THESIS

LYCOPENE ANALYSIS AND HORTICULTURAL ATTRIBUTES OF TOMATOES

Submitted by

Samuel E. Cox

Department of Horticulture

In partial fulfillment of the requirements for

the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2001

COLORADO STATE UNIVERSITY

March 9, 2001

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY SAMUEL E. COX ENTITLED LYCOPENE ANALYSIS AND HORTICULTURAL ATTRIBUTES OF TOMATOES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

Committee on Graduate Work

Michelle L. Jones

David A. Sampson

Cecil Stushnoff
Advisor

Steve Wallner
Department Head

THESIS ABSTRACT

LYCOPENE ANALYSIS AND HORTICULTURAL ATTRIBUTES OF TOMATOES

Tomatoes are the most popular home garden vegetables grown in the United States, and have one of the highest value/acre ratios of any commercially produced crop. Popularity of tomatoes, combined with competition among seed companies, have led to an abundance and often bewildering variety of cultivars intended for home garden cultivation. Thirty home garden cultivars marketed at local nurseries in northern Colorado were grown and evaluated. Plant health, morphology and size, subjective plant yield observations and objective taste measurements were all investigated in order to identify exceptional or unacceptable cultivars for this area.

Cellular damage from free radicals is a major cause of degenerative diseases and cancer. Lycopene is an open-chain hydrocarbon carotenoid found in abundance in tomatoes that has been shown to possess strong antioxidant activity in animal systems. This led to the discovery that several types of cancers are inhibited by its consumption. Research into the properties and health benefits of lycopene has been a relatively recent development and will undoubtedly continue to grow. Tomatoes have been shown to be

very high in lycopene relative to other fruits and vegetables, and are a very important source because of their widespread popularity. Producing cultivars with high lycopene has long been a goal of breeders, primarily because of the increased red color of such cultivars, but more recently because of the enhanced health benefit for humans. Studies which require accurate quantification of lycopene in tomatoes have traditionally utilized high performance liquid chromatography (HPLC) with some variation of fresh tissue extraction. Fresh tissue extraction imposes limitations on analysis due to variations in fruit water content and difficulty in processing large sample quantities simultaneously to avoid changes in fruit over time. Here is presented a new analysis method which utilizes freeze-dried tissue and HPLC to increase flexibility and accuracy by "freezing" cellular constituents at the same point in time so as to render them directly comparable and by eliminating fruit water content variation. This method increases analyses efficiency and should be a helpful tool for researchers. The time limits imposed by natural degradation of lycopene are relaxed relative to analysis of fresh tissue, but not entirely eliminated. Freeze-dried powder can be stored for at least 30 days at -20°C without significant degradation, but will degrade 50% or more in less than two weeks if stored at 4°C.

Utilizing the new method and protocols, garden tomato cultivars commonly grown in northern Colorado were analyzed for lycopene content. Tomato color was usually found to be a good indicator of lycopene content, as red cultivars contained more lycopene than orange, which contained more than yellow. Lycopene content of three black cultivars, darker than any traditional red cultivar, were compared with a traditional red cultivar. Two black cultivars had lower and one black cultivar had higher lycopene

contents than the red cultivar. This suggests that although one black cultivar had higher lycopene than a red cultivar, black cultivars *as a group* do not owe their significantly darker pigment to lycopene alone.

Standard procedure of field tomato production is harvesting fruit green-mature so that they will arrive at distant markets in peak condition. Green-mature harvesting has the disadvantage of reducing tomato fruit quality overall, including reduced sugar content and reduced pigmentation. An investigation of the effect of light on ripening green-mature tomatoes revealed that exposure to increased photoperiod significantly increased lycopene synthesis. Producers who wish to enhance tomato pigment may opt to expose fruit to light to achieve this.

Samuel E. Cox
Department of Horticulture
Colorado State University
Fort Collins, CO 80523
Spring 2001

ACKNOWLEDGMENTS

Inestimable thanks to my friend and advisor, Dr Cecil Stushnoff, who gave me the chance to develop my laboratory skills both as an undergraduate and a graduate. His insightful suggestions, cogent technical guidance and good humor have contributed immeasurably to this thesis and my graduate education overall.

I am indebted to my committee members Dr David Sampson for his enthusiastic assistance with the HPLC method development and Dr Michelle Jones for her positive encouragement and suggestions.

I appreciate the assistance from Ann McSay in coordinating laboratory facilities and equipment, Dana Christiansen for helping keep watch on the plants and for maintaining the irrigation schedule and Jackie Harris, Candice Kuykendall and Eric Hunter for their assistance with the more mundane sample preparation and analysis duties. Thanks very much.

Special tip of the cap to my grandfather, Olen Cox, for encouraging in me an interest in plants at an early age, and to Andra Berryman, for her unwavering friendship and companionship throughout.

Funding for this project was supplied by the Colorado Agricultural Experiment Station.

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CHAPTER 1

TOMATO HISTORY AND FREE RADICAL BIOCHEMISTRY
A Review of Current Literature

History of the Tomato

The United States Congress passed the Tariff Act of 1883, a rather innocuous piece of legislation requiring a 10% tax on imported vegetables, in response to growing international trade. Just a few short years later, a tomato importer evaluated the law closely and decided to challenge it on the botanical grounds that a tomato was in fact technically a fruit, not a vegetable, and should therefore be exempt from said tax. John Nix's case posed merit enough to land the case before the Supreme Court in 1893. In Nix vs Hedden, 149 U.S. 304 (1893), Justice Gray wrote, “Botanically speaking, tomatoes are fruits of a vine, just as are cucumbers, squashes, beans, and peas. But in the common language of the people...all these are vegetables, which are grown in kitchen gardens, and which, whether eaten cooked or raw, are, like potatoes, carrots, parsnips, turnips, beets, cauliflower, cabbage, celery and lettuce, usually served at dinner in, with or after the soup, fish or meats which constitute the principal part of the repast, and not, like fruits generally, as dessert” (Cutler 1998). The court rejected the botanical truth that the tomato is in fact a monstrously sized berry, and deferred to the culinary vernacular of

vegetable to describe it. Thus is tax yet paid on imported tomatoes.

The giant berry traded abroad has a colorful history, and the story above is typical of a fruit that originated in one continent, became popular in another, and jumped to yet another for intense breeding that produced the tomato now familiar to most people today. *Lycopersicon esculentum* now enjoys worldwide distribution and is integral to the culinary disposition of multiple cultures.

Where did the tomato come from? For more than a century, tomatoes have been grown in gardens from Kazakstan to California, and in many locales cultivation of the red fruit goes back centuries. Like most crops, pinpointing where it all began is not easy. Vavilov was a renowned Russian scientist who conceived the idea that if one wants to locate the very center of origin for any crop species, look for the area which still has the highest diversity of that crop (Harlan 1971). This is grounded in the idea that only a portion of the wild plant gene pool will be incorporated into a domesticated plant line, such that the cultivated crop will represent only a portion of the genetic variety found in the wild ancestors which presumably are still inhabiting the area, in more or less the same form, to this day. By that logic, one would look closely at the western coast of South America, in present day Peru (Fig 1.1), where eight species in the tomato genus still grow wild in the Andes Mountains (Table 1.1)(Gould 1983). The current range of wild tomato relatives extends from the northern tip of Chile on the south to Ecuador on the north and reaching inland from the Pacific 100-200 miles, also including the Galapagos Islands.

Tomatoes belong to the genus Lycopersicon, which is in the same family, Solanaceae, as potatoes. The morphological resemblance between leaves and flowers of

potato and tomato plants seems to validate this taxonomic grouping. Two members of the genus Solanum (the genus which potato is classified in) have been successfully hybridized with members of the Lycopersicon genus (Rick et al. 1990). These are S. *lycopersicoides* and *S. pennellii.* Wild tomato species have tiny fruits, and only the red ones are edible. Tomato plants do not tolerate frost, and grow as annuals in colder regions. In warmer regions they are perennial and flower regardless of daylength.

All members of the genus have perfect flowers. Cultivated tomato is self fertile, whereas all other members of the genus are self-incompatible, with the exception of *L. pimpinellifolium*, which undergoes various degrees of self-fertilization (Simpson & Ogorzaly 1986). The major feature of domestication, aside from increased fruit size, is the gradual shortening of the flower style length from very long and prone to outcrossing, to very short and outcrossing-inhibitive (Fig 1.2). Full enclosure of the pistil by the anthers, a feature which virtually guarantees self-fertilization, did not occur until 1965 in California, although early North American and European cultivars were close to this state (Rick 1995).

From Peru, an unidentified wild ancestor of tomato made its way north at some time several thousand years prior to the Spanish exploration of Central America in the early 16th century (Rick 1995). Tomatoes of the species *L. esculentum cerasiforme* were in wide cultivation throughout Central America when the first conquistadors arrived in the Yucatan area of what is now Mexico. *L. esculentum cerasiforme* is thought to be the direct ancestor of cultivated tomato based on its wide presence in Central America and the presence of a shortened style length in the flowers (Hancock 1992). Cultivated tomato,

L. esculentum, has since been classified into five botanical varieties (Table 1.2).

That the tomato originated in South America and was an important crop among New World Indians by the 15th century is supported by strong evidence. The riddle that has kept some botanists on edge for many years is the question of where and when the wild tomato became domesticated.

Most evidence supports Central American domestication. The strongest evidence is cultural. Pre-Columbian cultures in Peru were inclined to decorate textiles and pottery with depictions of crops and figures important to their well being. It may be significant that depictions of tomatoes on artifacts in that area have not been unearthed. If the tomato had undergone domestication there, one would expect to find tomato representations on artifacts (Rick 1995). Linguistic evidence also supports this theory. The Aztecs of Central America called it “xitomatl”, and wild Central American tribes called it “tomati” (Gould 1983). The writings of ancient Peruvian tribes fail to mention a tomato-like fruit as being an important part of the diet or even a word meaning tomato, while Aztec writings in Central America mention dishes comprised of peppers, salt and tomatoes, a concoction which seems likely to be the original salsa recipe (Cutler 1998). And finally, genetic evidence also exists in support of Central American domestication. Genetic analysis of old cultivars descended from the original stock brought out of the New World by the Spanish showed modern cultivars to be more closely related to a cultivar grown widely in Mexico at that time than any wild species in Peru (Rick 1995). This cultivar was subsequently named as a variety of the domesticated tomato, called *cerasiforme*, and is regarded to be the direct ancestor of the modern cultivated tomato.

The *cerasiforme* variety still grows in a somewhat wild state in Central America, producing small, cherry-like fruits on a creeping vine. Thus it is known commonly as the cherry tomato (Gould 1983). Since domesticates were known to be cultivated in Central America, the lack of a genetically similar cultivar in South America suggests that domestication took place only to the north. Taken together, it seems well founded that initial domestication of tomato occurred in Central America.

As for how it traveled to Central America, evidence is less conclusive. It could have spread as a weed of maize and beans cultivated by natives (Ucko & Dimbleby 1969). Many crops of worldwide importance, such as rye and oats, were considered weeds at one time or another. Over time, a weed in a crop production system begins to evolve under the same selection pressures as the crop, and soon becomes dependent on the irrigation and fertile soil provided such that it, too, becomes domesticated. Migrating natives feasiblely traded seeds of maize and beans, and could have spread seeds of the small but tasty tomato. The evidence on this point is simply inconclusive.

The Spanish explorer Cortez conquered the Aztec city of Tenochtitlan, later to be renamed Mexico City, in 1521. It is presumed that the tomato found its way across the Atlantic shortly after. The earliest mention of the tomato in European literature is found in an herbal written by Matthiolus in 1544 (Gould 1983). He described tomatoes, or as they were called in Italy, *pomi d'oro* (golden apple), and wrote that they were “eaten in Italy with oil, salt and pepper.” This provides evidence that the first tomatoes to reach the Old World were a yellow variety, and that they were introduced via the Mediterranean. Red tomatoes were said to be introduced to Italy by two Catholic priests

many years later (NMSU 2000). Although not specifically documented, early tomatoes were probably small fruited, since they most likely were of the small-fruited *cerasiforme* variety cultivated by the Aztecs. Additionally, later emphasis on breeding for smooth-skinned cultivars suggests that early cultivars had rough skin (Gould 1983).

Undoubtedly it was initially received in Spain, and the name *pome dei Moro* (Moor's apple) was probably among the first (Cutler 1998). Cultivation of perhaps several varieties became widespread in the ensuing decades in Spain, Italy, and in France, where it was called *pomme d'amour* (love apple), perhaps because of suspected aphrodisiac properties, but more likely the result of a corruption of the early Spanish name, *pome dei Moro* (NMSU 2000). Although used in a limited capacity as food in Mediterranean countries, northern European countries regarded the tomato as nothing more than a curiosity for over a century (Rick 1995). English authors referred to the tomato as a horticultural novelty as early as 1578 (Gould 1983). One such English cultivator wrote in 1596, “these love apples are eaten abroad”, but went on to describe them “of rank and stinking savour” (Cutler 1998). By 1623, red, orange and yellow cultivars were known (Gould 1983). The first cookbook to mention tomatoes was published in Naples in 1692 (Cutler 1998). By 1700, seven types are mentioned in one article, including a large red type (Gould 1983). In 1752, English cooks began incorporating tomatoes sparingly in soups (Cutler 1998). In 1758, a tomato recipe allegedly showed up in the popular British cookbook, *The Art of Cookery* by Hannah Glass (Cutler 1998). Earliest records of marketing tomatoes are from the early 1800's in Europe (Gould 1983).

The introduction of the tomato did not proceed peacefully in all areas of Europe.

Germanic cultures affiliated the tomato plant with poisonous members of the Solanceae family which bore morphological resemblance, including deadly nightshade, henbane and mandrake. Deadly nightshade in particular does indeed bear good resemblance to a tomato plant (Fig 1.3). *Atropus belladonna* is a poisonous plant which has been used as both a hallucinogenic drug and a beauty aid in different parts of Europe. The Latin name *belladonna* literally means beautiful woman, in reference to the practice of ladies in medieval courts who would apply a few drops of nightshade extract to their eyes to dilate their pupils, a look considered most fashionable at the time. The hallucinogenic properties of the plant, visions and the sense of flying, probably led to the association of nightshade with witchcraft. Old German folklore has it that witches used plants of the nightshade family to evoke werewolves, a practice known as lycanthropy (Fig 1.4). The common German name for tomatoes translates to “wolf peach”, and for obvious reasons, was to be avoided. In the 18th century Carl Linnaeus conjured up binomial nomenclature to identify species, and took note of this legend when he dubbed the tomato *Lycopersicon esculentum*, which literally means, “edible wolf peach” (Cutler 1998, Simpson & Ogorzaly 1986).

Plants were brought to North America with colonists early on as ornamentals from Britain, the fruits of which were reportedly most valued for pustule removing properties (Simpson & Ogorzaly 1986). In 1781, Thomas Jefferson brought tomatoes to his table, along with french fries (a visionary), and George Washington Carver, the man who made peanut butter a household item, strongly advocated tomato consumption to his poor Alabama neighbors in an effort to improve their woefully vitamin-deficient diet,

but the negative stigma held on (Jones 1999). Early efforts by merchants to peddle their crops were not highly successful. One account has it that the fruit was brought to the liberal hamlet of Salem, Massachusetts in 1802 by a painter who had difficulty even convincing people to *taste* the fruit, so shrouded in superstition was its reputation (Gould 1983). Although New Orleans cuisine is reported to have incorporated tomato by 1812, suspicion about the fruit remained in some areas (Gould 1983). Lingering doubts in New England about the safety of the tomato were supposedly put to rest in 1820, when Colonel Robert Gibbon Johnson announced that at noon on September 26, he would eat a bushel of tomatoes in front of the Boston courthouse. The story goes that thousands of eager spectators turned out to watch the poor man die after eating the poisonous fruits, and were shocked when he lived (Simpson & Ogorzaly 1986). The source of this story, an old farm journal, may be less reliable than it is entertaining. Nevertheless, around the western world, tomatoes began to steadily grow in popularity by the early 1800's.

Several cookbooks from the 1820's mention tomatoes in recipes (Cutler 1998). In 1835, tomatoes were sold by the dozen in Quincy Market in Boston. In 1847, Thomas Bridgeman listed four varieties in his seed catalogue: *Cherry*, *Pear*, *Large Yellow* and *Large Squash*. A seed merchant named Buist in 1858 commented on the tomato: "In taking retrospect of the last eighteen years, there is no vegetable on the catalogue that has obtained such popularity in so short a period as the one now under consideration. In 1828-29, it was almost detested; in ten years most every variety of pill and panacea was extract of tomato. It now occupies as great a surface of ground as cabbage, and is cultivated the length and breadth of the country." Buist listed eight cultivars in his

catalogue that year. In 1863, a popular seed catalogue listed 23 cultivars, among which was *Trophy*, the first modern-looking large, red, smooth-skinned variety which fetched five dollars for a packet of twenty seeds. Large scale breeding for particular traits became commonplace in the 1870's in both Europe and the US, and by the 1880's, several hundred cultivars had been named (Table 1.3). A study carried out at Michigan Agricultural College in the late 1880's revealed that 171 named cultivars represented only 61 truly different lines, many of which were only marginally different (Gould 1983). By the late 1800's, it was clear that the tomato had firmly implanted itself in western culture.

The original center of domestication was, as mentioned, Central America. However, further domestication on a much more intense level occurred throughout Europe in the 18th and 19th centuries, and later, in North America. Eastern Europe seemed to generate a particularly high number of good quality cultivars. Tomato plants are naturally self-pollinating, and a general characteristic of self-pollinating plants is that they become genetically homozygous after many generations. Since they do not naturally outcross very often, seeds of a tomato will most often produce plants resembling the parents. Early cultivars did not change much because of this property, and were kept in a family or community for long periods of time, thus earning the name heirlooms. Heirloom cultivars dating back over a hundred years are still grown today. Most heirloom varieties are unique in size, shape or color. Many are green, some have green stripes (Fig 1.5). Some are black, or dark purple, or red with black shoulders (Fig 1.6). Some are rainbow colored, or shaped like peppers (Fig 1.7). Of course there are orange and yellow cultivars also, and everything in between. Some are cherry size, some are over two pounds.

Many heirloom cultivars have colorful histories as well. Consider the story regarding the cultivar *Mortgage Lifter*. A West Virginian named Charlie owned a radiator repair shop that fell on hard times in the Great Depression as people abandoned their cars. He used the four largest fruited tomato plants he had and crossed them repeatedly among each other to create a plant that produced two pound fruits. He sold plants for a dollar each, claiming one plant would feed a family of six. Within four years, he had made enough money to pay off the four thousand dollar mortgage on his house (Heirloomseeds 2000).

Names of heirloom cultivars often reflect some of the history of the plant (Homegrowntomatoes 2000). *Polish* is a cultivar said to have been smuggled into the US on the back of a postage stamp in the late 1800's. *Soldacki* came to the US with Polish immigrants who settled in Ohio in the early 1900's. *First Pick* was grown by generations of the Baptiste family in Reims, France. *Picardy* has a history that dates back to 1890 in France. *Besser* arrived from the Freiburg region of Germany. *Schellenburg's Favorite* came from the Schellenburg family near Manheim, Germany. *Elbe* originated in the late 19th century near the Elbe River in Germany. *Amish Paste* is a cultivar that has been cultivated by the Amish in Pennsylvania since the 1870's. *Brandywine* was developed by Amish farmers near Brandywine Creek in Chester County, Pennsylvania in 1885. *Hillbilly* came from the hills of West Virginia. *Old Virginia* was grown by locals in Virginia since the early 1900's. *Jeff Davis* is an old cultivar from Alabama honoring the Confederacy's only president. *Ace* was introduced by the Campbell Soup Company in 1953, and is still popular for canning today. Stories of immigrants smuggling seeds into the United States

hidden in waistbands or hollow canes seem to pop up frequently in heirloom descriptions. Of course, the tendency toward exaggeration must be considered with all these stories. Nevertheless, the gene pool which assembled in North America paralleled human immigration, and became a melting pot of tomato germplasm from which would rise countless new varieties.

As with any homozygous crop, hybrid breeding can result in terrific gains in production and quality. When two homozygous lines are crossed, the resulting progeny inherit a high degree of genetic variability which leads to heterosis, or hybrid vigor, and perform much better and/or produce much more than either one of the parents. In this case, $1 + 1 = 3$. One of the first hybrid tomatoes, *Mikado*, was introduced in 1880 by Rice's Seed Company of New York (Fig 1.8) (Simpson & Ogorzaly 1986). Like most early hybrids, the *Mikado*'s claim to fame was increased fruit size. Soon, higher yield cultivars were unveiled. By the beginning of the 20th century, disease resistance, bush type and determinate growth habits were also found in hybrid cultivars. These traits were mostly incorporated into cultivated tomato by crossing with a wild relative, since all will hybridize with varying amounts of success (Rick 1995). Hybrid cultivars have come to dominate every area of tomato production, from large scale to backyard. One drawback as far as the home gardener is concerned is that hybrid seed or plants must be purchased every year. Seed from hybrid plants, if propagated, will produce an F_2 segregating generation, and plants will be very diverse and not at all like that parent. This is the very property that makes hybrids so attractive to seed producers since it ensures that customers must buy new seed each year. Heirloom cultivars grow true from seed, and are

still propagated by home gardeners. Many people argue that new hybrid cultivars bred for size and yield have overlooked the taste, and that the flavor of heirloom cultivars can't be beat. Hybrid cultivars have historically looked and tasted all very similar to each other. Heirlooms definitely present greater variety, but typically have lower yields and less disease resistance.

Tomato production began to soar in the early 1920's in western countries with the advent of mass canning. Canning of tomatoes was first documented by Harrison Crosby of Lafayette College in Easton, Pennsylvania (Gould 1983). Prior to 1890, all tomato canning was done by hand. Mechanized peeling tables were put into use in the 1890's. Juice extractors were invented in the 1920's. Shortly after, a young entrepreneur named Joseph Campbell found a ready market for canned tomato products, and went on to make millions after founding the Campbell Soup Company. High-solids cultivars have been introduced to maximize paste and solids for canning. *Roma* is a backyard favorite spanning half a century of cultivation which has been widely used for sauces because of its high solids content.

As the potential for introducing new traits into tomato cultivars through hybridization with wild relatives became more lucrative, the Tomato Genetics Cooperative was established at Cornell University in 1951 to collect and disseminate useful germplasm for breeding projects (Gould 1983). A prominent tomato breeder named Charles Rick heads up the Tomato Genetic Resource Center at University of California at Davis. In addition, tomato germplasm is kept in storage at the USDA National Seed Storage Laboratory in Fort Collins, Colorado.

Tomatoes attracted attention in Hollywood. The late sixties sci-fi flick *Attack of the Killer Tomatoes* featured vicious oversize killer tomatoes running amuck. Tomatoes have been subject to politicking as well. In 1981, the USDA chairman declared tomato ketchup to be a vegetable in order to justify Reagan administration budget cuts in the public school lunch program (Cutler 1998).

