DISSERTATION

EVALUATION OF ANTIOXIDANT AND SENSORY PROPERTIES OF SELECTED CULTIVARS OF COLORADO-GROWN LETTUCE (*LACTUCA SATIVA* L.)

Submitted by

Marisa Bunning

Department of Food Science and Human Nutrition

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 2007

UMI Number: 3266401

ProQuest Information and Learning Company
300 North Zeeb Road
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COLORADO STATE UNIVERSITY

January 10, 2007

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY MARISA BUNNING ENTITLED EVALUATION OF ANTIOXIDANT AND SENSORY PROPERTIES OF SELECTED CULTIVARS OF COLORADO-GROWN LETTUCE (*LACTUCA SATIVA* L.) BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

David A Sampson

Dr. David Sampson

Martha Stone

Dr. Martha Stone

Liangli Yu

Dr. Liangli Yu

Patricia Kendall

Dr. Patricia Kendall - Advisor

Cecil Stushnoff

Dr. Cecil Stushnoff - Co-Advisor

Chris Melby

Dr. Chris Melby - Department Head

ABSTRACT OF DISSERTATION

EVALUATION OF ANTIOXIDANT AND SENSORY PROPERTIES OF SELECTED CULTIVARS OF COLORADO-GROWN LETTUCE (*LACTUCA SATIVA* L.)

Epidemiological evidence has substantiated the health benefits associated with the consumption of vegetables, particularly leafy greens. Lettuce (*Lactuca sativa* L.) cultivars (varieties) have usually been selected based on shelf-life, transportability, and yield rather than nutritional or sensory traits. Information on the effects of seasonality and genetics on various characteristics of lettuce is limited. Nutritional, antioxidant, and sensory profiles of lettuce may vary considerably among cultivars and in response to environmental factors that may fluctuate widely throughout the growing season. Bitterness, an important flavor characteristic of lettuce, is generally thought to increase with higher growing season temperatures and may vary with phenolic content.

Total phenolic content, radical scavenging capacity, vitamin C levels, and sensory properties of multiple lettuce cultivars harvested early, mid-way, and late in the growing season were assessed in this study. Each lettuce crop was grown using standard organic methods and sampled with uniform harvest and postharvest procedures. Daily temperatures and radiation were monitored to determine the impact of climatic factors.

Total phenolic content and radical scavenging capacity were quantified in eight cultivars of lettuce grown at six different times over two growing seasons. 'Cimmaron' (red romaine), 'Crisp and Green' (green leaf), 'Crispino' (iceberg), 'Green Forest' (green romaine), 'Lochness' (butterhead), 'Nevada' (green batavia), 'Sierra' (red batavia) and 'Vulcan' (red leaf) lettuce cultivars were analyzed for total phenolic content using the Folin-Ciocalteu assay and radical scavenging capacity was estimated with an ABTS assay. Vitamin C levels of the eight cultivars grown at two different times were determined using HPLC.

Significant variation ($P < 0.001$) existed in both total phenolic content and radical scavenging capacity among the eight cultivars. With four cultivars, increases in total phenolic content were observed with higher summer temperatures but trends were not attributable to seasonality. Total phenolics varied from 13.1 mg gallic acid equivalents/gram dry weight in 'Crispino' to 48.2 mg GAE/gdw in 'Vulcan'. Radical scavenging capacity ranged from 160.3 μmole TEAC/100 grams fresh weight for 'Crispino' to 653.8 μmole TEAC/100gfw for 'Cimmaron'. Cultivars with red pigmentation exhibited higher levels than similar green cultivars and leaf lettuce exhibited the highest levels among the four types. Variation in vitamin C among these cultivars was not statistically significant ($P > 0.05$).

Thirty sensory panelists rated bitterness, appearance, flavor, texture, and overall acceptability of five cultivars: 'Crisp and Green' (green leaf), 'Crispino' (crisphead), 'Green Forest' (romaine), 'Lochness' (butterhead), and 'Vulcan' (red leaf) lettuce. There was considerable variation in sensory ratings among the 5 cultivars ($P < 0.005$) but few differences within cultivars across the growing season. 'Crispino' received higher scores for flavor, texture, and overall acceptability and was rated less bitter than other cultivars

($P < 0.01$). Mean scores for all attributes remained within the acceptable range, indicating that acceptable lettuce can be grown in this region during summer months.

These results demonstrate the diversity of antioxidant capacity among cultivars and suggest that genotype may have a significant influence on nutrient levels in this crop. As part of a food-based approach to improving nutrition, it is beneficial to identify the health-promoting potential of specific vegetable cultivars that also exhibit favorable sensory properties. Nutritional and sensory assessment of different types and colors of promising lettuce cultivars may improve the market competitiveness of Colorado-grown lettuce.

Marisa Bunning
Department of Food Science and Human Nutrition
Colorado State University
Fort Collins, CO 80523
Spring 2007

ACKNOWLEDGEMENTS

I would especially like to thank my co-advisors, Dr. Pat Kendall and Dr. Cecil Stushnoff, for their expert guidance, time, patience, and commitment to this project. I am also grateful for having an exceptional doctoral committee and wish to thank Dr. Martha Stone, Dr. Lucy Yu, and Dr. David Sampson for their comments and suggestions. I owe a special note of gratitude to Frank Stonaker for growing the lettuce and Ann McSay and Jeanette Stushnoff for training in lab procedures.

