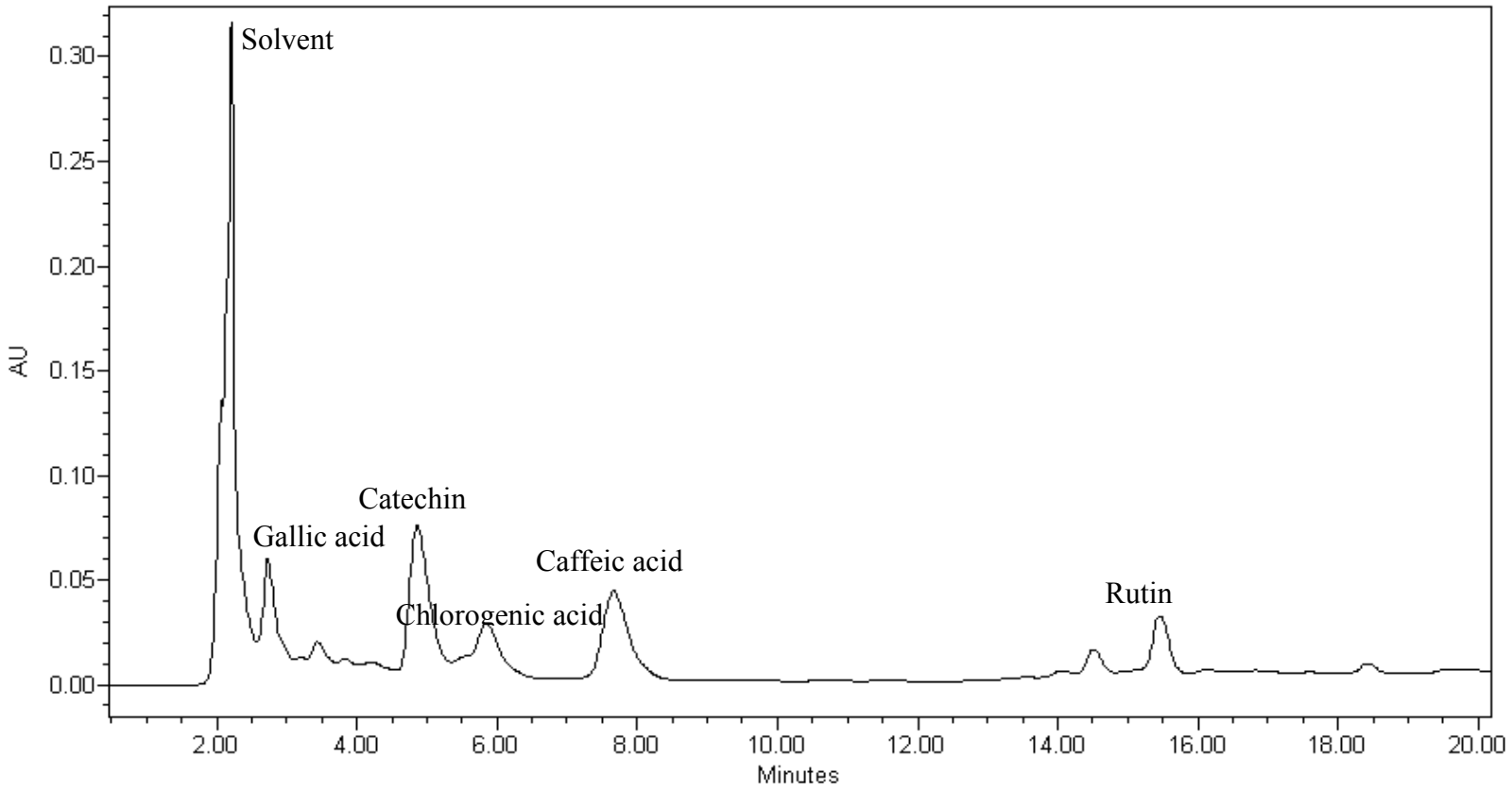
Carotenoid chromatogram for ‘Innovator’, microwaved sample, Dalhart 2003.