The most recent contribution to tomato breeding has been biotechnology. For years merchants have tried to balance a good tasting fruit with a tough, good-shipping fruit. Ripe tomatoes are very soft, bruise easy and begin to decline in quality after only a few days. Tomatoes ripen off the vine in response to the chemical ethylene, which is produced by the fruit as the development of the seeds nears completion. Traditionally, growers would pick the fruits in the green-mature stage just as the shoulders of the fruit lost their dark green color. The fruit would then be shipped to other locations, sometimes thousands of miles, and would resist bruising or rotting because of their immature stage. The fruits would be red by the time they reached their destination, or could be gassed with ethylene to hasten ripening, and could then be sold in peak condition. Consumers often complain that taste suffers because of this practice. So, in the 1980's a project was undertaken by Calgene Fresh, Inc. using biotechnology to tweak the tomato genetics to inactivate the gene responsible for softening the tomato during ripeness. Tomatoes turned red, but remained firm indefinitely. The practice of picking tomatoes green could be discarded, and everyone would be happy. They called this cultivar *Flavr Savr* and it hit the produce sections of stores in the US during 1993 (Cutler 1998). The *Flavr Savr* tomato was a financial and public relations failure. Industry executives severely

underestimated the public's concern over biotechnology, and failed to anticipate the backlash from consumers over this new and potentially risky technology applied to human food. Although evidence suggesting any danger over genetically engineered food is lacking, consumers are nervous about potentially unknown and unforeseen side effects. The antisense gene inserted into the tomato also had unanticipated negative impacts on fruit quality. The fruit had soft skin and an unusual taste. These drawbacks could have perhaps been overcome had the tomato been cheaper than normal tomatoes, but in fact it was many times more expensive, selling for two to three dollars a pound. Monsanto purchased the rights to the Flavr Savr tomato but has yet to reintroduce it.

The top five tomato producing countries of the world, in descending order, are the United States, China, Turkey, Italy and India. Within the US, Florida, California and Georgia are the top commercial producing states, with about 200 square miles under cultivation in 1997. An estimated 35 million backyard gardens across the country grow tomatoes as well. Per capita yearly consumption of tomatoes in the US increased from 16.6 lb in 1985 to 18.8 lb in 1995 (Jones 1999). Continued increase in this figure is expected due to the purported health benefits associated with tomatoes in the diet. Specifically, these include a ranking of 16^{th} among all fruits and vegetables as a source of vitamin A, 13^{th} in vitamin C and when adjusted for consumption, the most important provider of these two vitamins in the western diet. Tomatoes also contain significant amounts of lycopene, β-carotene, magnesium, niacin, iron, phosphorus, potassium, riboflavin, sodium and thiamine. A University of California Davis survey ranked the tomato as the single most important fruit or vegetable of western diets in terms of overall source of vitamins and minerals.

After only a few hundred years in western culture, the tomato has firmly implanted itself as a major player in diets of many nationalities. Italian cooking has become synonymous with tomato sauce. Pizza would be lost without it. Where would Mexican cuisine be without salsa? Tomato soup, slices on a burger and ketchup are all highly integrated uses for the versatile fruit in American culture. Additionally, millions of Americans grow tomatoes in their backyards each year. From one continent to another, the tomato has crossed through a variety of cultural barriers to become one of the world's foremost vegetables.

With an eye to the future, tomatoes may soon gain more notoriety for their health benefits than they have in the past. As antioxidant becomes an everyday word to consumers, people may begin to hear more about the tomato.

Free Radical Chemistry

Electrons are the glue that hold atoms together to form complex molecules. All atoms with an unpaired electron will attract other electrons to fill this void. Atoms just aren't content to go through life with unpaired electrons. Any molecule or atom with an unpaired electron is called a free radical. Electronegativity is the unequal sharing of electrons in covalently bonded molecules, and among the elements, oxygen ranks second only to flourine in the strength of electronegativity, giving it a much greater pull on electrons than any other element (Campbell 1995a). Oxygen atoms with an unpaired electron exert tremendous power on other atoms' electrons and may tear other molecules apart in order to get one. The loss of an electron is called oxidation, since whenever an

electron is lost, it is usually to oxygen. Molecules or atoms with unpaired electrons are also often called singlet oxygen species because oxygen atoms, either alone or in molecules, are often found lacking a stable complement of electrons. Free radicals are formed by normal respiratory processes, as well as contact with ionizing radiation which kicks electrons off atoms, usually of water molecules (Hickman et al. 1995). Evidence also exists for smoking as a cause of free radical formation (Morrow et al. 1995, Handelman & Packer 1996). Free radicals are common in living organisms.

Free radical formation is problematic in human systems because many molecules our bodies produce provide essential functions in normal physiological operation. Most molecules which come in contact with a free radical are destroyed or altered, and must be replaced or repaired. Free radicals are thought to be at the heart of cancer and degenerative diseases of major organs in mammals (Wang et al. 1996, Ames et al. 1993).

The human body has a remarkable ability to compensate for such losses and produce or repair damaged molecules, and it does so every minute of a lifetime. Young, vigorous and healthy mammalian systems have enormous potential for replacement of damaged parts and repair of molecules in the body, however, as time goes on, physiological processes slow down and that capacity for repair is diminished. Cells become less robust and change in organs becomes apparent. Free radicals are believed to accelerate the aging process by taxing the natural regenerative properties in mammalian cells (Cutler 1991). When a creature is young, it replaces cells quickly almost as soon as damage occurs. But as the body gets older this ability diminishes, and it is at this point that damage is noticed. Diseases which define the aging process are caused at least in part

by free radical activity (Bronson et al. 1999). The process of oxidation is familiar to most people in the burning of wood, or the browning of a cut apple. Oxidizing agents responsible for these processes are also at work on living systems, and often have similar results. Our bodies are constantly being torn down and rebuilt, and as we age, the rebuilding isn't as quick.

Free Radicals and Cancer

Deoxyribonucleic acid (DNA) is the primary source for all physiological function and an error in DNA coding could, if present in a vital section of DNA and not repaired, lead to faulty protein production, abnormal cellular function and/or cell death. Every cell division necessarily involves DNA replication and mistakes in translation and transcription of DNA occur regularly. Fortunately, such an important molecule has a complex replication safeguard system to ensure that errors rarely go unnoticed. Errors in replication occur spontaneously once every $10e^4$ to $10e^5$ base pairs, a remarkably low number considering the speed and scale of replication. DNA Polymerase I is the proofreading and repair enzyme for DNA. During replication, it follows the replication site checking for mistakes, and cuts out any incorrect base pairs placed by Polymerase III, the synthesis enzyme. Polymerase I activity reduces the mistakes during replication to 1 in $10e^9$ to $10e^{10}$ base pairs. This process is termed excision-repair (Cambell 1995c).

Excision-repair is also employed to keep DNA from mutating in response to mutagens which frequently invade the human body. Ionizing radiation like x-rays or gamma rays are constantly entering the human body from both extra-terrestrial and

terrestrial sources. They cause the production of free radicals by ejecting electrons from stable compounds. These damaged atoms are the vehicles which deliver destruction to cells. Free radicals damage DNA just as they damage other molecules, but the difference is that DNA is much more important. A free radical in proximity to DNA will outgun its opponent for any available electron, and in the process alter DNA structure and function. DNA Polymerase I usually repairs at the site of such reactions, but not always.

The few alterations to the genetic code which go unnoticed usually are not lethal, and in fact are the driving force behind evolution. However, mutations to DNA in a few very sensitive areas can have profound effects on cell function. In humans, for example, a small protein designated p53 has generated excitement for its apparent contribution to cancer suppression. Cell division depends on the activity of enzymes known as cyclin dependant kinases (cdk), which bind to cyclin to become active. In normal cells the gene coding for the p53 protein functions normally and produces the p53 protein. The p53 protein "turns on" the production of another smaller protein. This smaller protein binds to the cdk-cyclin complex, which is found in this state in normal cells. In cancerous cells, the smaller protein is conspicuously absent from the cdk-cyclin complex. This absence of the p53 protein is thought to be a significant contribution to cancer resulting from a disabling mutation in the gene coding for the p53 protein (Campbell 1995d).

Deleterious mutations to DNA are corrected only as efficiently as the capacity to do so by DNA Polymerase I. Healthy, young mammals have robust physiology that produces enough DNA Polymerase I to cope with DNA glitches, but as mammals age,

their physiological functions slow down, and DNA Polymerase cannot keep up with the constant rate of mutation. Higher mutations in DNA increase the probability that a vital section of DNA will be mutated, as in the gene coding for p53. This explains why cancer is a disease found in elderly mammals, and most usually in humans, since other mammals tend to die of other causes before they get old enough to have this sort of physiological slow-down.

As with most areas of science, the focus thus far has been bent towards humans, however, since DNA is fundamentally the same in plants, animals, fungi, etc., the same basic chemistry applies. Plant DNA and proteins are just as easily damaged by free radicals as their animal counterparts. Intense sunlight often causes the photon capture rate in the photosystem to exceed energy dissipation capacity, leading to an excess of excited oxygen molecules and a flux of free radicals that threaten to overwhelm and destroy vital compounds in the photosystem within plant chloroplasts (Asada & Takahashi 1987). DNA is damaged constantly, and just as in animal systems, rebuilt constantly. Plant cells are subject to mutation by radiation, and even to uncontrolled cell growth which we call cancer in animals. DNA Polymerase I functions to repair damaged DNA in the same manner as it does in animal systems.

Antioxidants

To counter the free radical threat, plants and animals produce molecules that intercept oxidizing agents before they react with vital molecules, like DNA. Both plants and animals produce these molecules, called antioxidants or free radical/singlet oxygen

quenchers. Antioxidants are agents with reducing capacity, that is, they donate electrons to oxidizing agents, and in the process they disarm the offending molecule. Antioxidants are damaged just like other molecules whenever they lose electrons, but they are expendable and are sacrificed to prevent damage to vital molecules. Usually this is accomplished by the high relative susceptibility of antioxidants to oxidation. Antioxidants are usually unstable, large molecules containing many double bonds or other easily oxidizable features which will yield free electrons when broken. The more double bonds an antioxidant has, the more effective it is as a free radical quencher (Miller et al. 1996). The free electrons liberated by the breaking of a double bond will join with the free radical to stabilize it, and the fragments of the antioxidant will be reabsorbed into the cytosol and recycled.

A well-studied antioxidant produced in living cells is a metal-complexing enzyme called superoxide dismutase. It quenches the reactive power of superoxide anions, which are a type of free radical formed when oxygen gas (O_2) reacts with an unreduced metal cation, like Fe^{2+}. The iron donates an electron to the oxygen molecule to form Fe^{3+} and a superoxide anion (O_2-). Superoxides form as a routine part of cellular metabolism and photosynthesis, and without the antioxidant poperties of superoxide dismutase, terrible physiological consequences ensue (Hopkins 1999).

The importance of free radicals is not neglected in any kingdom. Bacteria within mammals are hunted by white blood cells which envelope bacteria and isolate them in the lysosome, where the pH is then lowered and free radicals are injected to kill it. Bacteria can also produce superoxide dismutase to quench free radicals, however, as

mentioned, this enzyme requires metal cations to function. Thus, to counter the immune system response, the bacteria must have access to free metal cations. As a counter punch, white blood cells remove metal cations from the lysosome, leaving bacteria without ammunition to fight the free radicals which ultimately destroy them.

Other important antioxidants include the enzymes glutathione peroxidase and catalase (which quench hydrogen peroxide), as well as macromolecules like albumin, ceruloplasmin and ferritin (Wang et al. 1996).

Vitamin E (α-tocopherol) is a common antioxidant that has been shown to prevent oxidation of important compounds like Vitamin A (Campbell 1995b). Resveratrol is an antioxidant found in red wines, and is linked to reduction of heart disease in wine-drinkers (Cuevas et al. 2000). Vitamin C (ascorbic acid), β-carotene, isoflavones, flavones, anthocyanins, catechin and isocatechin, all frequent components of human diets, also demonstrate strong antioxidant activity (Bors & Saran 1987, Bors et al. 1990, Hanasaki et al. 1994, Scott 1992). Vitamin C, although the recipient of much popular press, accounts for less than 15% of the total antioxidant activity in oranges and grapefruits (Wang et al. 1996). Similarly, β-carotene was found to account for less than 14% of total carotenoids found in the serum of two human study groups, indicating that other carotenoids are also utilized by the body (Thompson et al. 1985, Thurnham et al. 1987). This demonstrates that there are many more beneficial components in plants than are currently recognized, and the variety of antioxidants is large.

Health Benefits of Antioxidants

Overwhelming evidence in favor of the positive health benefits gleaned from consuming fruits and vegetables rich in antioxidants has surfaced in the last 10 to 20 years. The evidence is so convincing that the National Cancer Institute recommends consumption of at least five servings of fruits and vegetables a day to ward off cancer (Challem 1999). Consumption of fruits and vegetables is linked to lower incidence and lower mortality rates of several types of cancer (Doll 1990, Dragsted et al. 1993, Ames et al. 1993, Willett 1994a). Animal experiments have confirmed that vegetables common in the human diet have antitumorogenic effects (Belman 1983, Bingham 1990, Bresnick et al. 1990, Maltzman et al. 1989, Stoewsand et al. 1988, Wattenberg & Cocciea 1991). Low blood antioxidant content is correlated to subsequent cancer mortality (Stahelin et al. 1991, Willett 1994b). Vegetable consumption is negatively correlated to heart disease mortality, and positively correlated to low blood pressure (Armstrong et al. 1975, Verlangieri et al. 1985). These positive effects on human health are attributed in large part to the antioxidant compounds found in high quantities in fruits and vegetables (Ames 1983, Gey 1990, Steinberg et al. 1989). A study conducted at Tufts University demonstrated that women who consumed at least 400 mg/day of Vitamin C for ten years had an 80% lower risk of developing cataracts (Challem 1999). More health benefits for specific antioxidant classes will be described in the following sections.

Production and Function of Carotenoids in Plants

Carotenoids are compounds present in all plants, algae and cyanobacteria (Cunningham 1999). Within plants, they are often located in the chloroplast membranes

or in chromoplasts of roots, stems, leaves, flowers and fruits of various plants (Hopkins 1999a). Carotenoids are unsaturated fatty acids generally of large size. Carotenoids are all structurally similar, being composed of carbon and hydrogen isoprenoid units, with some having two or four oxygen atoms (Salisbury & Ross 1992). Two types of carotenoids exist: Carotenes are pure hydrocarbons, whereas xanthophylls contain at least one oxygen molecule. Both contain 40 carbon atoms brought together by the fusion of eight 5-carbon isoprenoid units in a string of double bonds, and differ only in degree of saturation and the presence or absence of cyclic ends (Hopkins 1999a). Because of their nonpolar nature they are not water soluble, but dissolve in many organic solvents. Owing to the large number of double bonds, some carotenoids also act as chromophores, refracting light in different wavelenghs to produce various colors in the red-orange-yellow spectrum. Beta-carotene gives daisies and carrots a yellow/orange color, while lycopene confers red color to tomatoes.

Carotenoids are part of the isoprenoid pathway (Fig 1.9), the same pathway from which spring over 22,000 primary and secondary metabolites, including menthol, camphor, rubber, gibberellic acid, abscisic acid, plastiquinones, spearmint, clove oil and sterols (Dey & Harborne 1997, Salisbury & Ross 1992). All products resulting from reactions in this pathway are generally termed isoprenoids, terpenoids or terpenes, depending on the author. All isoprenoid units derive from acetate of acetyl CoA molecules via the mevalonic acid pathway. They are constructed with 5-carbon isoprene units. The isoprenoid pathway involves many intermediate compounds and enzymes which will not be discussed. For a detailed description of the pathway, see Dey &

Harborne (1997).

All carotenoids derive from gerynlgerynl pyrophosphate (GGPP), and are called tetraterpenoids because all have a 40 carbon backbone. Since all carotenoids are produced in the same pathway, some carotenoids are precursors to others, thus, certain carotenoids predominate at the expense of others (Fig 1.10). For example, tomatoes are high in lycopene, which is a precursor to β-carotene. In order for a tomato to have high lycopene, it must halt the pathway reactions before β-carotene is produced. Thus, red tomatoes have little β-carotene (Chalukova & Manuelyan 1991). Conversely, carrot roots are high in β-carotene but low in lycopene, as well as every other carotenoid.

The synthesis pathway of carotenes is well worked out (Fig 1.10). The first carotenoid in the pathway is phytoene, a colorless compound resulting from the condensation of two GGPP molecules by phytoene synthase, an enzyme associated with the chromoplasts and stroma of chloroplasts. The next step involves dehydrogenation of phytoene into phytofluene and β-carotene by phytoene desaturase. Further dehydrogenations are catalyzed by β-carotene desaturase to yield neurosporene and lycopene. Lycopene cyclase acts on lycopene, an open-chain structure, to add aromatic rings on each end to form γ-carotene (one ring) and β-carotene (two rings). The mechansisms behind the pathway beyond β-carotene to the oxidized xanthophylls are not as clear. The oxygen moieties added to the carbon chain originate from molecular oxygen, and the hydroxylation reactions may be catalyzed by a cyt P450-dependent mixed-function oxidase. Neurosporene, lycopene and all the carotenoids are subject to cyclization reactions at one or both ends of the open chain structure (Fig 1.11). The

enzymes involved in the cyclization reactions are not well known in all cases.

The genetic control of the carotenoid pathway is less clear. The carotenoid pathway is different in anoxygenic non-photosynthetic bacteria and fungi and oxygenic photosynthetic organisms (Armstrong 1994). The genes coding for phytoene synthase (CrtE (aka Psy)), phytoene desaturase (CrtB (aka Pds)) and lycopene cyclase (CrtL) have been sequenced and cloned from photosynthetic organisms (Dey & Harborne 1997, Miura et al. 1998). The gene coding for lycopene cyclase was sequenced and cloned from cyanobacterium (Synechococcus sp strain PCC 7942) and named CrtL by Cunningham et al. (1994). The same gene was cloned from tomato by Pecker et al. (1996), also named CrtL. A gene called CrtI has been cloned from *Erwinia uredovora* which codes for bacterial phytoene desaturase, which desaturates phytoene to lycopene in one step, bypassing ξ-carotene desaturase. It is this CrtI gene that was overexpressed in tomatoes by researchers at the University of London to produce fruit with elevated levels of β-carotene (Romer et al. 2000). It was also this gene which was overexpressed in rice to produce elevated β-carotene in the endosperm, leading to the celebrated "golden rice" (Xudong et al. 2000). The potential for bioengineering of metabolic pathways is enormous, as these examples illustrate.

Carotenoids play an important role in photosynthesis. One function demonstrated in algae, and probably at work in higher plants as well, is light harvesting in photosytem I and II, specifically, transferring absorbed light energy to chlorophyll (Hopkins 1999b). The second role they fulfill is related to protection of the photosystems. During periods of peak irradiance, many crop species will only use 50% of the light energy absorbed, and

evergreens may use as little as 10%. That extra light energy is potentially destructive as excited constituents of the photosystems, notably ferredoxin, react with water and air to produce superoxide radicals. These free radicals can cause photooxidation of any organic molecule contacted. Many carotenoids prevent photooxidation of chlorophylls, either by absorbing excess blue light (Frosch et al. 1979, Feirabend & Winkelhusner 1982, Reiss et al. 1983) or by combining with singlet oxygen species to become oxidized xanthophylls, although the exact mechanism has not been elucidated (Hopkins 1999a). This discovery was first made when it was observed that mutant maize plants which did not produce carotenoids would bleach out in high irradiance, but green up in low irradiance or low-oxygen atomspheres. Similiar results were observed when plants were treated with norflurazon, which chemically inhibits carotenoid synthesis (Hopkins 1999b). Although the mechanism is not yet understood, evidence suggests that the primary function of zeaxanthin is to accept the excess energy from photosynthesis in the form of reactive oxygen species. Despite the evidence surrounding functions of β-carotene, zeaxanthin, lutein and others, the function of many carotenoids remains unknown (Hopkins 1999a). Carotenoids are also important as precursors to the important plant hormone, abscisic acid (Dey & Harborne 1997).

More than 500 carotenoids are found in nature, although most are rare (Scott 1992). β-carotene is the most abundant carotenoid in higher plants, but lutein, a yellow xanthophyll, is present in more species. Although there are several hundred carotenoids known, only 20 have been found in human blood where they perform antioxidant duties (Thompson et al. 1985). Barua and Furr (1992) list the major carotenoids found in

human plasma as lutein, zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, lycopene, α-carotene and β-carotene.

Health Benefits of Carotenoids

The familiar carotenoid β-carotene is also known as provitamin A since it is metabolized into vitamin A by mammalian livers. Vitamin A is imperative for vision, thus, β-carotene is a very important molecule.

All carotenoids, by nature of their antioxidant properties, are highly associated with health benefits in humans. They appear to impart protection against lung and epithelial cancer (Micozzi et al. 1986, Calditz et al. 1985, Olson 1986, Moden et al. 1981). Beta-carotene has been linked to protection against lung cancer and stomach cancer (Stahelin et al., 1984; Menkes et al. 1986, Wald et al. 1988). Low β-carotene content has been associated with high cancer risk in a number of human studies (Sahelin et al. 1984, Nomura et al. 1985, Menkes et al. 1986). β-carotene supplementation in HIV-positive human patients increased the number of T-helper lymphocytes and total white blood cells, cells which are typically reduced as a symptom of the disease (Coodley & Coodley 1996). Terao (1989) reported that canthaxanthin and astaxanthin were very effective at preventing peroxidation of lipids by free radicals. Canthaxanthin has been proven to have anticarcinogenic attributes (Mathews-Roth 1982, Schwartz & Shklar 1988). High carotenoid diets are associated with decreased digestive tract cancers (Steinmetz & Potter 1991). Consumption of cruciferous vegetables is negatively

correlated with several types of cancer, and the credit is largely attached to the carotenoids within (Peto et al. 1981). The antioxidant activity of carotenoids is probably dependant on several factors: number of conjugated double bonds, end groups (acyclic or cyclic) and functional groups contained in the rings (Stahl & Sies 1996). Thus, the diversity of form found in carotenoid compounds is likely to translate into a diversity of function, and so far that seems to be true. A comparison of three carotenoids revealed their antioxidant potential as lycopene > α-carotene > β-carotene (Anguelova & Warthesen 2000).

Nature and Function of Lycopene in Plant Organs

Lycopene has been shown to be the most potent antioxidant produced by the carotenoid pathway (Di Mascio et al. 1989). The structure of lycopene was elucidated in 1930 by Karrer et al. Like every carotenoid, it is a very large molecule, $C_{40}H_{56}$, MW = 536g, and is deep red in color (Fig 1.12). This deep red color of lycopene gives many red fruits their color, including tomato, peppers, grapefruit, watermelon, guava, papaya, apricots and others (Table 1.4) (Ellis & Hammer 1943, Mangels et al. 1993). The open chain structure is held together by 11 double bonds, and 2,048 isomers are theoretically possible although only a few have actually been found in plants and animals (Fig 1.12) (Zeichmeister 1962). Ninety-five percent of lycopene found naturally in plants is of the all-*trans* isomer, also called (all-E)-lycopene, and the majority of the remaining isomers are of a *cis* configuration, which means simply that they are bent at one carbon, usually either carbon 5, 9 or 13 (Zechmeister et al. 1941, Deuel 1951, Schierle et al.

1997). The *cis* isomer is more highly reactive to oxidizing agents, and has a less intense red color (Boskovic 1979).

Lycopene is extremely sensitive to heat and light, and is easily degraded in their presence if not protected by the fluids inside the cell. Studies dealing with extraction and purification of lycopene warn of its lability and advise a range of precautions from low-light to gold light to freezing temperatures to prevent degradation during extraction or storage (Thurnham et al. 1988, Barua & Furr 1992, Scott 1992). Lycopene in the tomato, however, is very stable. Heating tomatoes at 80°C for 10 hours had no effect on lycopene, and heating at 100°C only reduced lycopene 10% in the same amount of time (Zanoni et al. 1999).

The function of lycopene in plants is not entirely clear, although because of its proximity and relationship to the photosystem, it probably protects photosystem molecules form oxidation by absorbing excess blue light (Hopkins 1999a). Lycopene is not present in chlorplasts like β-carotene and lutein. Instead, it is present mostly in the chromoplasts of fruits of plants, most notably in tomato (Table 1.5). Such a large molecule as lycopene is metabolically expensive to manufacture, therefore it seems almost impossible that it would not confer some evolutionary advantage. It may be that it acts as a colorant to advertise fruit to seed dispersers. Given the powerful antioxidant properties of lycopene, it may serve to protect the soft-fleshed tomato fruit from oxidation resulting from slight nicks and cuts on the fruit or as a photosystem antioxidant. Given that lycopene is also abundant in the inner tissue of pink grapefruit, papaya and watermelon, the latter explanation seems more likely. No decisive answer can be given

however, and it seems to be a subject entirely overshadowed by the excitement surrounding the function of lycopene in *human* systems.

Within tomatoes, lycopene levels vary based on cultivar, stage of ripeness and growing conditions (Table 1.6). Formation of lycopene is regulated by phytochromes in the fruit, as demonstrated by an experiment which showed lycopene production was stimulated by red light and reversed by far red light, regardless of ethylene treatments (Alba et al. 2000). During tomato ripening, chloroplasts undergo a transformation to chomoplasts, which hold the lycopene (Ellis & Hamner 1943, Hobson and Davis 1971, Kirk & Tilney-Bassett 1978). The optimum temperature for lycopene synthesis in tomato is 24°C (Vogele 1937). Lycopene synthesis is severely impaired above 30°C or below 10°C (Goodwin & Jamikron 1952; Tomes 1963, Mustafa 1989). Duggar (1913) reported that tomatoes maturing in sunlight produced more red color (lycopene) than those which were shaded. Initially, this was attributed to differential temperature, which has been shown to be a key factor. However, light quality/intensity has also been shown to influence lycopene synthesis. Shewfelt and Halpin (1967) discovered that tomatoes held at constant 22°C developed significantly more lycopene when exposed to any one of three types of flourescent light relative to dark controls. Furthermore, wide-spectrum bulbs induced significantly higher lycopene production than standard bulbs. These data are congruent with the known photosystem antioxidant properties of many carotenoids, and hint that lycopene plays an important role in photosystem protection as well. Variations in fertilizer (N-P-K) solutions and soil types did not significantly effect carotene (lycopene) content in tomatoes (Ellis & Hamner 1943). Confirming the suspicions of shoppers everywhere, Ellis and Hamner (1943) quantified a shortfall in

carotene content in tomatoes picked green-mature compared to vine-ripened fruit.

Tomatoes as a Source of Lycopene

Tomato (*Lycopersicon esculentum* L.) consumption is both widespread and on a massive scale. Tomatoes have one of the highest lycopene levels of any fruit, and certainly the highest content among common fruits and vegetables (Table 1.5). Lycopene is the principle carotenoid found in tomatoes (Ellis & Hamner 1943). Tomatoes and processed tomato products are the major worldwide sources for dietary lycopene intake (Stahl & Sies 1996). Fresh tomatoes alone account for 50% of total lycopene intake worldwide (Rao et al. 1998). Average lycopene intake from all sources in Spain, United Kingdom, Ireland and France was 3.5 mg/day in 1997 (Olmedilla et al).

Bioavailability of lycopene from tomatoes depends on processing practices and what other foods are consumed along with the tomato product. Heating tomatoes disrupts the cells and allows lycopene out of the cell, rendering it more readily available for absorption in the intestine. Additionally, since lycopene is highly lipophilic, eating low amounts of fat with tomato products will result in decreased uptake compared to high fat consumption (Gartner et al. 1997). Several types of dietary fiber reduce the bioavailablilty of lycopene when concurrently consumed (Reidl et al. 1999). Consumption at a rate of 18 g/day of Olestra, a non-absorbable oil added to some potato chips and snack foods to help people avoid weight gain, reduced carotenoid absorption by 27% (Koonsvitsky et al 1997, Schlagheck et al. 1997). These statistics show that overall diet is an important factor in lycopene uptake specifically, and vitamin uptake generally.

Far from being degraded when exposed to heat, one study demonstrated no change in lycopene content after tomatoes were cooked at 100°C for 16 minutes (Thompson et al. 2000). In fact, benefits from lycopene are realized even more from processed tomato products. Processing dehydrates the product, resulting in higher concentrations of lycopene (Table 1.5). One study determined that the isomeric configuration of lycopene was not significantly different from 95% *trans* in raw tomatoes, paste or juice (Gartner et al. 1997). A different study concluded the opposite, that heating tomato products changed *trans* lycopene to *cis* lycopene, a more readily oxidized and bioavailable form, and thus more beneficial (Stahl & Sies 1992). Therefore, some evidence suggests that processing tomatoes increases the health benefit by both concentrating lycopene and altering it to a more beneficial form.

Lycopene content varies by cultivar (Ellis & Hamner 1943). Breeding for increased lycopene content has been going on for decades, but not with the notion of improving the health benefits for humans. Since lycopene makes tomatoes red, breeders have been seeking to increase its abundance in tomato fruit purely for aesthetic reasons (Thompson et al. 1965, Ellis & Hamner 1943). Evidence suggests that the the very first tomatoes brought into cultivation in Europe were yellow varieties. Red varieties did not become popular until the 19th century (Gould 1983). A wild relative of cultivated tomato, *Lycopersicon pimpinellifolium*, produces 40 times more lycopene than the reddest modern cultivar, and has been used in breeding projects for the aim of introducing this deep color into cultivars (1.13) (Porter & Lincoln 1950, Wann & McFerran 1960). One USDA-ARS study found, serendipitously, that cherry tomatoes grown in tissue culture at low temperatures had leaf tissue ripen into fruit-like tissue, in addition to producing normal

fruits, a curious finding that may have significance for geneticists. More importantly, both tissues contained ten times more lycopene than normal cherry tomatoes, although the reason is not clear (Ishida 1999). The triggers for high lycopene production in tomatoes may be uncovered by this discovery.

Commercial organizations have taken interest in lycopene too. Numerous patents covering aspects of lycopene purification and utilization have been issued by the US government. VitaCost took out a patent on "An orally ingested composition for providing or treating macular degeneration, cataracts, elevated ocular pressure, diabetic retinopathy and glaucoma..." containing multiple carotenoids, including lycopene (Goersk 2000). Separate extraction procedures have been patented by Indena, Nippon Del Monte Corporation and Kemin Foods, LC (Bombardelli et al. 1999, Kawaragi et al. 1999, Ausich & Sanders 1999). CrtI, the gene encoding for phytoene desaturase, and a process for generating a carotenoid compound from the mevalonic pathway, have also been awarded US patents (Hirschberg et al. 1998, Kirin et al. 1995). Cashing in on the supplement market, an Israeli company called Lycored sells a lycopene supplement called "Lyc-O-Mato" worldwide (Anon 1998). All of the commercial activity surrounding lycopene manipulation indicates a willingness, perceived or otherwise, of consumers to consider health properties, such as antioxidant content, in foods that they eat. Lycopene has enormous potential in this area.

Lycopene and Human Health

The interest of lycopene for humans lies in its suggested health benefits. Lycopene

is the most abundant carotenoid found in human serum, and therefore, most important in terms of net antioxidant activity (Stahl & Sies 1996). Like other carotenoids, it has been implicated in the prevention of several types of cancer and degenerative diseases. It has been shown by several studies to be the most effective singlet oxygen quencher among all carotenoids (DiMascio et al. 1989). This is fortuitous for the health of Americans, since lycopene consumption in the US is increasing (Nebeling et al. 1997).

Uptake of lycopene from food into the body depends on the isomer present in the food as well as other constituents of the meal. Once in the body, lycopene is concentrated in various tissues: 1 nmol/g in adipose tissue, and up to 20 nmol/g in the testes, adrenal gland and prostate (Stahl & Sies 1996). It accounts for about 50% of all carotenoids present in serum, and is present at a concentration of approximately 0.5 μmol/L (Gerster 1997). The persistance of lycopene in the body varies widely between studies. Rock et al. (1992) reported half life of lycopene to be 12-33 days among twelve males, whereas Stahl & Sies (1992) reported it as 2-3 days. Degree of oxidative stress obviously plays a large role in the persistence of lycopene in an animal system.

In vivo research has revealed that lycopene inhibits carcinogenesis in animal and human cells (Wang et al. 1989, Kim 1995). Lycopene controlled mammary tumorogenesis in a high-tumor-strain of mice (Nagasawa 1995). Habitual consumption of lycopene via tomatoes has been demonstrated to reduce the incidence of digestive tract cancer among Italians. Slattery et al. (2000) concluded that increased consumption of tomatoes (among other vegetables) reduces the risk of colon cancer. A six-year study of 48,000 men conducted by Harvard Medical School showed that consumption of

tomato products at least twice a week decreased prostate cancer by 34% (Giovanucci et al. 1995). A study which compared the macronutrient supply values with prostate cancer in 28 countries was able to draw a negative correlation of $r^2 = 0.62$ between incidence of prostate cancer and consumption of tomato products, with lycopene being credited with this affect (Grant 1999). Similarly, three weeks of daily lycopene supplements (30 mg/day) reduced the size and malignancy of prostate tumors (American Assoc for Cancer Research 1999). New York women diagnosed with cervical cancer had significantly depressed amounts of lycopene in their serum (Palan et al. 1996). High tomato product intake was associated with a 50% reduction in cancer among elderly Americans (Franceschi et al. 1994). Lycopene was found to be more effective than β-carotene or α-carotene at preventing proliferation of epithelial cancer cells (Levy et al. 1995). Lycopene, as well as other carotenoids, was shown to reduce risk of lung cancer in one study, but failed to prevent lung cancer in another study from Uruguay (Michaud et al. 2000, DeStefeni et al.,1999). Serum levels of lycopene were not affected by smoking, unlike β-, α-carotene and vitamin C, according to Ross et al. (1995). This result seems to suggest that lycopene has no role in protecting against lung cancer, however, an earlier study did show that serum lycopene levels were reduced 21-29% in smokers compared to non-smokers (Pamuk et al. 1994). The cancer link is boosted by a report that age is highly correlated to decreased serum lycopene concentrations (Gerster 1997).

Although the implications are not clear, HIV-positive patients with CD4 counts < 400 cells/mm had significantly depressed serum lycopene content (Coodley et al. 1995, Lacey et al. 1996). CD4 cells are the first cells of the immune system to be attacked by

HIV. High lycopene content in the fat of 1,379 European men was highly correlated to low incidence of heart disease (Kohlmeier et al. 1997). This finding was echoed by a University of North Carolina study which also found a high correlation between consumption of tomato products and heart attack. Low density lipoprotein (LDL) oxidation is causally associated with coronary heart disease, therefore, it is significant that Agarwal and Rao (1998) found that lycopene supplementation of 19 human subjects significantly reduced LDL oxidation. A test group of middle-aged Finnish men exhibited a negative correlation between lycopene serum content and increased intima-media thickness of the common carotid artery wall, a condition which leads to early atherosclerosis (Rissanen et al. 2000). Some evidence indicates that lycopene from tomatoes can slow the development of a stroke, although the mechanism behind this was not investigated (Anon 1999). Patients with chronic renal failure had depressed levels of lycopene in their serum (Ha et al. 1996). Three- to four-fold reduction in lycopene serum content was linked to four types of liver disease (Leo 1993). Supplementing male smokers and non-smokers with lycopene yielded a significant decrease in the amount of lymphocyte DNA breakage (Pool-Zobel et al. 1997, Riso et al. 1999). Lipid peroxidation was reduced 86% in mammalian cell cultures by supplementation of lycopene (Matos et al. 2000). A study involving rats demonstrated that under certain dietary conditions, lycopene prevented cataract formation (Pollack 1999). Even asthma seems to ameliorated by lycopene: one study of twenty subjects with exercise induced asthma (EIA) reported that lycopene supplemetation of 30 mg/day protected 55% of the subjects from EIA (Neuman et al. 2000). Lycopene, as well as other carotenoids, was shown to significantly improve three key areas of immune response in the elderly in one

experiment, but had no effect on cell mediated immune response in another (Inserr et al. 1999, Watzl et al. 2000).

Lycopene presents some exciting possibilities for human health, however, not all studies find positive effects. A recent review by Giovanucci (1999) concluded that lycopene was proven to be effective in reducing/preventing cancers of the prostate, lung and stomach, but regarded claims of reducing cancers of the pancreas, colon, esophagus, oral cavity, breast and cervix as being suggestive, yet unproven. Some go further, for example, Garcia et al. (1999) concluded from their study of over 1,500 human subjects that no carotenoid, including lycopene, provided any protection against bladder cancer. Any claims for a cure-all should be approached with healthy skepticism. If something sounds too good to be true, it may well be. Extraordinary claims of anti-cancer properties should not be translated into a promotion of massive lycopene supplementation, lest a fiasco like the β-carotene supplement issue be repeated (β-carotene was also given strong credit for decreasing cancer in the early 1990's, and immediately β-carotene supplements became popular until it was shown that in high amounts β-carotene actually acts as a *pro*-oxidant and *causes* lung cancer!) (Herbert 1994). Time will tell if lycopene truly is the anticancer vitamin of the century, or just another passing fad.

Thesis Objectives and Justification

1. To identify some horticultural properties of commonly available tomato cultivars.

Taste, plant growth and fruit characteristics will be investigated. Many cultivars are available from local nurseries and many more through seed catalogs. Direct comparison of commonly available cultivars will be useful to those seeking top quality plants and fruit.

2. To develop an efficient lycopene analysis protocol utilizing HPLC. This is prerequisite to any meaningful study involving lycopene or any organic compound.

3. To quantify lycopene concentration among the major garden tomato cultivars of northern Colorado. This information will be useful to gardners interested in the nutritional quality of the food they grow, and to breeders who are seeking to find sources of germplasm for tomato breeding projects aimed at boosting fruit lycopene concentration.

4. To determine the effect light may have on lycopene production in fruit picked green-mature. Since most tomatoes sold in markets in the United States originate from field operations which harvest fruit green-mature, any strategy which might enhance the quality of these tomatoes would be useful for growers and consumers alike.

Table 1.1 The Genus Lycopersicon (Gould 1983, Hancock 1992)

Subgenera	Species	Common name	Chromosome number
Eulycopersicon (red-fruited)	*L. esculentum esculentum*	Tomato	24
	L. esculentum cerasiforme	Cherry Tomato	24
	L. pimpinellifolium	Currant Tomato	24
Eriopersicon (green-fruited)	*L. peruvianum*	Wild species	24
	L. hirsutum	Wild species	24
	L. cheesmanii	Wild species	24
	L. chilense	Wild species	24
	L. chmielewskii	Wild species	24
	L. glandulosum	Wild species	24

Table 1.2 Botanical varieties of cultivated tomato, *L. esculentum*

Botanical Variety	Common Name
commune	common tomato
cerasiforme	cherry tomato
pyriforme	pear tomato
grandifolium	potato-leaved tomato
validum	upright tomato

Table 1.3 Some popular early cultivars in the United States (Gould 1983)

Cultivar name	List Dates
Ferry's Improved	1868-1888
Tilden's	1868-1878
General Grant	1871-1883
Red Pear	1872-1936
Trophy	1872-1926
Canada Victor	1874-1892
Acme	1879-1930
Essex Early Hybrid	1881-1912
Turk's Turban	1880-1882
Favorite	1883-1907
Golden Queen	1886-1936
Early Michigan	1889-1930
Mikado	1889-1902
Buckeye State	1895-1915
Matchless	1901-1922
Earliana	1904-1936
Globe	1906-1936
Bonny Best	1916-1936
Avon Early	1921-1936
Cooper's Special	1926-1936

Table 1.4 Plant sources of lycopene (Ngyuen & Scwhartz 1999)

Common name	Type	Common name	Type
Aglaonema aroid	fruit	Mango	fruit
Apricot	fruit	Palm	oil
Arbutus	leaf	Papaya	fruit
Berry	fruit	Peach	fruit
Bitter melon	fruit	Peach	fruit
Bitter nightshade	fruit	Pepper	fruit
Calendula	flower	Pepper berry	fruit
Carrot	root	Persimmon	fruit
Citrus	fruit	Plum	fruit
Cuckoo pint	tuber	Pumpkin	fruit
Cowberry	fruit	Ramanas rose	plant
Cloudberry	fruit	Red byrony	plant
Cranberry	fruit	Rosa mosqueta	plant
Damask rose	fruit	Rose	fruit
Date palm	fruit	Rutabaga	root
Eggplant	fruit	Saffron	seed
European nettle	plant	Tea	leaf
Gazania	plant	Tomato	fruit
Grape	fruit	Turnip	root
Grapefruit	fruit	Watermelon	fruit
Guava	fruit	Yew	fruit

Table 1.5 Lycopene (mg $100g^{-1}$ fresh weight) content in selected foods common in human diets

Food	mg Lycopene $100g^{-1}$ fresh weight
Fresh Apricots	0.005[a]
Canned Apricots	0.0065[a]
Dried Apricots	0.86[a]
Canned Chili	1.08-2.62[a]
Fresh Pink Grapefruit	3.36[a]
Fresh Guava	3.34[a]
Ketchup	16.60[a]
Red Papaya	2.00-5.30[b]
Canned Pizza Sauce	12.71[a]
Cooked Pizza Sauce (from pizza)	32.89[c]
Canned Rosehip Puree	0.78[a]
Canned Salsa	9.28[d]
Canned Spaghetti Sauce	17.50[d]
Fresh Red Tomatoes	3-11[e]
Canned Red Tomatoes	11.21[c]
Canned Tomato Juice	7.83[c]
Canned Tomato Soup	3.99[c]
Canned Tomato Paste	30.07[c]
Fresh Red Watermelon	4.1[a]
Vegetable Juice	7.28[c]

[a] USDA 1998
[b] Mangels et al. 1993
[c] Ngyuen & Schwartz 1998a
[d] Ngyuen & Schwartz 1999
[e] Table 1.6

Table 1.6 Published lycopene content in fresh tomatoes.

mg lycopene/100 g fresh weight tomato	Reference
3.35	Al Wandowi 1985
3.1	Heinonen et al. 1989
3.92	Khachik 1992
3.1	Mangels et al. 1993
9.27	Tonucci 1995
11	Sharma et al. 1996
5.6	Gartner et al. 1997
6.4	Shi et al. 1999
3.1 - 7.7	Ngyuen et al. 1999

Figure 1.1 Map of South America

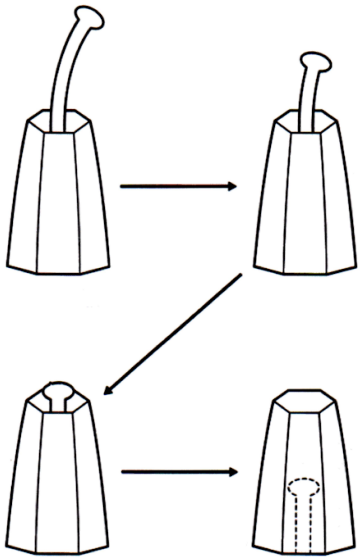


Figure 1.2 Modification of flower style length by domestication

Figure 1.3 *Atropus belladonna*, Deadly Nightshade

Figure 1.4 Lycanthropy woodcut from present-day Germany, 1493

Figure 1.5 Green Zebra heirloom tomato cultivar

Figure 1.6 Black Krim heirloom tomato cultivar

Figure 1.7 Federle heirloom tomato cultivar

Figure 1.8 Mikado hybrid tomato advertisement, 1880

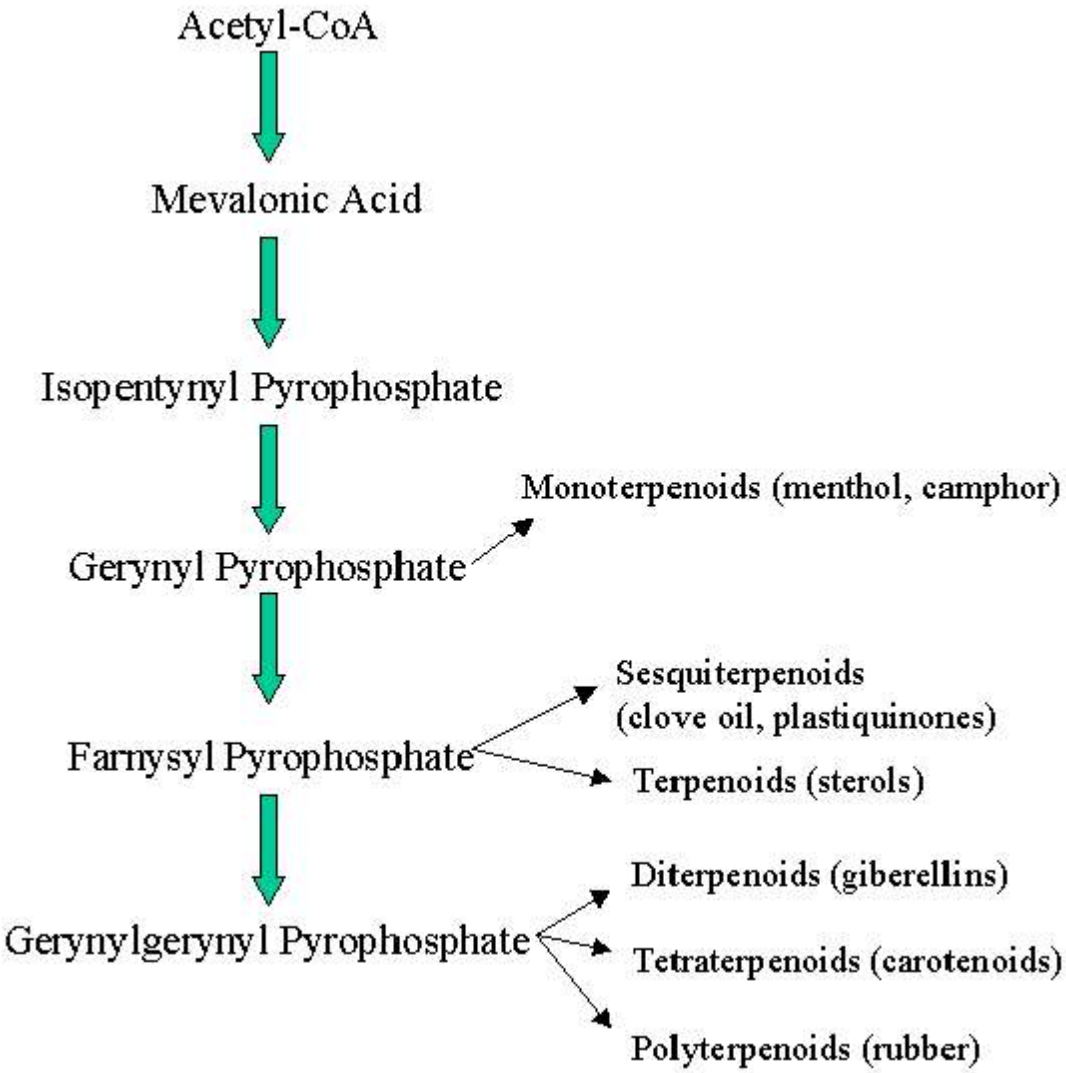


Figure 1.9 Isoprenoid pathway with major classes of terpenoids

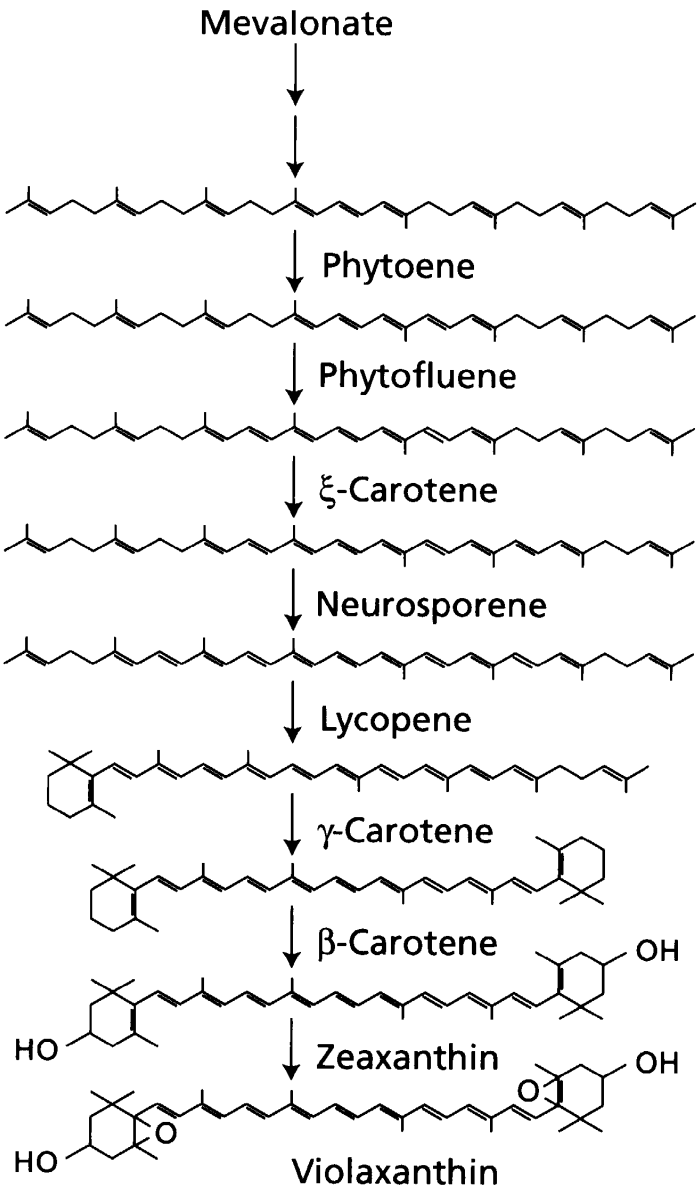


Figure 1.10 Carotenoid synthesis pathway (Dey & Harborne 1997)