APPENDIX B



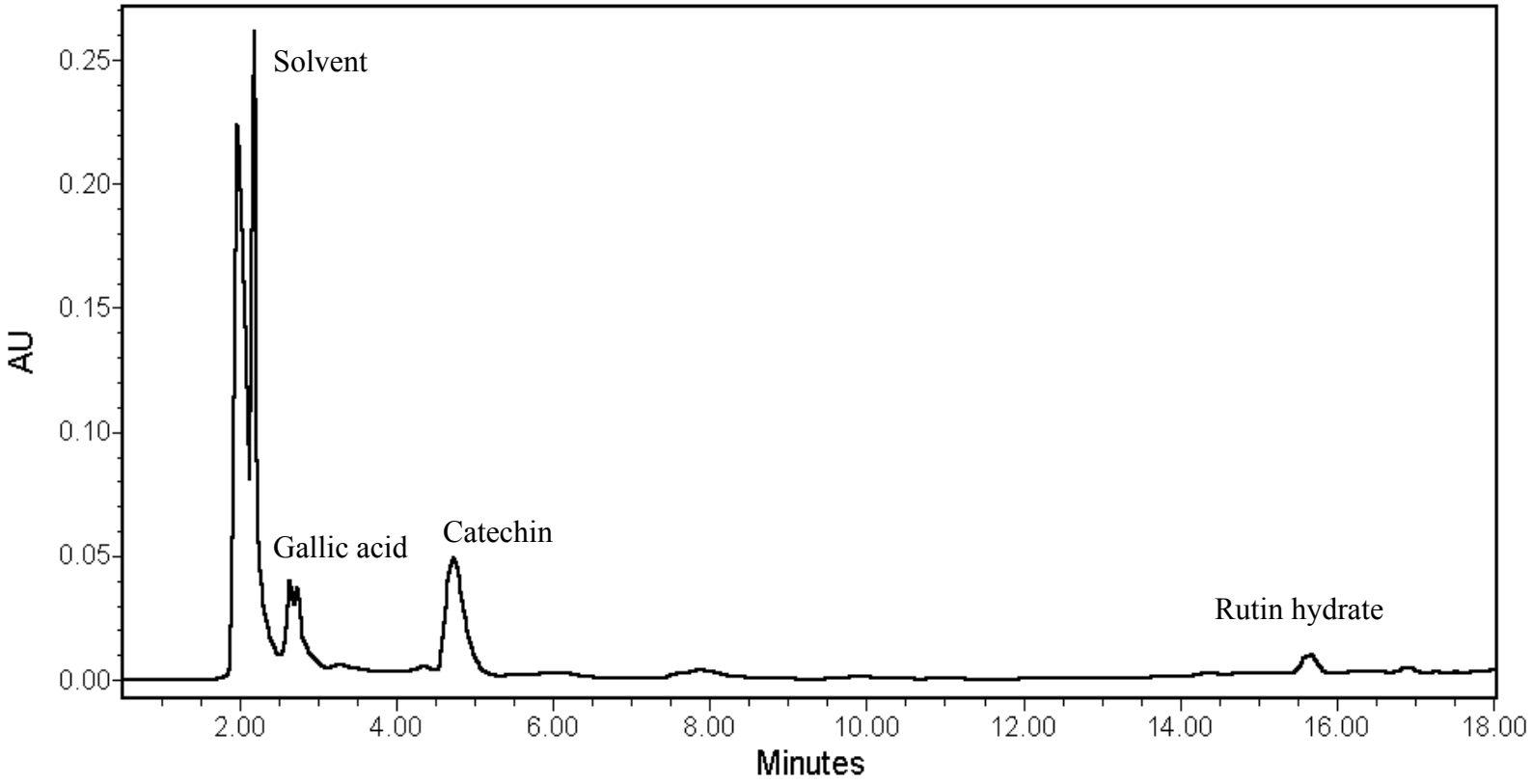
Carotenoid chromatogram for 'Atlantic', exterior surface, storage 10 days at 4 ^{o}C, 200 Gy Electron beam, Springlake 2004.

APPENDIX C



Phenolic chromatogram for 'Russet Burbank', microwaved sample, McCook 2003.

APPENDIX D



Phenolic chromatogram for 'Santana', stored at 4 ^{o}C for 110 days, raw sample, Dalhart 2003.

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PUBLICATIONS:

Blessington, T., A.L. Hale, D.C. Scheuring, and J.C. Miller, Jr. 2005. Effects of cooking, storage, and gamma- irradiation on antioxidant activity in potato (*Solanum tuberosum* L.). Am. J. Potato Res. 82: (Abstr.).

Blessington, T., A.L. Hale, D.C. Scheuring, and J.C. Miller, Jr. 2004. Effects of cooking and storage on antioxidant activity in potato (*Solanum tuberosum* L.). HortScience 39:879. (Abstr.).

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Warrington, R.T., A.L. Hale, D.C. Scheuring, D.W. Whitaker, T. Blessington, and J.C. Miller, Jr. 2002. Variability for antioxidant activity in cowpea (*Vigna unguiculata* L. Walp.) as influenced by genotype and postharvest re-hydration. HortScience 37:738. (Abstr.).