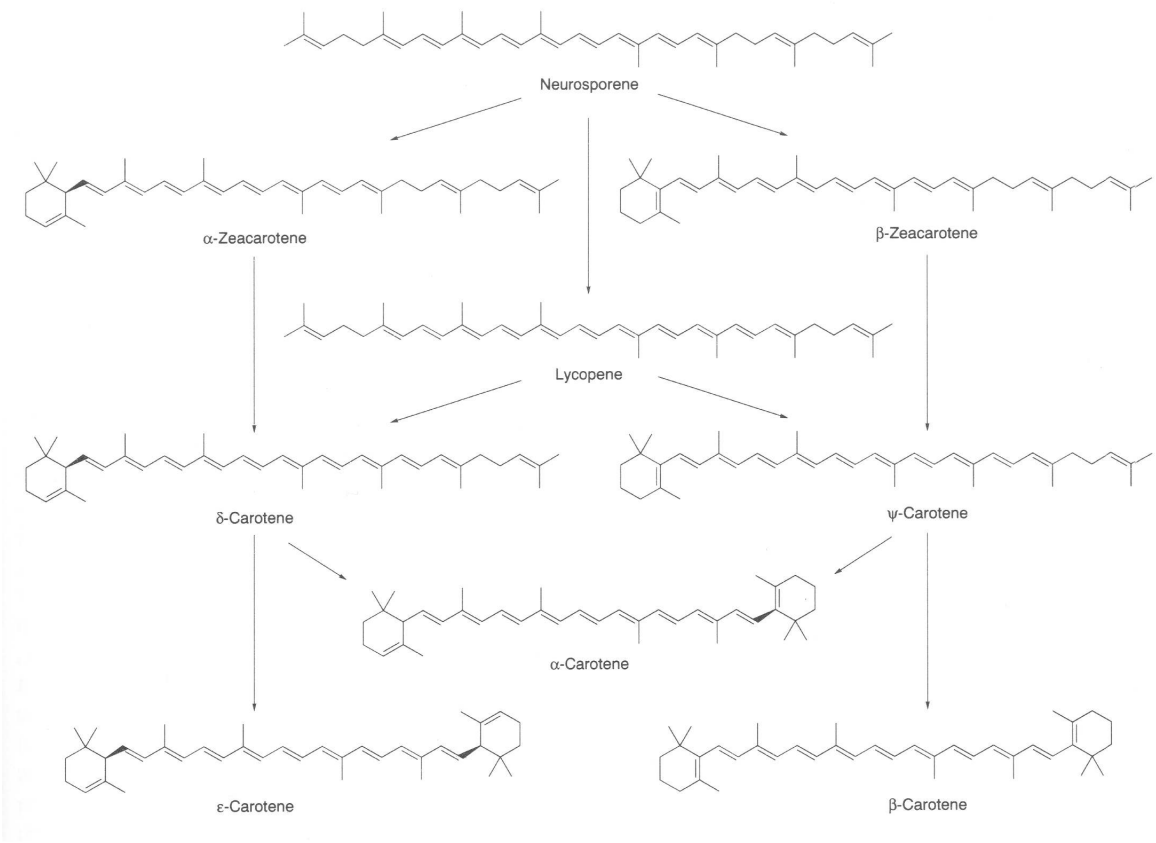


Figure 1.11 Cyclization reactions of carotenoids (Dey & Harborne 1997)

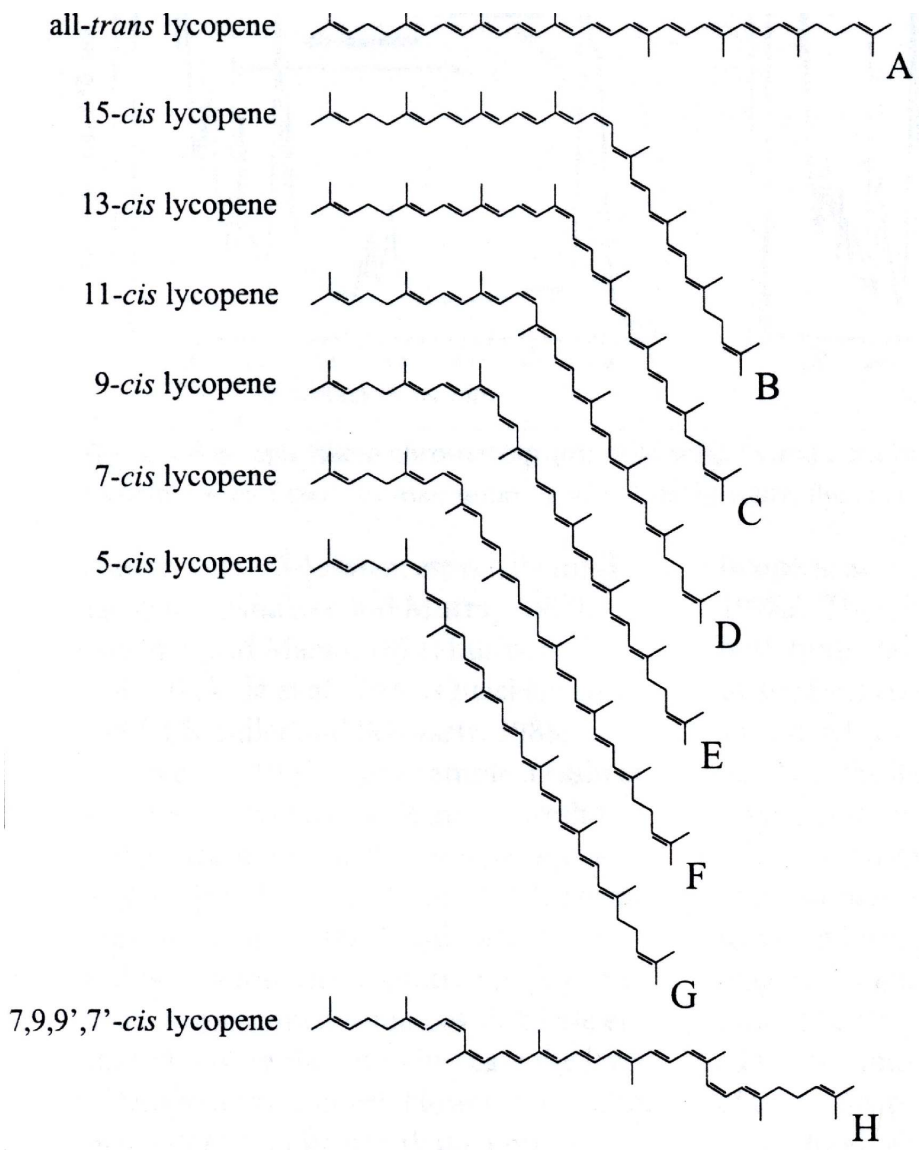


Figure 1.12 Common lycopene isomers (Ngyuen & Schwartz 1999). Structure A is the predominant structure found in plants (≈95%). Structures B, C, E and G are found in human serum, making up approximately half the total lycopene content. Structure H is the naturally occurring form of lycopene in fresh Tangerine-type tomatoes.

Figure 1.13 *Lycopersicon pimpinellifolium*, wild tomato relative with high amounts of lycopene

CHAPTER 2

HORTICULTURAL PROPERTIES OF NORTHERN COLORADO GARDEN TOMATOES

INTRODUCTION

Tomatoes are the most popular garden vegetable grown in the United States (Jones 1999). They are relatively easy to grow, produce large yields of fruit and are a flavorful addition to many meals. So popular is the tomato in home gardens that several thousand varieties are available either through local nurseries or via mail order. Choosing appropriate cultivars for northern Colorado can be difficult because of unique climatic conditions which include dry, hot summer days and cool nights within a short growing season. Tomato plants are generally not especially drought-tolerant, but some will perform better than others under drought conditions. Likewise, the cooler nights of Colorado summers inhibit high yields in some cultivars which perform well in southern areas where nights stay warm. An important consideration when choosing a cultivar for home gardening purposes is growing season length. Typical home gardens yield 4-10 lb/plant. Many cultivars require a longer growing season for optimal yield than northern Colorado can provide. Thus, it is wise to plant only cultivars which produce heavily within a short time, or begin with large transplants.

Hybrid cultivars have dominated commercial markets for over 50 years, but non-hybrid heirlooms, which produce fruit with unusual and unique characteristics, remain popular among many gardeners. Colors beyond the traditional red, orange and yellow are often encountered, as well as shapes of all types. Heirloom cultivars are not products of modern breeding. Consequently, disease resistance and yield are usually lower in these cultivars.

Tomato fruit quality is the result of interaction between flavor, appearance and texture. Flavor is perhaps the most important component of tomato fruit quality when judged by consumers. A typical ripe tomato is about 95% water, with the remaining 5% composed of roughly 50% sugar (glucose and fructose) and about 15% acid (citric and malic) (Davies & Hobson 1981). Flavor is imparted by the interaction of sugar and acid. A combination of high sugar and high acid imparts a flavor that most people find pleasing. High acid combined with low sugar gives the tomato a tart flavor, and high sugar coupled with low acid results in a bland flavor. Any fruit with low sugar and low acid is generally considered unpleasant (Peet 1996). Extensive research isn't required to conclude that the general population regards vine-ripened, home-grown tomatoes as vastly superior to their grocery store counterparts picked green-mature. Nonetheless, researchers have documented this (Jones 1999). When fruit are picked green-mature, the carbohydrate source is cut off, and the fruit must ripen with whatever carbohydrate is then inside. Vine-ripened fruit continue to import carbohydrates from the leaves up until ripeness. It makes perfect sense then that the flavor of vine-ripened tomatoes would be superior.

A major contributing factor to the perceived inferior taste of supermarket tomatoes is the emphasis on breeding cultivars for commercial production that will ship well, stay firm, avoid disease and look good. Taste is not a primary concern. USDA grade standards for tomatoes do not include evaluations of flavor, only evaluations of color, firmness, disease and external blemishes (Gould 1983). Likewise, consumers in a grocery store have no way to evaluate flavor by looking at a tomato, so they must select fruit based on outward appearance, employing the same basic guidelines used by the USDA. Flavor just doesn't sell tomatoes.

On the other hand, home-grown tomatoes are all about flavor. They need not be firm, determinate, deep red or particularly disease-resistant. Shelf life is meaningless for home gardeners. Thus, popular garden cultivars have become so principally because of their flavor.

Texture is important too, although inferior texture is often forgiven in light of outstanding flavor, but rarely the other way around. Overripe tomatoes will take on a loose, watersoaked texture, sometimes grainy. This is generally regarded as unpleasant. Underripe tomatoes will be very firm and stiff. The ideal texture of a tomato is slightly firm and smooth.

Appearance, as mentioned, is very important to a tomato eater before they have tasted the tomato. An ugly tomato has little chance of redeeming itself through good flavor or texture since the taster's mind is biased before the first bite. Conversely, excellent looking specimens may garner more praise than deserved based on all factors. Taste tests sometimes employ a method of masking a tomato’s appearance through the use of colored or dim lighting to minimize the appearance effect on flavor ratings.

Thirty local garden cultivars were grown and evaluated on plant growth, required season length and fruit characteristics including soluble solids, water content, size, color and taste. Relationships between fruit characteristics and lycopene content were also investigated. This information will be useful to home gardeners, nurserymen and tomato breeders.

The hypotheses of this experiment were that some cultivars would be more suitable to northern Colorado growing conditions based on plant growth and fruit production, and that a taste test of multiple cultivars would show one or more to be superior when rated by a diverse panel of tasters.

MATERIALS AND METHODS

Tomato plants were grown in the field and in the greenhouse to identify morphological characteristics. Fruit from these plants were harvested for taste testing, lycopene analysis and identification of morphological and physiological features.

Field Plant Growth

Three 2" pot transplants of thirty common cultivars of tomato, *Lycopersicon esculentum*, were purchased on May 13, 1999 from Gully Greenhouse and Bath Nursery (Fort Collins, CO). Cultivars selected included orange, yellow and red-fruited as well as small, medium and large-fruited (Table 2.1). Plant height ranged from 3-12". A twelve day outdoor hardening period in shade with limited water was followed by field planting on May 25 at the Colorado State University Horticulture Research Farm, northeast of

Table 2.1 Cultivars commonly grown in Northern Colorado home gardens selected for field study. Legend - Size: L = large, M = medium, S = small; Color: R = red, O = orange, Y = yellow

Cultivar	Size	Color	Cultivar	Size	Color
Amish Paste	M	R	Husky Cherry Gold	M	O
Beefmaster	L	R	Husky Gold	L	O
Better Boy	L	R	Juliet	S	R
Better Bush	M	R	Keepsake	M	R
Big Beef	L	R	Large Red Cherry	S	R
Brandywine	L	R	Lemon Boy	M	Y
Brandywine Yellow	L	Y	Miracle Sweet	M	R
Big Girl	L	R	Park's Whopper	L	R
Celebrity	L	R	Patio	M	R
Champion	L	R	Red Robin	S	R
Early Cascade	M	R	Roma	M	R
Early Girl	M	R	Sweet 100	S	R
Flor-America	M	R	Sub Arctic Maxi	-	-
Golden Boy	L	O	Super Fantastic	L	R
Heartland	M	R	Yellow Pear	S	Y

Fort Collins. Approximately 85g Osmocote (14-14-14) was mixed with loose soil in 1' deep holes spaced 3' apart along a linear row graded up for furrow irrigation. Roots were planted deep enough so that stems were half below ground, with a furrow around the base to collect water. Plants were hand irrigated every 3-4 days for two weeks, then furrow irrigated roughly once per week as needed. Most plants were staked on June 15 using 3' cane poles and zip ties to secure the central leader. Axillary shoots were pinched off every 3-4 days. By July 1, the plants had outgrown the cane poles, and were re-staked using 5' lengths of #40 polyvinylchloride (PVC) pipe hammered 1' deep in the ground. The pesticide Bravado was applied on August 10 to control psyllids. Tomato hornworms were routinely picked off by hand. Large weeds were pulled as needed.

Greenhouse Plant Growth

Seeds of the black heirloom cultivars Black Krim, Black Tula and Black Plum were purchased from Seed Savers Exchange (Decorah, IA). One package of the common F_1 hybrid cultivar Celebrity was purchased from Sutherlands Hardware Store (Fort Collins, CO). On March 17, 2000, seeds were planted in peat/perlite media in jumbo six-cell packs at a rate of two seeds/cell with each cultivar occupying one six-cell pack. Seeds were germinated in a growth chamber held at 26°C with a 16/hour flourescent light period. Germination was near 100%. After germination, seedlings were thinned to one per cell. Seedlings were grown in the chamber for about 30 days, until April 13, at which point the plants were 6-8" tall and ready for transplanting. The seedlings were placed in a greenhouse for two days as a short dehardening period, then transplanted into

peat/perlite mix in five gallon paint buckets with drainage holes punched in the bottom. At planting time, 85g Osmocote slow release fertilizer (14-14-14) was mixed into the potting medium. Four replicates of each cultivar were planted and identified by plastic pot labels. Tomato cages were inverted and placed over the pots to support the plants as they grew. No temperature data are available for this greenhouse, but relatively speaking, it got very hot, probably well over 100°F (38°C) on many occasions. Because of this heat, plants required hand watering every day. Weekly liter-volume fertilizer treatments with Miracle Grow (24-18-18) plus 1g/L $CaNO_3$ followed until fruit was picked. Axillary shoots were pinched off at the bud stage to allow a central leader to dominate. Fertilization of the flowers was accomplished by jostling the peduncles and bumping the wooden table on which the plants rested. Due to greenhouse demolition, all plants were moved in late June to a new facility which had automatic irrigation capacity as well as a more efficient cooling system. Extremes in temperature as well as drought stress were eliminated with the move. Fully ripened tomatoes, free from bruises and rot, were collected from every plant between June 29 and July 12, 2000. Since plants were grown in a greenhouse environment, growing season was not an issue. Plant and fruit growth characteristics are reported, however black cultivars were not included in the taste test.

Morphological and Physiological Characteristics

Water content from field and greenhouse-grown tomatoes was determined by calculating weight difference between fresh and freeze-dried fruit. Oven drying was not employed since the fruit tissue was also to be assayed for lycopene content. For field-

grown cultivars, the date on which ripe fruit were first available was noted for each plant. Growing degree day base 10 (GDD_{10}) measures the number of hours the temperature was above 10°C, the general threshold for warm season crop growth. This figure is a more precise measure of growing season since it is based on temperatures and not time alone. Growing degree day data was retrieved from archived weather files for the NE Fort Collins station at http://ccc.atmos.colostate.edu/cgi-bin/coag_raw.pl. Refractive index is a measure of soluble solids and in the case of tomatoes measures primarily soluble carbohydrates content. Fluid from tomato was placed on a Bausch and Lomb Low Range Refractometer 33.45-30 for determination of refractive index (Rochester, NY).

Taste Test

On September 19, several ripe tomatoes from each of the field-grown plants were picked and put into paper bags. Plant mortality and lack of ripe fruit decreased the number of plants sampled to 88. The tomatoes were stored at room temperature for five days. On September 24, the tomatoes were laid out for an eight-hour taste test. Each bag was assigned a number corresponding to a master list identifying each cultivar. Each sample was washed, dried and placed on a paper plate. Samples were lined up on either side of long wooden tables in a large classroom. Testers were recruited and came in intermittently throughout the day. Volunteers were students, faculty and staff. Each tester was given a knife with which to cut portions of the tomato for sampling, a cup to fill with water to cleanse the palate between tasting and a score sheet to record evaluations.

The score sheet contained five categories: appearance, taste, flavor, texture and overall. These were rated on a four point scale (1 = worst, 4 = best) for each tomato (Fig 2.1). A fine line distinguishes taste from flavor, and this was a source of some confusion among tasters. Taste represents one of the four fundamental sensations perceived by the tongue, which are sweet, sour, salt and bitter, whereas flavor represents the overall sensational quality of the fruit. Most people have an idea of the ideal tomato flavor, which includes taste, aroma and other intangible combinations. Flavor is more general than taste as it describes an almost intangible element of the fruit.

Since 88 tomato samples were available for tasting, it was assumed that most tasters would not be physically able to taste them all. Therefore, to achieve an approximately equal sample size, tasters were first told to start at random points and work either direction around the tables. Later in the day, tasters were assigned to begin in a specific area with previously low testing frequency. Four tasters sampled all tomatoes. Twenty-one other volunteers sampled some tomatoes.

Statistics

The general linear model and correlation procedure in SAS v8 (SAS Institute, Nashville, TN) were used for statistical analyses of data.

Sensory Evaluation of Garden Tomato Cultivars Based on a Hedonic Preference Scale Evaluator's name_____ Date_____																				
	1. Dislike very much 2. Dislike slightly 3. Like slightly 4. Like very much																			
#	Appearance (color, bright, dull)				Taste (sour, sweet, salty, bitter)				Flavor (aroma, mild, strong, typical, atypical)				Texture (firm, soft, tough)				Overall			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4

Figure 2.1 Taste test evaluation sheet, grid truncated here. Sheets were printed to fill 8.5 x 11 inch paper

RESULTS AND DISCUSSION

Field Plant and Fruit Characteristics

Water content of some cultivars is listed in Table 2.2. Of the cultivars listed, Red Robin had the lowest water content at 83.5% and Celebrity had the highest at 93.8%. Jones (1999) simply states that tomatoes are generally more than 90% water, and that the availability of water during fruit enlargement is the primary non-genetic influence on water content. The water content of cultivars listed appeared low, primarily because the field conditions were very sunny and dry most of the time. Low water content is desirable for processing tomatoes since the solid matter is what is ultimately used for products like paste or sauce. Water content was negatively correlated to fruit size (R^2 = -0.7211, $p = 0.0081$, $n = 12$), meaning that larger fruit have a larger proportion of water than smaller fruit.

Date of the first ripe fruit for every cultivar is listed in Table 2.3. Days to ripening is often standardized because of varying local climates to the growing degree day (GDD) scale. Instead of counting each day of a growing season, hours within each day that exceed the minimum temperature required for growth are recorded. Thus, a crop will require the same number of growing degree days whether it grows up north or down south. Cumulative GDD from the time of transplanting is also presented in Table 2.3.

One Sweet 100 plant produced fruit just 71 days (541 GDD) after transplanting. Other cultivars that produced fruit in less than 100 days (900 GDD) after transplanting were Park's Whopper, Early Cascade, Super Fantastic and Yellow Pear. Every other

Table 2.2 Water content of selected cultivars determined by calculating the difference between fresh weight and freeze-dried weight

Cultivar	Mean (% H2O)	Standard Deviation	Sample Size (n)
Beefmaster	91.3	3.8	4
Black Krim	93.3	1.3	4
Black Tula	94.0	0	3
Brandywine	93.5	0.7	2
Celebrity	93.8	0.5	5
Champion	91.0	1.4	2
Early Cascade	87.8	3.5	8
Heartland	87.3	2.1	4
Juliet	88.3	3.1	9
Keepsake	85.7	3.2	3
Large Red Cherry	91.0	4.2	2
Red Robin	83.5	3.5	2
Sweet 100	84.3	5.4	7
Yellow Pear	86.7	5.4	6

Table 2.3 Date of first ripe fruit for each cultivar with days after planting in parentheses. Mean growing degree days base 10°C (hours of temperature above 10°C) in far right column (n = 3) with SD.
* indicates plant death prior to fruiting
- indicates live plant which bore no useable fruit

Cultivar	Rep I	Rep II	Rep III	GDD
Amish Paste	9/6 (114)	8/30 (107)	8/30 (107)	919 ± 27
Beefmaster	8/30 (107)	8/30 (107)	8/18 (95)	862 ± 73
Better Boy	8/30 (107)	8/27 (104)	8/27 (104)	884 ± 17
Better Bush	8/26 (103)	8/18 (95)	8/30 (107)	866 ± 46
Big Beef	8/18 (95)	8/18 (95)	8/18 (95)	778 ± 0
Brandywine	9/15 (123)	8/30 (107)	8/30 (107)	938 ± 59
Brandywine Yellow	9/15 (123)	9/8 (116)	-	986 ± 28
Big Girl	8/30 (107)	9/8 (116)	8/30 (107)	925 ± 36
Celebrity	8/30 (107)	9/6 (114)	8/30 (107)	919 ± 27
Champion	8/18 (95)	8/23 (100)	8/30 (107)	837 ± 63
Early Cascade	8/18 (95)	8/13 (90)	8/18 (95)	761 ± 29
Early Girl	8/26 (103)	8/18 (95)	8/2 (79)	755 ± 119
Flor-America	8/30 (107)	8/30 (107)	8/30 (107)	904 ± 0
Golden Boy	9/6 (114)	8/26 (103)	9/23 (131)	954 ± 95
Heartland	8/30 (107)	9/8 (116)	8/13 (90)	866 ± 123
Husky Cherry Gold	9/6 (114)	9/23 (131)	8/30 (107)	968 ± 75
Husky Gold	8/30 (107)	8/18 (95)	8/30 (107)	862 ± 72
Juliet	8/9 (86)	8/23 (100)	8/13 (90)	749 ± 71
Keepsake	8/18 (95)	9/8 (116)	8/26 (103)	868 ± 94
Large Red Cherry	8/26 (103)	8/18 (95)	8/26 (103)	833 ± 48
Lemon Boy	8/26 (103)	9/8 (116)	8/18 (95)	868 ± 94

Miracle Sweet	8/18 (95)	8/23 (100)	8/18 (95)	795 ± 29
Park's Whopper	8/18 (95)	8/18 (95)	8/18 (95)	778 ± 0
Patio	8/30 (107)	8/30 (107)	8/26 (103)	890 ± 25
Red Robin	8/30 (107)	9/8 (116)	9/8 (116)	945 ± 36
Roma	8/18 (95)	8/30 (107)	9/8 (116)	883 ± 96
Sweet 100	7/25 (71)	8/8 (85)	8/13 (90)	649 ± 97
Super Fantastic	*	8/18 (95)	8/10 (87)	739 ± 55
Yellow Pear	8/9 (86)	8/16 (93)	8/13 (90)	726 ± 35

cultivar had at least one plant which took more than 100 days (900 GDD) to bear fruit. The extended length of time required by some cultivars to produce fruit renders them unsuitable for cultivation in northern Colorado. For example, a killing frost wiped out the plants on September 28, and many cultivars had been harvested for the first time only days before (Table 2.3). Production potential of most plants was never fully realized because they were killed before most of the green fruit could ripen or reach the green-mature stage. Growing seasons in northern Colorado can vary drastically from year to year due to late spring and early fall snowfall or freezes. Cultivars requiring long seasons are not suited for cultivation in northern Colorado. Some examples of such late-producing cultivars are Brandywine Yellow (mean = 120 days, 986 GDD), Husky Cherry Gold (mean = 117 days, 968 GDD), Golden Boy (mean = 116 days, 954 GDD), Red Robin (mean = 113 days, 945 GDD) and Big Girl (mean = 110 days, 925 GDD). When planting these cultivars in this region, large transplants should be used and planted early. Growing degree day requirement was not significantly correlated to fruit size, color, water content or refractive index.

Sub Arctic Maxi proved a very poor cultivar for this area as all three plants died within one week of planting. The reason behind this rapid demise is not known for certain, but is suspected to be poor Fusarium wilt resistance. After two weeks, one replicate of Super Fantastic died from Fusarium wilt, as identified by the Colorado State University Plant Disease Clinic. Roma and Red Robin were lackluster growers, never achieving a height more than two feet. This may be ideal for container planting, however they produced very little fruit, and some Roma replicates produced no fruit at all. While

quantitative yield measurements were not taken, it was obvious that Sweet 100, Yellow Pear, Juliet and Early Cascade were the heaviest producers and formed large plants. Yellow Pear produced the tallest plant at well over 6' before it suffered from lack of support. Better Bush produced a well-rounded and attractive determinate plant of about three feet in height which did not require staking, however the yield was low. Park's Whopper produced the best looking and largest fruit, and is apparently very well suited for this area. Brandywine and Brandywine Yellow produced unattractive cat-faced fruit, which is a wrinkled condition due to incomplete fertilization (Jones 1999).

Soluble solids is a rough measure of the soluble carbohydrate content, which in tomatoes is predominantly composed of fructose and glucose (Davies & Hobson 1981). Soluble solids values for many cultivars are given in Table 2.4 where means were separated using F-protected LSD. Lorenz & Maynard (1988) list the carbohydrate content in ripe tomatoes as 4.3%. More than half of the tomatoes grown for this study exceeded that estimate. Ironically, Miracle Sweet was in the group with the lowest sugar content.

Comments on Black Cultivar Plant and Fruit Morphology

Relative to Celebrity, all three black cultivars exhibited more vigorous growth and greener shoots and leaves throughout the experiment. All three black cultivars were strongly vining and indeterminate. Vegetative growth was apparently strong at the expense of fruit production in these three cultivars. This conjecture is backed up by personal observation of these black cultivars growing in the field in Fort Collins, CO and

Table 2.4 Refractive Index (Soluble Solids) measurements for all field-grown cultivars. ANOVA $p < 0.0001$. Means associated with same letters are not significantly different. $\alpha = 0.05$ LSD = 0.93

LSD Grouping	Mean	N	Cultivar
A	6.0	2	Brandywine Yellow
B A	5.6	3	Sweet 100
B A C	5.3	3	Better Bush
B A C	5.3	3	Better Boy
B D C	5.0	3	Brandywine
B D C	5.0	3	Big Girl
B D C	5.0	2	Husky Gold
B E D C	4.7	2	Husky Cherry Gold
E D C	4.6	3	Heartland
E D C	4.6	3	Juliet
E D C	4.6	3	Champion
F E D C	4.5	3	Lemon Boy
F E D C	4.5	3	Flor-America
F E D C	4.5	3	Early Girl
F E D C	4.5	3	Park's Whopper
F E D	4.3	3	Large Red Cherry
F E D	4.2	2	Super Fantastic
F E D	4.1	3	Amish Paste
F E D	4.1	3	Keepsake
F E D	4.1	3	Early Cascade
F E	4.0	2	Golden Boy
F E	4.0	3	Beefmaster
F E	4.0	3	Red Robin
F E	4.0	3	Big Beef
F E	4.0	3	Yellow Pear
F	3.6	3	Miracle Sweet
F	3.6	3	Roma
F	3.6	3	Patio
F	3.6	3	Celebrity

Fort Worth, TX where, in relation to traditional red cultivars, fruit yield was substantially lower but vegetative growth was substantially higher (Olen Cox, personal communication). Flowering occurred generally later than on Celebrity. Black Plum produced ripe fruits earlier than the other cultivars, but since it is a small fruited plum-type cultivar, this was not surprising. All three black cultivars produced fruit which was much darker in color than Celebrity (Fig 2.2).

All three black cultivars were much more susceptible to blossom end rot, as well over half of the fruit produced in the original greenhouse location were soon rotten from this condition, as opposed to the virtual absence of the condition in Celebrity tomatoes. Blossom end rot is a physiological condition (not bacterial or viral) manifested as brown, soggy tissue caused by rotting dead cells. Cell death is induced by a lethal calcium deficiency in the rapidly expanding cells near the blossom end (Jones 1999). Fertilizer applications were anticipated to remedy the blossom end rot problem by adding calcium to the soil, but the recurrent drought stress brought on by high-respiring large plants in small pots in a hot greenhouse in June inhibited movement of adequate amounts of calcium from the root zone to the fruit. Thus, fruit ripened much earlier than late June on almost every plant, but it was not until then that intact, healthy fruit were available. Since environmental stress influences synthesis of secondary metabolites, using damaged fruit for analysis of lycopene would be inappropriate (Foster et al. 1992). The problem became almost obsolete once the plants were transferred to the newer facility in late June and most tomatoes produced afterward were free of blossom end rot.

A

B

Figure 2.2 A Black Krim on the left and Celebrity on the right
B Black Krim on the left and Celebrity on the right

An interesting morphological feature of Black Plum was its profligate production of adventitious roots on all stems above ground. Roots as long as a centimeter lined all sides of the primary and secondary stems.

Taste Test

A preliminary analysis of variance for unpooled data was performed to test the significance of various factors. The effect of the block planting was insignificant ($p = 0.5660$). The order that each tester tasted fruit was also found to be insignificant ($p = 0.6088$). Simply interpreted, it didn't matter which planting block the tomato came from, and tomatoes tasted near the beginning were not given higher ratings by each tester. Since block and order could be eliminated as factors, the replicate data for each cultivar was pooled to increase the sample size of each cultivar tested. Sample sizes ranged from 19 to 36, despite efforts to equalize them by assigning testers to certain samples. A second ANOVA with this pooled data was run. "Overall" and "Texture" ratings did not show significant mean separation ($p = 0.2861$, $p = 0.4349$, respectively). "Taste", "Flavor" and "Appearance" were all highly significant ($p < 0.0001$). Fischer's F-protected LSD was used to determine mean separation for these three variables. Complete LSD groupings of all cultivars for each variable are given in Tables 2.5-2.7. Because of high variance, few significant differences were detected. At best, cultivars were separated into two or three groups. It is puzzling that taste, flavor and appearance could be separated into significantly different classes by a group of tasters who would give them all essentially the same overall score. A larger rating scale may have highlighted

Table 2.5 Flavor Ratings. Means associated with the same letters are not significantly different. Solid line separates high and low rated groups.
$\alpha = 0.05$ LSD = 0.6595
** indicates cherry type cultivar
+ indicates yellow or orange cultivar

LSD Grouping	Mean	N	Cultivar		
A	2.94	18	Sweet 100		**
B A	2.90	19	Large Red Cherry		**
B A C	2.86	21	Brandywine		
B D A C	2.83	31	Champion		
B D A C	2.80	35	Big Beef		
E B D A C	2.77	31	Early Girl		
E B D A C	2.76	21	Big Girl		
E B D A C	2.75	32	Park's Whopper		
E B D A C	2.71	35	Better Boy		
E B D A C	2.68	22	Brandywine Yellow	+	
E B D A C F	2.64	22	Flor-America		
E B D A G C F	2.63	32	Better Bush		
E B D H A G C F	2.57	35	Early Cascade		
E B D H A G C F	2.55	31	Celebrity		
E B D H A G C F	2.41	29	Patio		
E B D H A G C F	2.38	29	Amish Paste		
E B I D H A G C F	2.37	35	Beefmaster		
E B I D H A G C F	2.37	30	Super Fantastic		
E B I D H G C F	2.27	26	Heartland		
E B I D H G C F	2.27	30	Golden Boy	+	
E B I D H G C F	2.25	16	Juliet		**
E I D H G C F	2.20	35	Husky Cherry Gold	+	
E I D H G F	2.19	36	Husky Gold	+	
E I H G F	2.13	30	Lemon Boy	+	
I H G	1.97	36	Miracle Sweet		
I H G	1.97	29	Keepsake		
I H	1.93	15	Yellow Pear	+	**
I	1.71	7	Red Robin		**

Table 2.6 Taste Ratings. Means associated with the same letters are not significantly different. Solid line separates high and low rated groups. $\alpha = 0.05$ LSD = 0.699

** indicates cherry type cultivar

\+ indicates yellow or orange cultivar

LSD Grouping	Mean	N	Cultivar		
A	2.94	18	Sweet 100		**
B A	2.89	19	Large Red Cherry		**
B A	2.88	32	Park's Whopper		
B A	2.86	22	Flor-America		
B A	2.86	21	Brandywine		
B A	2.81	22	Brandywine Yellow	+	
B A C	2.81	31	Champion		
B A C	2.81	31	Early Girl		
B A C	2.80	35	Big Beef		
B A C	2.80	35	Better Boy		
B D A C	2.71	35	Early Cascade		
E B D A C	2.67	21	Big Girl		
E B D A C	2.59	32	Better Bush		
E B D A C	2.56	16	Juliet		**
E B D A C	2.50	30	Golden Boy	+	
E B D A C	2.48	31	Celebrity		
E B D A C	2.48	29	Amish Paste		
E B D A C	2.37	30	Super Fantastic		
E B D A C	2.33	30	Lemon Boy	+	
E B D A C	2.31	35	Beefmaster		
E B D A C	2.31	29	Patio		
E B D A C	2.31	26	Heartland		
E B D A C	2.28	36	Husky Gold	+	
E B D F C	2.20	35	Husky Cherry Gold	+	
E D F C	2.11	36	Miracle Sweet		
E D F	2.03	29	Keepsake		
E F	2.00	16	Yellow Pear	+	**
F	1.57	7	Red Robin		**

Table 2.7 Appearance Ratings. Means associated with the same letters are not significantly different. Solid lines separate high, medium and low rated groups. $\alpha = 0.05$ LSD = 0.644
** indicates cherry type cultivar
+ indicates yellow or orange cultivar

LSD Grouping							Mean	N	Cultivar		
				A			3.61	18	Sweet 100		**
	B			A			3.44	16	Juliet		**
	B			A			3.41	22	Brandywine Yellow	+	
	B			A		C	3.37	19	Large Red Cherry		**
	B	D		A		C	3.36	36	Husky Gold	+	
	B	D		A		C	3.30	30	Golden Boy	+	
E	B	D		A		C	3.19	31	Early Girl		
E	B	D		A		C	3.14	35	Early Cascade		
E	B	D		A		C	3.14	35	Husky Cherry Gold	+	
E	B	D		A		C	F	3.12	16	Yellow Pear	+	**
E	B	D		A		C	F	3.09	32	Park's Whopper		
E	B	D		A		C	F	3.09	35	Big Beef		
E	B	D		A	G	C	F	3.06	35	Better Boy		
E	B	D		A	G	C	F	3.06	36	Miracle Sweet		
E	B	D		A	G	C	F	3.03	31	Champion		
E	B	D		A	G	C	F	2.97	32	Better Bush		
E	B	D			G	C	F	2.97	30	Lemon Boy	+	
E	B	D	H		G	C	F	2.90	30	Super Fantastic		
E	B	D	H		G	C	F	2.87	31	Celebrity		
E		D	H		G	C	F	2.73	26	Heartland		
E		D	H		G		F	2.72	29	Patio		
E	I		H		G		F	2.64	22	Flor-America		
E	I		H		G		F	2.60	35	Beefmaster		
	I		H		G	J	F	2.48	29	Amish Paste		
	I		H		G	J		2.43	21	Big Girl		
	I		H			J		2.31	29	Keepsake		
	I					J		2.05	21	Brandywine		
						J		1.86	7	Red Robin		**

differences better. In hindsight, it would probably have been better to limit the number of tomato cultivars tested so that each tester could feasiblely sample every cultivar. This high variance, not wholly unexpected in a human opinion experiment, makes conclusions about the data difficult.

Sweet 100 captured the highest mean score for taste, flavor and appearance, although these values were not significantly different from many others (Fig 2.3). Likewise, Red Robin scored lowest in all three categories, but again, the differences were not significant from all others (Figure 2.4, Table 2.5-2.7). This could be a statistical fluke, but it would be a very consistent fluke. Nonetheless, more testing is required for this assessment to be accepted as legitimate.

Mean ratings for all tomatoes were: Taste = 2.52, Flavor = 2.47, Appearance = 2.96. The relationship between flavor and taste was strong ($R^2 = 0.91$), such that future tests could easily combine these two categories for simplicity. Fruit appearance, size and color did not significantly influence effect on taste or flavor ratings ($R^2 < 0.34$). Likewise, flavor, appearance and taste were not correlated to growing degree day requirement or water content ($R^2 < 0.48$). The overall taste quality of a tomato is an interaction between sugar and acid, so it is not surprising that sugar alone, measured by soluble solids, did not correlate to flavor or taste ($R^2 < 0.45$).

The astuteness of the tasters was in some ways validated by some of the close ratings of similar cultivars. For example, when rating taste, Brandywine was ranked just above the related cultivar Brandywine Yellow, with a difference of only 0.039 on a four point scale, despite the fact that these cultivars look nothing alike (Table 2.6). Also on

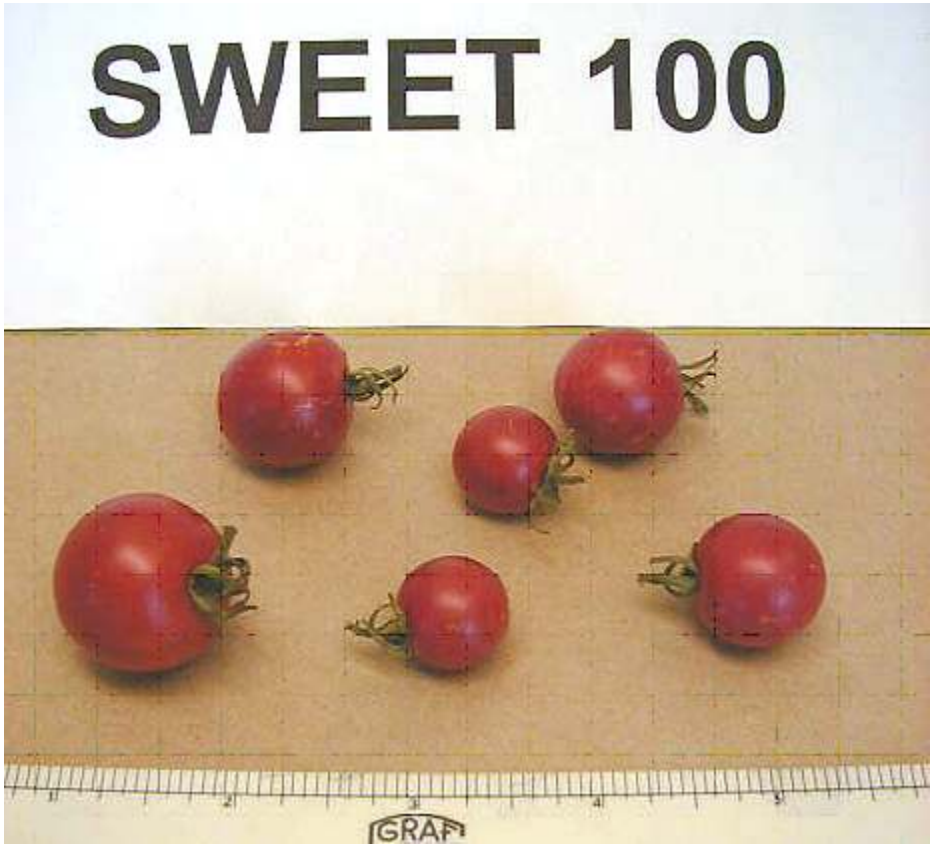


Figure 2.3 Sweet 100 tomato cultivar

Figure 2.4 Red Robin tomato cultivar

the taste ratings, Husky Gold ranked just one above Husky Cherry Gold, with a difference of 0.078. In the flavor ratings, Husky Cherry Gold ranked just one higher than Husky Gold, with a difference of just 0.0166 (Table 2.5). It is unlikely that the two cultivars were ranked so closely in two categories based on chance. It is remarkable that two cultivars which look different but come from similar backgrounds would be rated so closely on flavor and taste, especially since their mean appearance ratings differed by 0.2182 (as compared to the low differences in taste and flavor ratings). One can't help but conclude that the tasters were diligently rating the cultivars.

Based on the LSD separation, the cultivars could be divided into two approximate groups for flavor and taste, and three groups for appearance. Most cultivars tested were traditional medium to large sized red fruited types, and only a handful were a color or size other than this. These warrant special consideration.

Cherry Type Tomatoes

Five cultivars produced cherry type tomatoes: Sweet 100, Large Red Cherry, Juliet, Yellow Pear and Red Robin. In the flavor ratings, cherry type tomatoes occupied the two highest and the two lowest spots, with a total of 40% in the high rated group, and 60% in the low rated group. In the taste category, the situation was nearly the same: the two highest and two lowest spots were occupied by cherry-type tomatoes, with 60% in the high rated group. The appearance rating was skewed more in favor of cherry type tomatoes, with 80% in the high rated group, 0% in the medium rated group and 20% in the low rated group. Additionally, the top and bottom slots were reserved for cherry

types. As far as taste and flavor are concerned, there is no difference between cherry type fruit and traditional size fruit, however, it does appear that people consider the appearance of cherry-type fruit above average. The only exception is Red Robin, which was rated poorly in all categories, and whose appearance rating was perhaps downgraded in light of its despicable flavor. Overall size (including medium tomatoes) was not significantly correlated to any taste test variable.

Orange and Yellow Tomatoes

Another small subsection of the field grown cultivars that warrant individual consideration are the non-red cultivars. This six-member group is comprised of orange cultivars: Golden Boy, Husky Gold and Husky Cherry Gold; and yellow cultivars: Brandywine Yellow, Lemon Boy and Yellow Pear. The flavor of these cultivars was generally rated poor, as five of the nine lowest-rated cultivars were from this group. Only Brandywine Yellow was in the high rated group. In the taste rating, the story is almost the opposite. Of these cultivars, 66% were in the high rated group, and 33% in the low, although Husky Gold and Husky Cherry Gold are both on the dividing line between the two groups. Appearance of these cultivars was rated favorably, with all but one in the high rated group, and even then the lone standout, Lemon Boy, was the highest rated of the medium rated group. No yellow or orange cultivars were placed in the low rating group, although admittedly, this group is very small because of the LSD groupings. In no case was one color or the other shown to be superior. Of all six cultivars, Brandywine

Yellow scored highest in all three categories, although this difference was not always significant. Fruit color overall was not correlated to appearance, flavor or taste ($R^2 < 0.1$), despite apparent relationships described above.

CONCLUSIONS

Some cultivars commonly grown in northen Colorado are better suited to the growing season and conditions than others. Yield, water content and refractive index of fruit varied by cultivar.

Black Krim, Black Plum and Black Tula expend more energy on vegetative growth than fruit production relative to Celebrity. All three black cultivars are highly susceptible to blossom end rot, but this can be remedied by regular watering and fertilizer application.

Variance in taste tests runs very high, and very large sample sizes are required in order to achieve abundant significant mean separation. This study was able to produce some mean separations, and some cultivars were shown to be better than others. Although not statistically significant, Sweet 100 had the highest score and Red Robin had the lowest score in all categories with significant differences. Yellow and orange cultivars were rated above average on appearance, but well below average on flavor. Neither orange nor yellow was shown to be better, in any way, than the other. Cherry type cultivars were rated very favorably in appearance, but were polarized strongly on taste and flavor ratings, occupying the two highest and two lowest spots.

CHAPTER 3

HPLC ANALYSIS OF LYCOPENE IN TOMATO UTILIZING FREEZE-DRIED SAMPLES

INTRODUCTION

Lycopene is an open-chain unsaturated hydrocarbon carotenoid present in certain organs of many plants which has a structure similar to vitamin A (Ngyuen & Schwartz 1999). The conjugated double bonds serve as chromophores, and several carotenoids are yellow to red in color. Lycopene is orange-red in color, and is the major pigment associated with the red color of tomato, hence the name (Lyco as in *Lycopersicon esculentum*) (Wann & McFerran1960, Schunk 1903).The importance of lycopene was originally assigned to aesthetics only, but has recently been shown to confer a variety of health benefits to humans, including prevention of several types of cancer and reduced risk of heart disease (Frankceshi et al. 1994, Giovanucci et al. 1995, Kohlmeier et al. 1997). Lycopene is a powerful antioxidant which quenches free radicals and thus prevents oxidative damage to phospholipids of cell membranes, vital proteins and DNA (Di Mascio et al. 1989). Studies of lycopene require sophisticated analytical techniques due to the difficulty in accurately separating carotenoids (Bushway & Wilson 1982). The ability to accurately quantify the

amount of lycopene present in any plant product is vital for further enhancement of this desirable carotenoid.

Breeding high-lycopene tomato cultivars has been a goal of many plant breeders, both for enhanced health benefit and for the increased red pigment conferred to such cultivars (Thompson et al. 1967). Recently, genes controlling key carotenoid synthesis enzymes have been cloned and utilized in the transgenic enhancement of β-carotene, a carotenoid derived from lycopene, in rice and tomato as well as increased lycopene content in *E. coli* (Xudong et al. 2000, Romer et al. 2000, Farmer et al. 2000). To monitor such advancements, a multitude of lycopene analysis methods have been published, with the majority focusing on high performance liquid chromatography (HPLC) because of its precision and ease of use relative to previous analytical techniques (Bushway & Wilson 1982).

Lycopene analysis often employs time and labor-intensive analysis techniques including homogenization (Khachik et al. 1992), vacuum-filtration (Alba et al. 2000), saponification (Heinonen et al. 1988), rotary-evaporation (Schierle et al. 1997) and a number of other time-intensive procedures. No single method has been adapted and proven superior to others, and new techniques are constantly being designed. Most published methods are suitable for accurate and careful analysis of a limited number of samples (Zakaria et al. 1979, Heinonen et al. 1988, Gartner et al. 1997). Lycopene degrades very easily in the presence of light, heat and oxygen (Ngyuen & Schwartz 1999). Fresh tissue analysis requires the experiment to be carried out within a very narrow time frame, often of only one or two days, before natural degradation of the product confounds results. In agricultural studies, however, when fruit ripens all at once, analysis must either be done quickly on a massive

scale, or some method for preserving samples until they can be analyzed must be introduced. The logistical problems associated with rapid analysis on a massive scale require the implementation of an alternative approach to accommodate hundreds of samples.

There has been a definite focus in carotenoid research on analysis of fresh tissue, but for several reasons an alternative using freeze-dried tissue may be advantageous. First, working with replicates of a fresh product forces accurate analysis to be carried out within a narrow time period to avoid product deterioration. A fresh sample cannot be re-analyzed at a later date. In addition to the logistical problems associated with fresh-tissue analysis, considerable effort must be made to break down the intact cellular matrix when extracting cellular constituents (Zakaria 1979). Additionally, fresh samples vary widely in water content, and this can be a large source of variation in the analysis of a particular compound when analyzed on a per fresh-weight basis, as lycopene usually is.

Here we present a method for analysis of lycopene in tomatoes which utilizes freeze drying to stabilize samples so that they may be stored and processed over a longer period of time than would normally be feasible with fresh fruit. It solves the problems of analysis bottlenecks at peak times of the year, variation due to cultivar water content differences, degradation of lycopene in fruit waiting to be analyzed and difficulty in extraction from an intact cellular matrix.

MATERIALS AND METHODS

Sample preparation

Fresh tomatoes were freeze-dried to dryness in a Genesis 25LL Lyopholizer (Virtis Co., Gardiner, NY, USA) before being finely ground and sieved through a 100 μm pore-size screen with a sieve shaker (CSC Scientific, Fairfax, VA, USA). The freeze-drying protocol varied due to batch sizes and the fruit size, but generally consisted of one week of ramping the temperature up steadily from -40°C to 25°C under vacuum. The resultant fine powder was stored in # 4 dram glass vials under nitrogen to prevent oxidation, with a foil-lined lid at -20°C until analysis.

Lycopene extraction

Approximately 0.05 g of tomato powder (weight measured exactly to 1/1000 g) was added to 10 ml of ACS grade acetone in a 250 ml glass beaker. The solution was agitated gently for 1 minute, followed by vacuum-filtration through a 5.5 cm Whatman #2 paper filter. The filter paper was transferred to the original 250 ml beaker along with 10 ml acetone. The solution was agitated and filtered as before. This step was repeated for a total of 4 filtrations of the powder. A total of 40 ml acetone was used, but evaporation caused the final solution to be less than 40 ml. Volume of solution was noted before injection into the HPLC. All steps were performed in subdued light, as suggested by Thurnham et al. (1988).

HPLC system parameters

Samples were run with a Dionex DX-300 Series HPLC equipped with a 15 μl automatic injection loop, a Zorbax RP300-C18 reversed phase column (particle size: 5 μm;

25 cm × 4.6 mm I.D.), RP300-C18 guard column and a variable wavelength detector (Dionex, Sunnyvale, CA, USA). Wavelength for lycopene detection was 472 nm, as used in other studies (Hess et al. 1990, Zakaria et al. 1979, Schierle et al. 1997, Stakowicz-Sapuntikas et al. 1987, Sharma & LeMaguer 1996, Baura & Furr 1992). The column was kept at 25°C with an Eppendorf CH-460 column heater (Brinkman, Westbury, NY, USA). All other connections were made with polyetheretherketone tubing and connectors (Dionex). Four chemicals comprised the mobile phase: acetonitrile, acetone, hexane and methanol (Fisher, Fair Lawn, NJ, USA). The eluent program was as follows: From 0-4 min, acetonitrile, methanol and hexane (75:15:10) were run isocratically, followed by a linear increase in acetone from 0% to 100% of the eluent mix from 4-5 minutes. Acetone was run isocratically at 100 % from 5-8 minutes, then ramped down to 0% by minute 9 with the original isocratic mix replacing the acetone at the end of 9 minutes, and running until the end time of 10 minutes. Flow rate was 1.0 ml min^{-1} throughout.

Quantification of lycopene

An external standard method was used to quantify lycopene in samples. Pure lycopene (Indofine Chemical Co., Somerville, NJ, USA) was prepared as a standard in acetone at 1 x 10^{-4} M, from which dilutions were prepared in ten molar concentrations: $5e^{-5}$, $1e^{-5}$, $7.5e^{-6}$, $5e^{-6}$, $2.5e^{-6}$, $1e^{-6}$, $7.5e^{-7}$, $5e^{-7}$, $2.5e^{-7}$ and $1e^{-7}$. All standards were immediately injected three times into the HPLC. Peak areas calculated by AI-450 Chromatography Software (Dionex, Sunnyvale, CA, USA) were used to construct a regression curve using the reg procedure in SAS v7 (SAS, Nashville, TN, USA). Subsequent runs were quantified using

the regression equation in Quattro Pro v7 (Corel, Mississauga, ON, Canada).

Validation of extraction method

The effects of adding quantities of acid to the extraction solvent were investigated by adding acetic acid to the acetone used for extraction. 1M and 4M acid solutions were prepared. Triplicate extractions of the tomato cultivar "Sweet 100" were analyzed with HPLC. Petroleum ether was also tested against acetone for extraction efficiency, again with triplicate extractions of the cultivar "Sweet 100". In addition, the number of acetone flushes required to extract 99% of lycopene was evaluated by running each individual 10ml acetone flush on the HPLC and calculating the percent of total lycopene.

Recovery was tested by spiking a dry powder tomato sample with pure lycopene and analyzing the resultant HPLC data to determine % recovery, calculated with the equation: %Recovery = ((S - U) x 100) / Sp , where S = analyte concentration in spiked sample, U = analyte concentration in unspiked sample and Sp = concentration of spike. Dry tomato powder of the common cultivar “Champion” was used for all recovery experiments. The amount of lycopene in the sample to be spiked was quantified, and that amount was artificially added to the sample prior to extraction in 1X and 3X quantities. The amount of lycopene in the sample to be tested was 50 μg (n = 5), so the 1X spike was 50 μg and the 3X spike was 150 μg. Five replicate extractions of unspiked, 1X spike and 3X spike were performed within the same week and injected four times each into the HPLC using the aforementioned method.

Sharma & LeMaguer (1996) published an efficient method for extraction of lycopene

from fresh tomatoes with similarities to the dry powder method reported here, thus making it suitable for direct comparison to our method. Instead of freeze-drying their tomato samples, they canned them for later analysis, thus solving the problem of analyzing large quantities of perishable and degradation-prone samples. A comparison of the two methods was carried out with three replicate extractions of a store-bought tomato, cultivar unknown, with four replicate injections for each extraction (n = 12). The store bought tomato was divided into two halves. The first half was analyzed with the published method and the lycopene calculated as mg/g fresh weight. The second half, weighing 117.3 g, was freeze-dried and analyzed using our method to report lycopene concentration in mg/g dry weight. To compare fresh weight concentration with dry weight concentration, they were each multiplied by fresh weight and dry weight, respectively, to normalize lycopene into mg, thus rendering the two values directly comparable.

Stability of the freeze dried tomato powder was tested with the same sample used for method comparison. Three replicate extractions using the proposed method on one sample were injected four times each in the HPLC (n = 12). The powder was then stored under nitrogen at -20°C. Thirty days later, the process was repeated exactly on the same sample. Stability of freeze-dried powder was also tested at 4°C for two weeks using four tomato samples which were run before and after the two week period.

Lycopene concentration of extracts from five samples stored at -20°C in plastic parafilm-sealed tubes was evaluated at 3 and 6 months after extraction to assess the stability of lycopene in the extract. Five sample extracts were run 3 months after extraction and another five were run 6 months after extraction. Volume loss due to evaporation did not

occur.

Statistics

T-tests of mean differences for all experimental data sets were carried out using the ttest procedure in SAS v7 (SAS Institute, Nashvillle, TN).

RESULTS AND DISCUSSION

Quantification of lycopene

Elution of lycopene occurred at 2.5 ± 0.2 min. (Fig 3.1). Lycopene was the only peak detected at 472 nm from the tomato extract. Few other carotenoids are present in tomatoes, with the only other significant carotene being β-carotene, at ≈14% of total carotenoid content (Romer et al. 2000). Related carotenoids α-carotene and zeaxanthin are not found in tomatoes (Romer et al. 2000). β-carotene absorbs at 450 nm, and eluted at a different time using this method (data not reported), therefore, accidental measurement of β-carotene as lycopene did not occur. Lycopene makes up 95-100% of

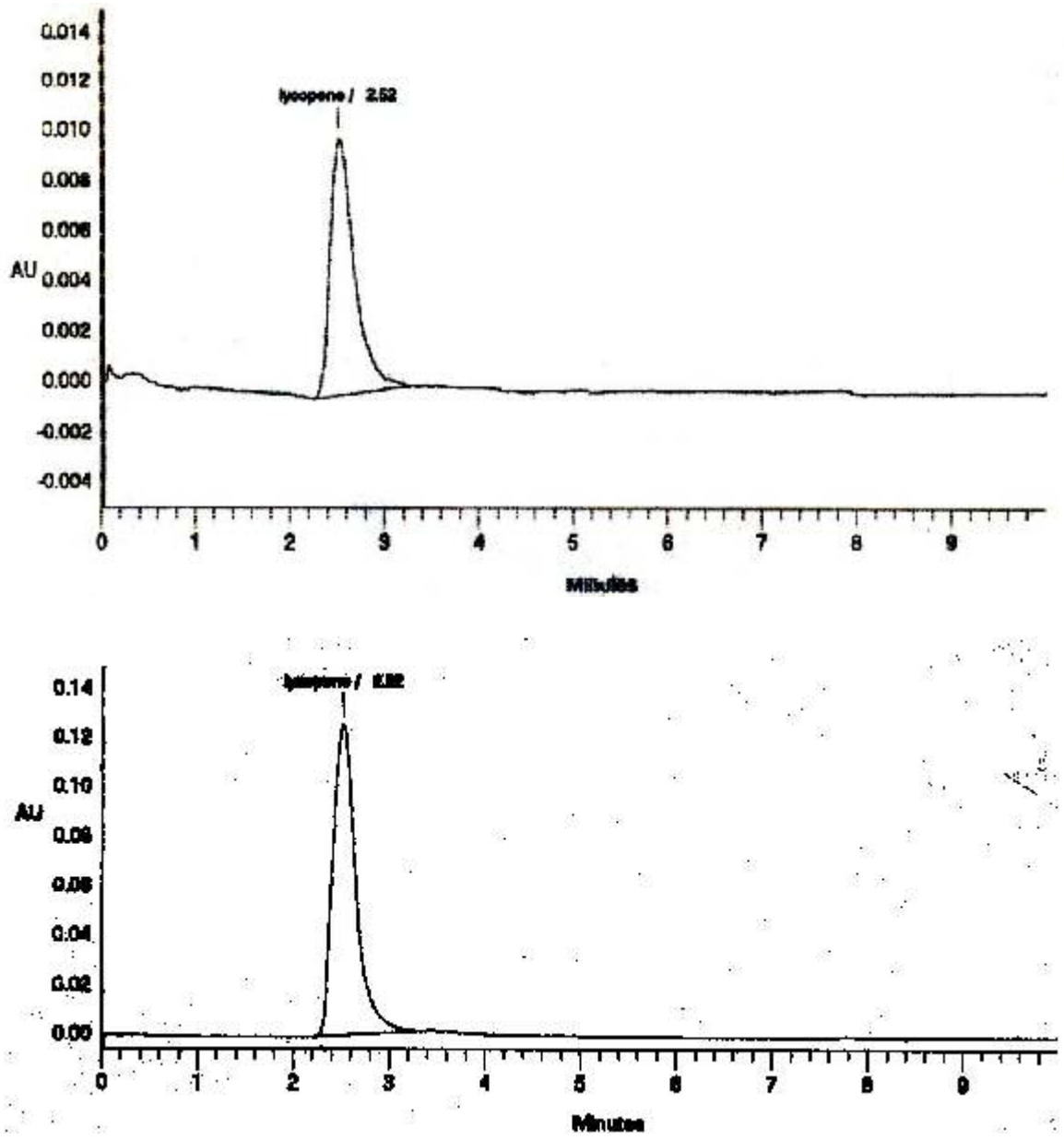


Figure 3.1 HPLC chromatogram of lycopene peak from tomato sample prepared using the dry-powder method (top, peak mass = 0.35mg) and purified lycopene standard (bottom, peak mass = 0.46mg).

the total carotenoid content in the currant tomato, *Lycopersicon pimpinellifolium* (Nyguen & Schwartz 1999). Using this method, it is impossible to rule out other carotenes being present in the sample, however, they would not absorb strongly at 472 nm, thus we are reasonably assured that the peak is primarily lycopene. The isocratic mobile phase mix without acetone eluted lycopene in the same manner, but the acetone was found to be necessary to eliminate residual lycopene on the column prior to the next run.

The R^2 for the regression of analyte peak area on molar concentration of the standards was 0.9948 with a coefficient of variation (cv) of 10.5% over the entire range. Over the range that all tested tomato samples fell into, $1e^{-6}$ to $1e^{-5}$ M, the cv was 5.06%. For comparable methods, cv of 2.1, 6.4, 7.4 and 15.8 were reported (Hess et al. 1990, Stacewicz-Stapuntikas et al. 1987, Thurnham et al. 1988, Barua & Furr 1992). The resulting line equation for all 30 experimental samples was: $x = (y + 742.1) / 2.22e^{9}$, where x = molar concentration of lycopene and y = analyte peak area. The strong linear response of lycopene in the HPLC system provided a sturdy platform by which to quantify the analyte.

The sensitivity of the system produced quantifiable lycopene peaks at 0.1 μg/L, which is several times more sensitive than required for analysis of yellow tomatoes, which have lower lycopene content than red cultivars. While not as impressive as the sensitivity of Zakaria et al. (1979) which could detect lycopene at 0.00395 ng/L, it is greater than a similar effort by Gartner et al. (1997) which could detect as low as 10 μg/L.

Validation of method

Adding acid to the acetone had no significant effect on extraction efficiency ($p = 0.1557$, $n = 3$). Extraction with petroleum ether was not significantly different from that with pure acetone ($p = 0.1154$, $n = 3$). Published methods typically utilize a variety of chemicals, such as ammonium acetate, 1-4 Dioxane (Hess et al. 1990), dichloromethane (Zakaria et al. 1979), tetrahydrafuran (Bushway & Wilson 1982), methanol, hexane (Tomes 1963), isopropanol, diethyl ether (Heinonen et al. 1988), petroleum ether (Ellis & Hamner 1943), potassium hydroxide (Heinonen et al. 1989) and acetone (Schierle et al. 1997). No single solvent has proven to be superior to another for extraction of carotenoids. These results help explain why multiple solvents are still used in carotenoid extraction. Acetone is preferred over others because it is less toxic and can be distilled and reused to reduce hazardous waste treatment costs.

Recovery with 1X spike samples was 108.6% ± 9.97% ($n = 5$), while recovery with the 3X spike samples was 92.9 ± 7.70% ($n = 5$). Overall recovery was 100.75% ($n = 10$). This suggests that there was no loss of lycopene during freeze-drying or extraction, a finding consistent with that of Shi et al. (1999). Comparable methods reported recoveries of 97.3% and 90-94% for tomato lycopene recovery (Hess et al. 1990, Schierle et al.1997). There is some concern that stainless steel frits and injection loops interact with carotenoids and degrade them (Nierenberg & Lester 1986, MacCrehan & Schonberger 1987). One study concluded that while steel frits inhibited recovery of carotenoids, steel injection loops did not (Scott 1992). As a precaution, our system uses only polyetheretherketone frits and tubing, excluding the steel-jacketed column.

Table 3.1 Published values for lycopene content in tomatoes (mg $100g^{-1}$ FW)

Lycopene (mg $100g^{-1}$ FW)	Reference
8.74	Zakaria et al. 1979
3.35	Al Wandowi et al. 1986
3.1	Heinonen et al. 1989
3.92	Khachik et al. 1992
4.2	Mangels et al. 1993
9.27	Tonucci et al. 1995
5.6	Gartner et al. 1999
6.4	Shi et al. 1999
3.1 - 7.7	Ngyuen & Schwartz 1999

Our analysis of the cultivar "Sweet 100" yielded a lycopene content of 4.37 ± 0.22 mg $100g^{-1}$ FW. This figure falls within published values for tomatoes (Table 3.1), although differences in cultivars, ripeness of fruit and growing conditions would tend to introduce variation. "Sweet 100" is a cultivar of average solids content, and should be roughly comparable to most cultivars used in other studies. Sharma & LeMaguer (1996) reported a lycopene concentration of 12.46 ± 0.37 mg $100g^{-1}$ FW, which is much higher than ours or those listed in Table 3.1, but was derived from high-solids processing cultivars which would logically contain more lycopene. An inherent advantage of our method, and that is the elimination of analysis variation due to water content. Tomato cultivars vary widely in their solids content, and tomatoes with higher solids content will contain more lycopene per unit fresh weight. By freeze-drying the tissue first, lycopene can be measured per unit dry weight, and cultivar variability due to water content will be eliminated. A report of lycopene content in terms of dry weight may be more useful to processors since tomatoes are most often dehydrated to some extent during processing.

Final yields of lycopene from the same fruit were significantly different ($p = 0.0018$, $n = 12$) when comparing the dry powder method with that of Sharma & LeMague (1996). Our method extracted 19.5% more lycopene from the same sample (Table 3.2). This could be due to using finely-ground powder which would logically facilitate extraction. Excluding lyopholization time, our method requires less time for extraction than does Sharma & LeMaguers' (five minutes vs 40 minutes). Similarly, the analysis time using our method is less than most other published methods (Zakaria et al.1979, Heinonen et al.

Table 3.2 Lycopene content analyzed by a dry powder method compared to a freshfruit method. Lycopene content was calculated in mg/g from the external standard-generated regression equation. One fresh tomato weighing 117.3 g was analyzed using the fresh tissue method, then freeze-dried to 6.9 g before analysis with dry tissue method. The FW and DW concentrations were multiplied by FW and DW respectively to normalize data into mg which could be directly compared. Final lycopene means were significantly different ($p = 0.0018$, $n = 12$).

Lycopene (mg g^{-1})	Original Sample wt. (g)	Total Lycopene (mg $sample^{-1}$)	Mean ± S.D. (mg)
Dry Powder Method			
0.984		6.78	
0.847	6.9	5.84	6.12 ± 0.57
0.833		5.75	
Fresh Fruit Method			
0.043		5.04	
0.042	117.3	4.93	5.12 ± 0.24
0.046		5.39	

($p = 0.1531$, $n = 12$). This validates the stability of the freeze-dried powder, stored as described, for at least 30 days. On the other hand, lycopene in powder stored at 4°C for two weeks degraded significantly in four samples ($p < 0.005$, $n = 12$) (Fig 3.2). From this it is clear that 4°C is not suitable for freeze-dried powder storage. Zanoni and Nani (1999) demonstrated that lycopene within the tomato is stable during 4 hours of drying at 80°C, and will only degrade 12% when heated for 4 hours at 110°C. If lycopene does not degrade under this regimen, it will likely be stable during freeze-drying. Short term exposure to above-freezing temperatures did not adversely effect lycopene to a significant degree, but long term exposure was deleterious. The problem is bypassed altogether by keeping samples below freezing as much as possible. This is consistent with a study by Anguelova and Wathesen (2000) which examined degradation of lycopene in tomato powder and used powder stored at -18°C under nitrogen in the dark as a control, thus assuming that powder stored in such a way does not undergo significant lycopene degradation. They concluded that oxidation was the predominant mechanism of lycopene degradation in tomato powders, and temperature played the most important role in promoting oxidation. Maintaining stored samples below freezing is critical to lycopene stabilization.

Unike the lycopene stored in a dry vial, lycopene in acetone solution was very unstable. After 3 months of storage at -20°C, four of the five samples had experienced significant reduction in lycopene, with an average decrease of 11% ($p < 0.05$). After 6 months, four out of five samples also experienced significant reduction with a mean decrease of 32% ($p < 0.005$). Even while stored at low temperature and sealed from the

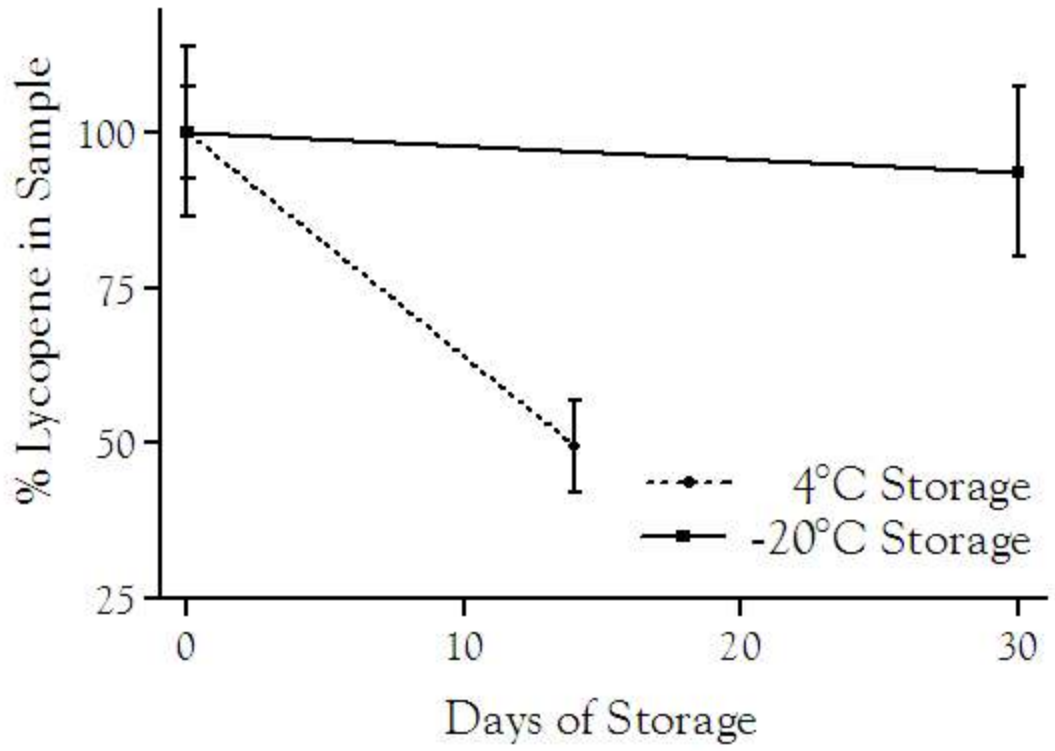


Figure 3.2 Stability of lycopene in freeze-dried tomato powder over time at -20°C and 4°C. Degradation was not significant after 30 days at -20°C ($p = 0.1531$, $n = 12$), but was highly significant after 14 days at 4°C ($p < 0.005$, $n = 12$).

atmosphere, samples appear to be unsuitable for long term storage as an extracted solution. Storage under nitrogen, however, may have significantly reduced lycopene loss. Scott (1992) reported that stock solutions of lycopene in chloroform degraded 20% in 20 days at -20°C. The addition of butylated hydroxy toluene (BHT) lessened that degradation to 11%. Stacewicz-Sapuntzakis et al. (1987) reported conflicting results of lycopene stability in solution: 3 weeks in diethyl ether without significant degradation. Hess et al. (1990) reported that stock solutions of lycopene in benzene could not be held at -20°C for longer than one week due to the tendency of the lycopene to crystallize.

This method was able to detect the presence of lycopene in preliminary work with grapefruit, but not with red potato.

CONCLUSIONS

Tomato powder can be stored for at least 30 days at -20°C under nitrogen without significant loss of lycopene. Extracted lycopene in acetone, however, will degrade even at -20°C within 90 days. The high recovery of the dry powder method, stability of freeze-dried tomato powder, plus evidence of enhanced extraction efficiency compared to extraction from fresh tissue demonstrates this method's analytical validity, and potential for research and large-scale applications.

CHAPTER 4

LYCOPENE DEGRADATION

INTRODUCTION

Lycopene is a very labile compound and, in its pure form, will break down quickly in the presence of light, heat and oxygen (Ngyuen & Schwartz 1999). Within living organisms, lycopene is more stable. The mechanism governing this property is not entirely known, but is most likely the result of water preventing oxygen and heat buildup within cells as well as the presence of even more reactive compounds which absorb the destructive power of light, oxygen and heat before they react with lycopene. Degradation of lycopene in samples presents serious difficulties for researchers seeking an accurate value for lycopene content.

Samples for the lycopene analysis of cultivars were run as quickly as possible, but due to the large number of samples, well over 250, analysis was spread out over more than seven months. Observation of data indicated that lycopene content in replicates of certain cultivars declined over time. Storage conditions of the tomato powder which included darkness, sub-zero temperatures and sealed oxygen-free glass vessels were considered to be more than adequate to forestall oxidation of lycopene. Indofine

Chemical Company (Somerville, NJ), from which lycopene standard was purchased, ships and advises storage of their product in the same manner. That the storage of the powder would lead to degradation of lycopene was not anticipated, and not tested beforehand so unlikely did it seem.

This set of experiments was designed to provide information on suitable storage conditions and degradation patterns of lycopene in freeze-dried tomato powder as a follow-up to the method development of the previous chapter. Additionally, the effect of temperature on degradation was examined. The hypotheses formed through qualitative observation were that the tomato powder was not stable for longer than thirty days, bags were less suitable than glass tubes for sample storage, and lycopene degradation would accelerate at 4°C relative to -20°C.

MATERIALS AND METHODS

Lycopene Degradation

Two samples, Champion and a store-bought tomato (cultivar unknown), were each analyzed for lycopene, stored at -20°C for thirty days, then analyzed again and compared to their previous lycopene value in order to assess if any degradation occurred during the thirty day interval. This experiment is detailed in Chapter Three, but will be included here as well because of its relevance.

Suspected degradation of lycopene in samples while stored in plastic bags was investigated by splitting ten samples known to have spent minimal time in plastic bags after freeze-drying (1-3 months) into two groups: one half continued to be stored in a

glass tube as described above, while the second half was replaced in a plastic bag. Both sets of samples were stored in a box at -20°C for 90 days, then analyzed as described above.

A third experiment was initiated to determine the stability of lycopene in dried powder at 4°C for two weeks. Samples were analyzed, stored in tubes under nitrogen and placed in a 4°C dark cooler for two weeks. Follow up analyses took place on the 15th day, and were compared to the original values.

Statistics

SAS v8 (SAS Institute, Nashville, TN) and Graph Pad Prism (Intuitive Software, San Diego, CA) were used for statistical analysis and graphing, respectively.

RESULTS AND DISCUSSION

The observation of lycopene degradation in dried tomato powder was followed up by examination of the data. Collection of over 15 replicates of Juliet, Sweet 100 and Early Cascade allowed a more objective study. Each cultivar had one or more replicates analyzed for lycopene in March while the majority of replicates for all three cultivars were analyzed in August or September. Comparing the lycopene content of both early and later analyses, using samples from the same cultivar (grown under the same conditions and picked during the same month) showed clearly that lycopene in the dried tomato powder declined significantly over time for all three cultivars ($p < 0.0001$, $n \geq 16$) (Fig 4.1).

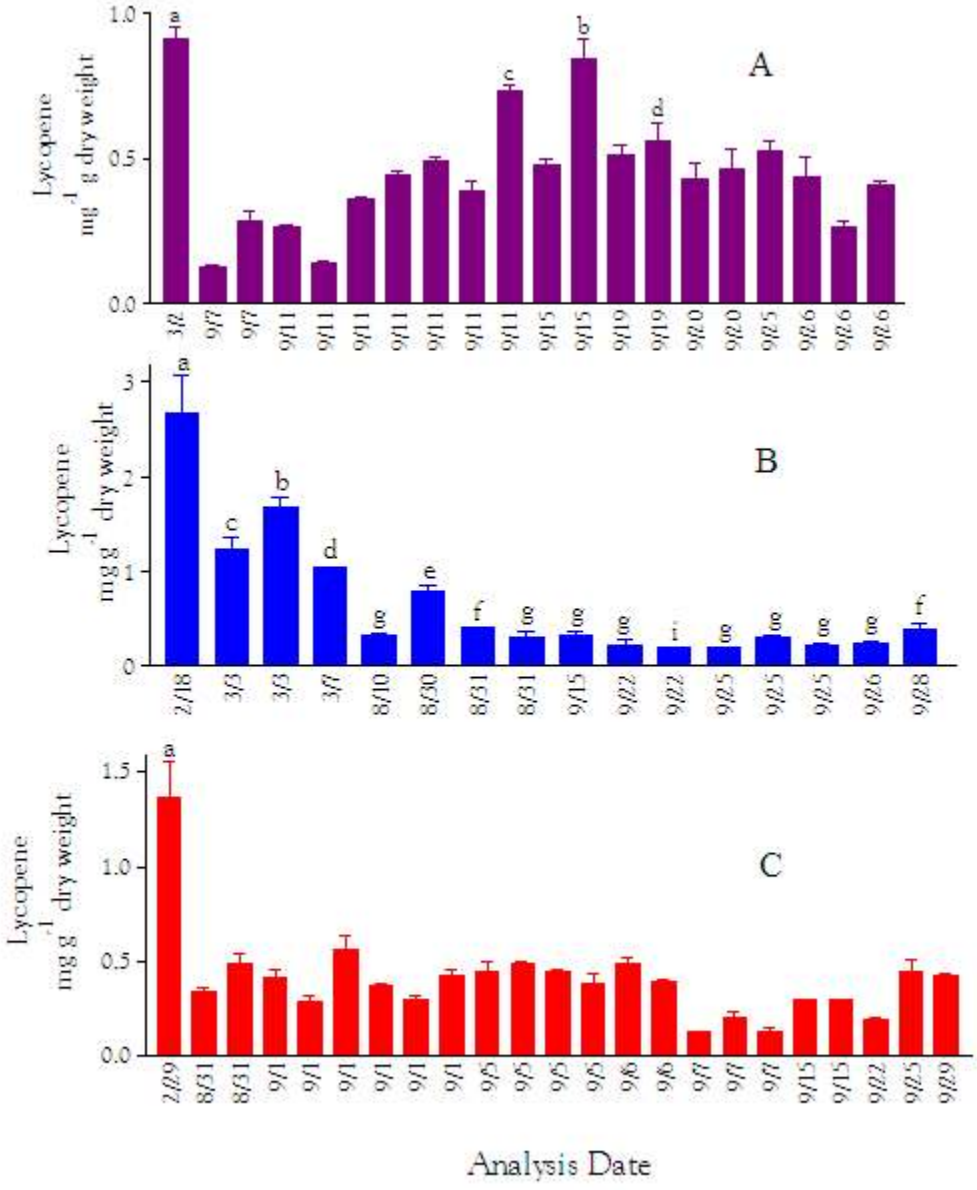


Figure 4.1 Lycopene content of Juliet (A), Sweet 100 (B) and Early Cascade (C) (with SD) over analysis date. All samples were tomatoes picked in August or September, 1999 from a field planting. All samples were analyzed in 2000, with the precise analysis date noted on the X-axis. Analysis of variance shows significant differences among samples in all three groups ($p < 0.0001$). Lower case letters over means indicate significant difference from other letters. In some cases, lower means are not labeled, but are nonetheless significantly lower than labeled means.

This result was not due to variation within the HPLC system since samples of Celebrity grown in 2000 and analyzed after only a couple of months showed lycopene peaks significantly larger ($p = 0.0267$) than samples from the same cultivar grown in 1999 and analyzed in 2000 (Fig 4.2). The two Celebrity 1999 samples analyzed on July 7, 2000 were much higher in lycopene than the last replicate analyzed on September 9, 2000. Another comparison would be to compare the last 1999 replicate with the 2000 samples since all five were analyzed at roughly the same time. If this is done, the difference between the two years is even more dramatic. Had the sensitivity of the HPLC diminished, then the 2000 samples of Celebrity would have shown peaks of the same size as the older samples. This indicates that the difference in lycopene content between the two years' samples cannot be attributed to the HPLC system.

To prove the observation that reduced lycopene levels in stored samples was caused by degradation, an experiment was run in which the lycopene content of Champion and a store-bought tomato (stored as described) were compared to their own lycopene content thirty days later. Champion exhibited significant degradation after thirty days ($p = 0.0087$, $n = 12$) in comparison to the store-bought sample which showed none ($p = 0.1531$, $n = 12$). The conflicting results may be due to the Champion sample being utilized heavily in the recovery tests (as described in Chapter Three) with inadequate nitrogen refilling between tests. This would lead to oxidative damage to the lycopene. The store-bought sample was opened only once, allowing little opportunity for oxygen to enter the sample tube. Another reason to suspect the Champion sample is that it had been stored for over seven months while the store bought sample had been stored

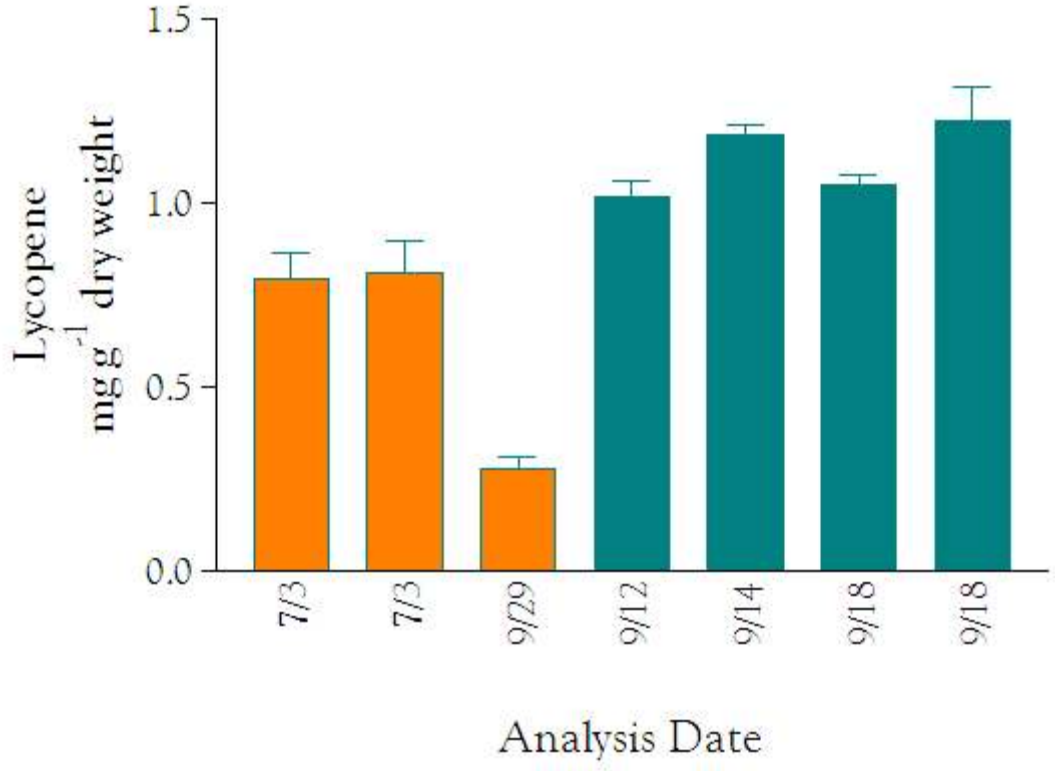


Figure 4.2 Lycopene levels of Celebrity tomatoes picked within a two week window in 1999 from a field planting (orange) and 2000 from a greenhouse planting (blue). Error bars are SD. All samples picked in 1999 and 2000 were analyzed in July and September of 2000, as noted on the X-axis. Means for the 1999 ($n = 3$) and 2000 ($n = 4$) samples are significantly different ($p = 0.0267$). This shows that lycopene degraded in samples stored for a year prior to analysis, and that the HPLC system had not lost sensitivity over time.

less than one. This suggests that lycopene may be stable for thirty days at the beginning of storage, but that degradation rapidly accelerates afterwards. This would explain why the Champion sample experienced high degradation relative to the store bought sample over the same time frame. The results obtained from using the Champion sample may be inaccurate to describe early storage degradation, but no reason exists to consider the store-bought sample results invalid. These results show that lycopene is indeed stable in tomato powder for at least thirty days, but may not be free of degradation for long periods of time. Time constraints prohibited a more thorough investigation of this.

Sample freeze-drying did not occur simultaneously, nor did grinding or sieving, therefore, different samples were held for varying amounts of time in several places. Some remained frozen for double the time others did. Some remained in their bags after freeze-drying for triple or even quadruple the time others were before being ground and stored in glass tubes. Scant records were kept on freeze-drying and grinding dates, since there was no foreseeable need for this information. It is known that some samples were freeze-dried, ground and stored in glass tubes by early February 2000, while other samples weren't ground and stored in tubes until mid September, and for most of that time, were stored already freeze-dried in plastic bags at -20°C. If glass tubes allowed enough oxygen, light and moisture to enter the sample and cause degradation, as previous results suggest, then plastic bags would be even more suspect. A comparison of bagged and tubed samples was carried out. This experiment was confounded by an inadvertent movement of the samples from -20°C to 4°C by a third party after ten weeks of the outlined twelve week storage. Thus, for two to three weeks, the samples were stored above freezing, and the

results from this study are suspect as well, but what they reveal is very surprising if valid. Contrary to what was expected, bagged samples showed significantly less lycopene degradation relative to tubed samples in eight of the ten samples (the other two were not significant) (Table 4.1). Previously it was demonstrated that tubed samples were stable for at least thirty days. These results indicate that the powder is not stable for ninety days, but this cannot be concluded definitively because the storage protocol was altered. No explanation can be given for why samples in tubes would experience higher degradation than samples in bags, but this point ought to be investigated further.

Immediately after the disruption of storage was noticed, four samples were run twice, two weeks apart, with storage in tubes at 4°C in the interim to assess degree of degradation at this temperature. Earlier results indicated that the powder was stable in tubes for at least thirty days at -20°C. The results of this experiment showed that significant degradation of lycopene approaching 60% had occurred in just two weeks of storage at 4°C ($p < 0.01$) (Table 4.2). With this information in hand, it is difficult to validate the results of the three month storage study. Bagged and tubed samples may have reacted differently to the change in storage temperature such that they cannot be compared even on a relative scale. No conclusion can be made regarding the effect of storage vessel on lycopene degradation in dried powder. However, it can be concluded with confidence that 4°C is not an appropriate storage temperature for freeze-dried tomato powder.

Table 4.1 Paired T-tests of degradation of lycopene in samples stored in bags or tubes for 3 months. Percent degradation is compared to the bagged sample. In all cases where a significant difference existed, the tubed sample degraded more.

Sample	p-value	% Degradation
Black Plum I 6-29-00	< 0.0001	24
Black Tula I 7-6-00	0.0330	26
Celebrity III 7-6-00	0.0017	26
Celebrity I 7-12-00	0.4226	ns
Celebrity IV 7-12-00	< 0.0001	60
Black Tula IV 7-12-00	0.0058	25
Black Tula III 7-12-00	0.0005	25
Celebrity IV 7-23-00	0.3286	ns
Black Krim III 6-29-00	< 0.0001	11
Black Plum III 7-6-00	< 0.0001	38

Table 4.2 Effect of 4°C for 15 days on lycopene degradation in powdered tomato samples. n = 4

Sample	p-value	% Degradation
Black Plum I 6-29-00	0.0053	6.7
Black Tula I 7-6-00	< 0.0001	48
Celebrity I 7-12-00	0.0003	60
Celebrity III 7-6-00	< 0.0001	50

CONCLUSIONS

Lycopene is a very labile compound, but will remain stable in a dried tomato powder matrix under nitrogen at -20°C for a minimum of thirty days. No conclusion can be made regarding the efficacy of plastic bags versus glass tubes for storage of dried tomato powder. Lycopene will degrade very rapidly in dried tomato powder stored at 4°C. Evidence exists for a nonlinear degradation pattern, stable in the beginning, then accelerating after thirty days. Further investigation of lycopene degradation could clarify these results.

CHAPTER 5

FRUIT COLOR AND LYCOPENE CONTENT

INTRODUCTION

Tomatoes are very popular vegetable in gardens across the world. They are simple to cultivate, pleasant to eat and new research has shown that they are very healthful additions to the diet, not only due to their high vitamin content, but to high antioxidant potential as well. Positive publicity surrounding antioxidants derived from fruits and vegetables, combined with a growing interest in the quality of foods among the public has created a climate where gardeners will be interested in the nutritional variety available among traditional garden fruit and vegetable cultivars.

Lycopene is a powerful antioxidant found in many fruits, especially abundant in tomatoes, which has been linked to reduced frequency and severity of several types of cancer and heart disease (Giovanucci et al. 1995, Grant 1999, Franceshi et al. 1994, others). Lycopene's multiple double bonds give it a red color, which in turn colors many fruits (Fig 1.12) (Ngyuen & Schwartz 1999). Lycopene is the primary carotenoid found in tomatoes, where it is located in the chromoplasts (Fraser 1994). Lycopene is the primary

pigment associated with tomato's red color. In many plants, anthocyanins and chlorophyll mask the color of less abundant carotenoids (Salisbury & Ross 1992). Lycopene may be equally plentiful in yellow, orange and red tomatoes, but masked in varying degrees by other pigments. An objective measure of lycopene in different colored cultivars could help reveal more information about tomato pigment.

Another variable among garden cultivars is fruit size. Sharma & LeMaguer (1996) showed that lycopene concentration in tomato skin was up to five times higher than in the inner tissue. If this is true with all tomatoes, then smaller cultivars with a higher skin/inner-tissue ratio should have higher overall lycopene content.

Red, yellow, orange and shades intermediate are the commonly encountered colors found among tomato cultivars of *Lycopersicon esculentum*, the domesticated tomato. Rare "black" cultivars of tomato are encountered occasionally in heirloom seed catalogs. In appearance they are not in fact black, but are dark purple to black on the shoulders, while the lower portion of the fruit is deep magenta or purple (Fig 5.1). Black cultivars, being heirloom types, are not hybrids like the majority of tomato transplants sold in nurseries.

Breeding programs have long sought to increase lycopene content in tomato cultivars and identification of high-lycopene cultivars for use as parent stock is desirable (Wann & McFerran 1960). Additionally, since lycopene has been shown to be a powerful antioxidant and implicated in the prevention of cancers and heart disease, high lycopene cultivars have potential health applications (Nyguen & Schwartz 1999).

Figure 5.1 Black Krim

The aim of this experiment was to quantify lycopene concentration among the major garden tomato cultivars of northern Colorado as well as several black heirloom cultivars. The hypotheses were that lycopene concentration would be greatest in black cultivars, greater in red cultivars than orange, greater in orange than in yellow, significantly varied among red cultivars and negatively correlated to fruit size. The benefit of such information lies in providing knowledge regarding everyday food for the general public in the form of outreach bulletins to utilize as they wish and to perhaps identify tomato cultivar candidates for inclusion in a breeding program motivated to enhance lycopene content in already established cultivars or their parent germplasm.

MATERIALS AND METHODS

Growth of tomato plants is detailed in Chapter Two. Red, orange and yellow cultivars were grown in the field in 1999 while the black cultivars were grown in the greenhouse in 2000, thus they will not be directly compared except in the case of black cultivars with Celebrity, which was grown in the greenhouse as a control red cultivar. Analysis protocol of tomato tissue is outlined in Chapter Three, but will be summarized here. Individual tomatoes were picked, frozen at -20°C, freeze-dried for one week, ground and sieved through a 100 μm screen. Tomato powder was stored under nitrogen in glass tubes sealed with foil-lined lids at -20°C in darkness until it was extracted with acetone and analyzed using HPLC. For this experiment, four injections for each replicate of each cultivar were run for a total of $n = 16$. HPLC analysis began in February 2000 and lasted until October.

Cultivar lycopene mean differences were analyzed using the general linear model procedure in SAS v8 (SAS Institute, Nashville, TN). Data were graphed with GraphPad Prism 3.0 (Intuitive Software, San Diego, CA).

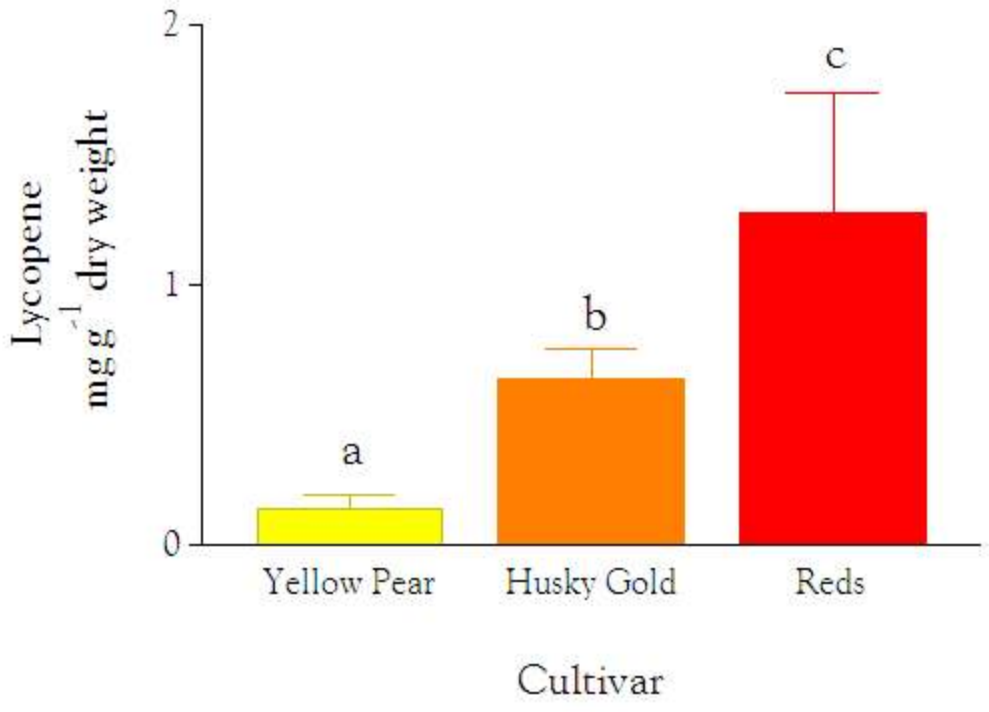
RESULTS AND DISCUSSION

At issue when conducting cultivar screening for lycopene is the ultimate manipulation of high lycopene germplasm for breeding projects. Wann & McFerran (1960) noted that crosses between the high lycopene tomato species *Lycopersicon pimpinellifolium* (lycopene = 18.92 mg $100g^{-1}$ fresh weight) and the domesticated tomato *L. esculentum* cv Rutgers (lycopene = 6.09 mg $100g^{-1}$ fresh weight) yielded F_1 fruit with a lycopene content of 9.75 mg $100g^{-1}$ fresh weight, intermediate between the two. Thus, high lycopene lines are useful for increasing lycopene content in selected cultivars. Finding high lycopene levels in black cultivars would be useful in breeding projects just as *L. pimpinellifolium* has been. Screening for high lycopene cultivars is not a novel idea. Ellis & Hamner as far back as 1943 analyzed lycopene content of three cultivars and found significant differences between all three, ranking them as Stokesdale > Rutgers > New York State, although actual quantities of lycopene were not reported. Thompson et al. (1967) compared lycopene levels in Campbell 146 (5.66 mg $100g^{-1}$), Eastern States 24 (3.88 mg $100g^{-1}$) and High Crimson (8.78 mg $100g^{-1}$). Again, crosses between cultivars yielded F_1 which produced fruit with intermediate lycopene content. Sharma & LeMaguer (1996) analyzed lycopene in five processing cultivars and reported that some showed significant difference.

Field-Grown Tomatoes

The lycopene comparison of all thirty cultivars was affected by degradation findings as outlined in Chapter Four, and most cultivars were omitted from lycopene analysis. Nevertheless, several conclusions can still be made with regard to lycopene content in yellow and red cultivars. Using the early data from February and early March, which was subject to minimal degradation and for which lycopene degradation would have occurred roughly proportionally in all samples, a significantly lower lycopene content was apparent in the yellow cultivar Yellow Pear and the orange cultivar Husky Gold relative to the red cultivars in general ($p < 0.05$) (Figure 5.2, 5.3). This supports the hypothesis that yellow cultivars contain less lycopene than orange cultivars which in turn contain less lycopene than red cultivars. The hypothesis that differences would exist among red cultivars cannot be proven as a result of sample degradation, coupled with low sample sizes of red cultivars in early analyses. Likewise, size correlation cannot be calculated.

Comparing the lycopene content from the early samples (February-March) with published values of several other studies (Table 1.6) illustrates that the experimental values were indeed somewhat lower. The range of early data on red cultivars was 0.8-2.7 mg g^{-1} dry weight compared to the published range of 3.1-11 mg g^{-1} dry weight. This indicates that a small amount of degradation did occur, and the lycopene content of these cultivars is most likely higher than shown.



	Reds	Husky Gold
Yellow Pear	0.0005	0.0025
Husky Gold	0.0318	

Figure 5.2 Comparison of lycopene in Yellow Pear and Husky Gold relative to several red cultivars. Error bars are standard deviation. Means associated with different letters are significantly different. P-value summary given below graph. n = 3 for Yellow Pear and Husky Gold, n = 18 for Reds (Sweet 100, Better Boy, Beefmaster, Early Cascade, Patio, Brandywine, Juliet, Heartland, Roma and Champion).

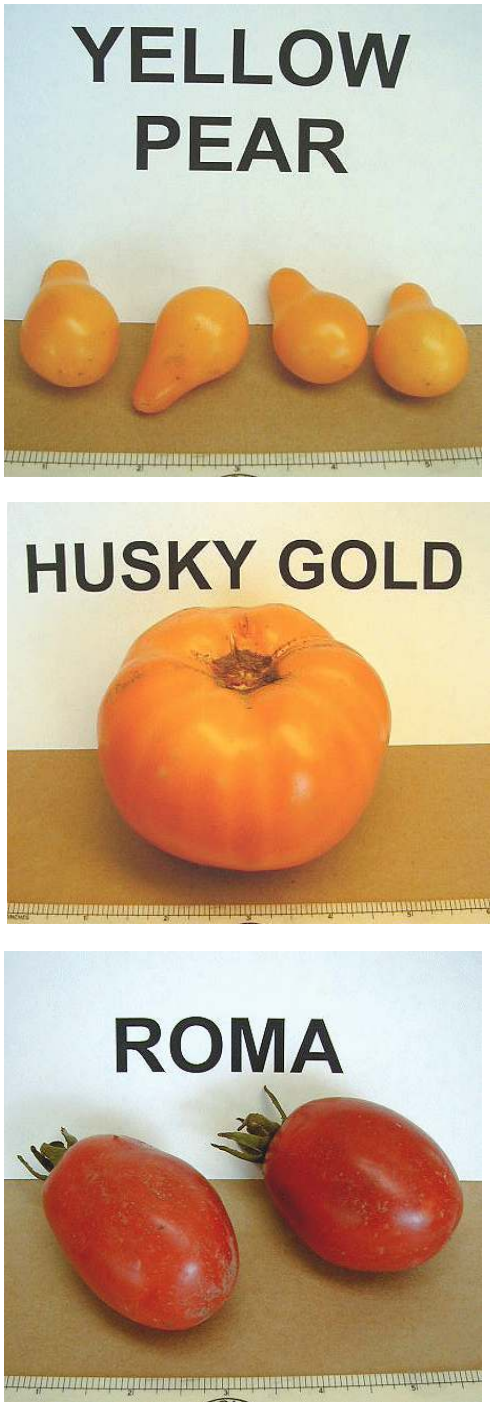


Figure 5.3 Tomato cultivars of varying colors: Yellow Pear (yellow), Husky Gold (orange) and Roma (red). Lycopene content was highest in red cultivars (including others in addition to Roma) and higher in Husky Gold than in Yellow Pear.

Black Cultivars

Analysis of variance for black cultivars and Celebrity was positive for significant differences ($p < 0.0001$). Fischer's protected least significant difference (LSD) was used for mean separation. Every cultivar exhibited a lycopene content which was significantly different from all others (Fig 5.4). Quantitative lycopene content was as follows: Black Krim > Celebrity > Black Plum > Black Tula. Since lycopene concentration is as much as five times higher in the epidermis than within a tomato, it is surprising that Black Plum had a lower lycopene concentration than Black Krim, since the latter has a much higher surface/volume ratio (Sharma & LeMaguer 1996).

The hypothesis that black cultivars contain higher amounts of lycopene than standard red cultivars is clearly not supported by the data. Although Black Krim shows a higher lycopene content, the other two black cultivars do not. Celebrity, the control red cultivar, is bracketed by black cultivars, indicating that the large difference in pigment does not translate to a large difference in lycopene content. The deeper color of the black cultivars could be imparted by anthocyanins, which give many fruits and flowers their red to purple color (Salisbury & Ross 1992). Whatever it is that imparts the intensely dark color to the three black cultivars studied, it isn't lycopene.

CONCLUSIONS

Tomato color among traditionally pigmented tomatoes is related to lycopene content in a "what you see is what you get" fashion as red cultivars exhibited higher lycopene content than Husky Gold which likewise had a higher lycopene content than

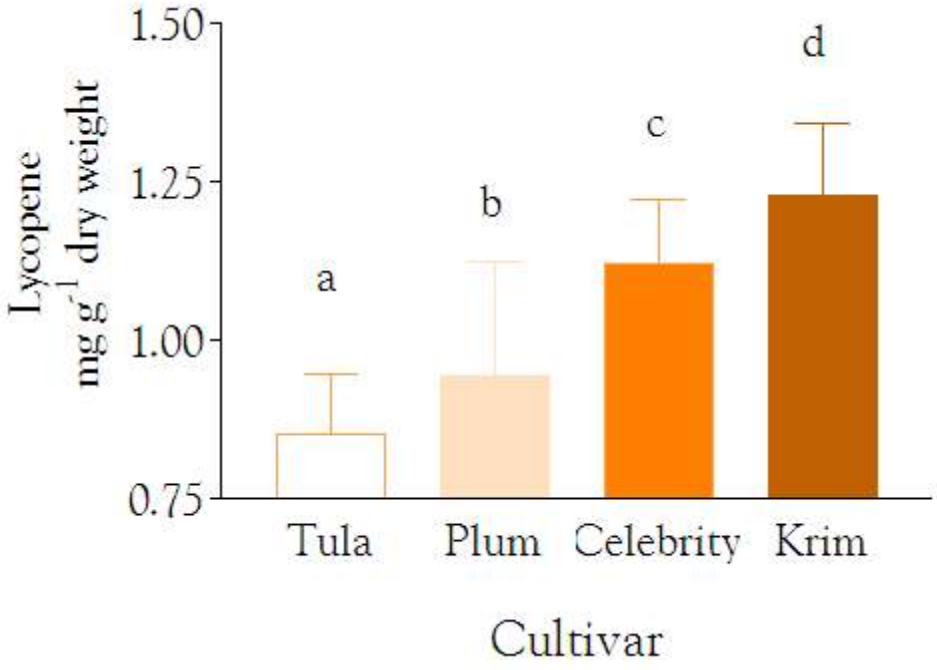


Figure 5.4 Mean lycopene content with standard deviation in three black cultivars compared to one red (Celebrity). Means with different letters are significantly different at $\alpha = 0.025$ ($n = 16$).

Yellow Pear. For the consumer of tomatoes interested in obtaining high amounts of lycopene, or the breeder interested in high lycopene parent stock, the reddest tomatoes are superior. No conclusion can be made regarding variation in lycopene content among red cultivars, or to lycopene's relationship to fruit size.

Despite findings that color was related to lycopene content in field grown tomatoes, the black cultivars *as a group* did not show an elevated level of lycopene relative to Celebrity. Lycopene concentration was highest in Black Krim and lowest in Black Tula. The darker color of these black cultivars relative to a familiar red cultivar is not imparted by lycopene. It is, however, entirely possible that the heavily pigmented cultivars are very effective in contributing antioxidants in addition to lycopene. Many anthocyanin phenolics found in fruits and vegetables are now known to be very effective as radical scavengers, and quenchers of lipid peroxidation (Lister, 1999). Thus combined with lycopene, these cultivars may yet prove to be very interesting in the quest for food antioxidants as an intervention strategy. Additional research, based upon assays to test interaction with reactive oxygen species would be required to test this question.

CHAPTER 6

LIGHT AND LYCOPENE SYNTHESIS

INTRODUCTION

The majority of tomatoes produced commercially in the United States are field-grown, with a small portion contributed by greenhouse operations (Jones 1999). Because tomatoes are a warm-season crop, field-grown tomatoes can only be produced in warmer climates for much of the year. California, Florida and Georgia, respectively, led the US in fresh market production from 1960-1990 (Jones 1999). Since the bulk of field-grown tomatoes in the US are grown in southern coastal states, shipping the fruit to market areas in the north and central states is difficult. This problem is universal. Tomatoes deteriorate rapidly after ripening, becoming very soft and easily bruised. To overcome this, field-grown tomatoes are usually picked green and shipped unripe. The green-mature stage is defined by Rubatzky & Yamaguchi (1997) as "Bright to whitish green; well rounded, skin with waxy gloss; seeds embedded in gel and not easily cut when fruit is sliced; seeds mature and can germinate; fruit ripening under proper conditions". Green-mature stage is the point just before pink begins to show on the blossom end. Green- mature fruit will turn red-ripe in 15 to 25 days when stored at 20°C, but fruit

picked prior to the green-mature stage will never ripen. The value of the US fresh market crop in 1995 was 891 million dollars, so proper timing of harvests for optimum ripeness at markets is very critical (Jones 1999). Ripening occurs during transit or shortly after arrival to the destination. Reduced flavor quality is often associated with this practice, but economically it is the best method for supplying tomatoes to distant markets.

The alternative is greenhouse tomato production. Since greenhouses can be built virtually anywhere, long distance shipping of fruit is eliminated. Thus, fruit need not be picked unripe, but instead can be vine-ripened and marketed immediately. Vine-ripened tomatoes are generally associated with superior flavor quality and enhanced shelf life, the latter likely due to reduced pathogenic soil contaminants (Jones 1999). The drawback to greenhouse production is cost. Heating and lighting greenhouses, in addition to the capital investment of the houses themselves, is often financially inhibiting for growers. The high cost of production is reflected in the higher market value of greenhouse-produced tomatoes. Economics dictate that the bulk of tomatoes be field-grown, but greenhouse tomatoes are often superior in quality.

Quality enhancement of tomatoes picked green-mature is important to growers. Evidence indicates that lycopene production is influenced by environmental factors even after harvest. This study examined the effect of light on tomato fruit picked green-mature. Since lycopene is contained in the chromoplasts, which are transformed chloroplasts, it seems probable that light quality will in fact effect lycopene synthesis. Thus, the hypothesis for this experiment was that longer photoperiods will increase

lycopene production in fruit picked green-mature relative to similar fruits exposed to a shorter photoperiod.

MATERIALS AND METHODS

This experiment was carried out from fall 1999 to summer 2000. Sixty four green-mature tomatoes of the medium-sized red cultivar Heartland (Fig 6.1), grown at the Colorado State University Horticulture Research Farm near Fort Collins, CO, were divided into two equal groups and placed into two different growth cabinets on September 27. Both cabinets were set at a constant 25°C, the optimum temperature for lycopene synthesis and chlorophyll degradation (Al-Mughrabi 1989). The first cabinet had a programmed 24h photoperiod, while the second cabinet had an 8h daily photoperiod. Both cabinets were lighted by cool white flourescent bulbs to an approximately equal intensity. Five fruit from each cabinet, of approximately equal size (60 g) and equal color, were removed and used for analysis on October 20. The 8h photoperiod treatment chamber was often a couple of degrees cooler than the light treatment chamber, likely due to the heat given off by the lights. The temperature was never more than 2°C different, so the effect is not given serious consideration.

Fruits were weighed, frozen, freeze-dried, ground and sieved through a 100 μm screen with the resultant powder stored in glass vials under nitrogen with a foil-lined lid in a -20°C dark freezer. Lycopene from samples was extracted with acetone washes on July 29 and 31 of the following summer (nine months after freezing). Extracts were analyzed four times each using a Dionex DX300 High Performance Liquid Chromatograph (HPLC) with a spectrophotometric detector set at 472 nm. Details of the storage, extraction and analysis procedures are outlined in Chapter Three.

Mean differences were analyzed using SAS v8 (SAS Institute, Nashville, TN).

Figure 6.1 Heartland tomato cultivar utilized in the ripening study

RESULTS AND DISCUSSION

Previous studies in this work (Chapter Four) indicate that lycopene is not stable enough to be stored for nine months, under any condition, without some degradation of the compound. The fruits in this experiment were all treated, processed, stored, extracted and analyzed at the same time. Therefore, despite invalidation of the absolute lycopene values obtained through analysis due to lycopene degradation, the relative values are still valid.

The mean lycopene content for light ripened tomatoes was 0.5159 ± 0.06 mg g^{-1} dry weight ($n = 20$). Mean lycopene content for tomatoes ripened under 8h photoperiod was 0.2938 ± 0.06 mg g^{-1} dry weight ($n = 20$). Mean variance was not significantly different between the two treatments ($p = 0.9325$). A student T-test showed that light treatment significantly altered lycopene synthesis between the two groups ($p < 0.0001$), with the fruit ripened under an 8h photoperiod averaging 35.8% less (Fig 6.2).

Duggar (1913) observed that tomato fruit grown on plants outdoors ripened quicker on the sun-facing sides. This was initially attributed to the higher temperature of the sunny sides of the fruit. This temperature effect theory was later proven unequivocally by many researchers (Rosa 1926, MacGillivray 1935, Sayre 1954, Ayres & Pierce 1960, Al-Mughrabi 1989). Denisen (1948) reported that light was not required for the ripening of fruit picked green-mature. This may be true in so far as tomatoes will turn red even in complete darkness, but results of Smith & Smith (1931) showed that intensity and duration of light influenced lycopene synthesis, such that dark-ripened tomatoes never turned quite as red as those exposed to light. Shewfelt & Halpin (1967) reported that

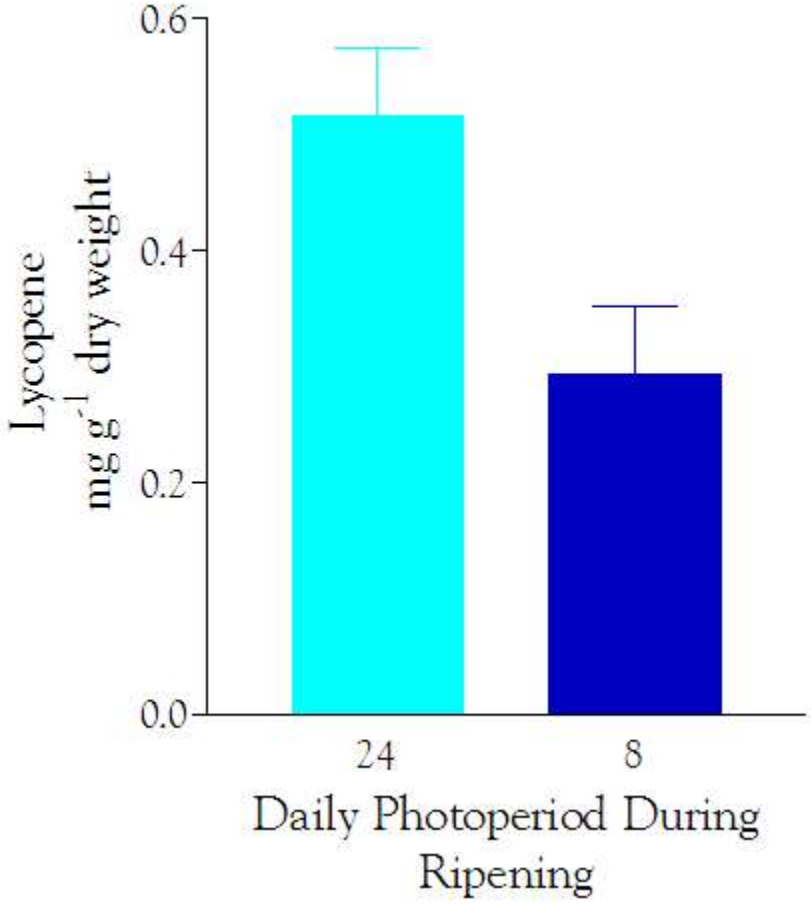


Figure 6.2 Mean lycopene content with standard deviation of green-mature tomatoes ripened under 24 and 8 hour photoperiods for 23 days until fully ripe. Means are significantly different ($p < 0.0001$, $n = 20$).

light quality effected ripening as well. Fruit ripened under cool white flourescent bulbs were 16 times more red and fruit ripened under wide spectrum bulbs were 32 times more red than dark-ripened controls. In the case of tomato fruit color, redness translates almost exactly to lycopene content (Fraser 1994, Sharma & LeMaguer 1996). My data are therefore in agreement with the conclusions of these past studies.

Alba et al. (2000) was able to explain the light/lycopene connection by identifying phytochromes which regulate lycopene synthesis, an idea first proposed by Khudairi (1971). In their experiment, red light induced lycopene formation while far red light actually reversed it. The phytochromes acted independently of ethylene treatments and were not involved in physical changes accompanying ripening, such as the softening of the pericarp or in accumulation of sucrose, fructose, glucose, citrate or malate. This is consistent with studies of the *hp-2* mutant of tomato, which has increased pigment content due to altered proteins in the phytochrome transduction chain (Mustilli et al. 1999). Ethylene regulates the production of phytoene synthase, which forms the first carotenoid, phytoene, from gerynlgerynl pyrophosphate (Dey & Harbourne 1997). Phytochromes probably regulate lycopene synthesis further down the pathway (Alba et al. 2000).

Many studies have identified optimal storage conditions for ripening. For optimal air quality, humidity should be 90-95%, oxygen 3-5% and carbon dioxide 0-3% (Lorenz & Maynard 1988). Optimum ripening tempaerature is 25°C (Rosa 1926, Al-Mughrabi 1989). Oddly enough, no standard exists for light quality/quantity during ripening. This is most likely due to the impracticality of lighting storage areas, since tomatoes are often

boxed up and shipped in dark freight carriers. Nevertheless, the effect of light on ripening is significant enough that some attention ought to given to it. If practical, green-mature tomatoes should be exposed to sunlight, or some other wide-spectrum light source, as much as possible during ripening.

Interestingly, extracted lycopene is quickly degraded in the presence of strong light, but is enhanced under the same conditions when inside the fruit (Ngyuen & Schwartz 1999). This is most likely due to two factors. Primarily, the stabilizing effects of water and other antioxidants inside cells prevent autooxidation of lycopene which will occur rapidly when exposed to reactive oxygen species. Secondly, light is likely a trigger for lycopene synthesis in plants just as UV radiation triggers melanin production in our skin.

CONCLUSION

Exposure to increased photoperiod significantly increased lycopene synthesis during ripening of green-mature tomatoes at 25°C.

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