I extend many thanks to my colleagues and friends in the Department of Food Science and Human Nutrition, especially Janice Brown, Heather Troxell, Cristina Munteanu, Ruth Inglis-Widrick, Jen Brunning, Jena Lenhart, Lyutha Khalfau Al-Subhi, Tricia DiPersio, Stephanie Wallner, Dawn Clifford, Laura Bellows, Alena Clark, Linda Quaratino and Mary Schroeder. I would also like to acknowledge the faculty, staff, and students of FSHN that served on the lettuce sensory panel.

I appreciate the support provided by the Colorado Agricultural Experiment Station for this research.

Finally, I'd like to extend my heartfelt thanks to my family, Mike, Abbey, Josh, Luke, Walt, Matt, and Becca for their continual support, encouragement, and understanding. They have been partners in this endeavor from the beginning and I cannot thank them enough.

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CHAPTER I

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is an important vegetable crop in the U.S. with an annual farm value of over 1.98 billion dollars. Several different types of lettuce are commonly available to consumers, selected from thousands of lettuce cultivars (varieties) accessible to farmers and home gardeners. Substantial changes have occurred in the salad crop industry in the last decade, including increased awareness of the nutrient and antioxidant content of leafy vegetables (Ryder, 2002). The growing popularity of diverse and colorful salad mixes reflects the demands of more knowledgeable and health-conscious consumers, as well as the increased availability of high-quality produce (Labensky et al., 2003). Freshness and appearance have been the standard qualities used to judge fruits and vegetables but other factors, like nutritional composition, agronomic practices, and growing location, are gaining importance with consumers (Grimme and Dumontet, 2000).

The association between botanical foods and health benefits continues to grow stronger (Lampe, 1999; Thompson et al., 2006) and appears to be related to numerous antioxidative compounds and phenolic-based metabolites produced by plants (Kris-Etherton et al., 2002; Morello et al., 2002; Manach et al., 2003). Plant compounds may modulate the activity of a wide range of enzymes and cell receptors or may act as antioxidants and inhibit oxidative damage that contributes to many chronic disease processes (Mathers, 2006). There are many different aspects in the antioxidant picture that need to be elucidated before there will be a clear understanding of this complicated issue.

Health-promoting benefits associated with phenolic plant compounds, like prevention of cardiovascular diseases and cancer, have also been gaining recognition (Manach et al., 2005; Scalbert et al., 2005). Phytonutrients are becoming accepted as part of a nutritious diet that promotes health and prevents disease (Birt, 2006). The assessment of phytochemical content of dietary plants is still in the early stages, with many components of various crops yet to be identified and measured. The chemical and nutrient composition of vegetable crops varies considerably and may be profoundly influenced by genetics, growing method, region, developmental stage, and climatic conditions (Lee and Chichester, 1974; Wien, 1997). Epidemiological studies, in vitro assessment of compounds in plant material, and in vivo studies are all contributing to the overall goal of understanding how dietary intervention can be used to realize health benefits.

Leafy vegetables are particularly good sources of bioactive compounds since, in addition to being principal photosynthetic sites, leaves are accrual areas for various phytochemicals with antioxidant, light-filtering, antimicrobial, antiherbivorial, and other defensive properties (Tarwadi and Agte, 2003). Lettuce is the most widely consumed salad crop (van Wyk, 2005) and a year-round source of vitamin A, vitamin C, beta-carotene, lutein, calcium, folate, and fiber (USDA Nutrient Data Base, 2005). Along with the increasing availability of bagged salads, consumption of various types of lettuce is increasing (Ryder, 1999; Zind, 2005). Nutritional profiles may vary considerably among lettuce types (Simonne et al., 2002) and climatic conditions, which fluctuate widely throughout the long growing season of lettuce, can be expected to influence chemical and sensory profiles (Hertog et al., 1992; Inzé and Van Montagu, 2002).

Lettuce is an important specialty crop in Colorado (Colorado Department of Agriculture, 2005) and regional environmental conditions, particularly higher altitude and light intensity, may create stresses that shift the pattern of phytochemical synthesis and could influence the chemical and organoleptic attributes of this crop (Inzé and Van Montagu, 2002). Lettuce exhibits distinctive family and species characteristics that make it an interesting model for the investigation of climatic effects on sensory and chemical properties. Throughout different stages of growth, lettuce is very responsive to changes in temperature and light (Wien, 1997) and lettuce belongs to the plant family, Asteraceae, known for its production of novel chemical compounds (Heywood et al., 1977). Bitter taste in lettuce has been associated with higher growing temperatures and the longer days of summer (Rubatzky and Yamaguchi, 1997).

Second to food choice, cultivar may be the most important variable affecting the phytochemical content of our diets (Kalt, 2005). Research that investigates the attributes of specific cultivars and the effects of environmental conditions can improve our ability to better utilize food crops and assist in providing the highest quality produce (Fennema, 1996). Liu and others (2005) reported that variations in phenolic content of lettuce were influenced by cultivar, type, and pigmentation with red leaf cultivars exhibiting the highest levels. As part of a food-based approach to improving health, it would be beneficial to identify specific vegetable cultivars that are good sources of phenolic and antioxidative compounds (Gopalan and Tamber, 2003). Nutrition education strategies aimed at diet improvement need to consider sensory response (Drewnowski, 1997) so it is also important to determine which cultivars exhibit favorable sensory properties. Studies are needed that investigate the effects of environmental variation on total phenolic content and antioxidant capacity among various cultivars, colors, and types of lettuce to better evaluate potential salutary benefits.

The goals of this study were to assess the effects of seasonal variation in regional growing conditions on sensory and antioxidant properties of multiple cultivars of lettuce and to investigate possible correlations between bitterness, total phenolic content, antioxidant capacity, and selected environmental factors. The chosen cultivars have exhibited promising production characteristics and represent the lettuce types most commonly available to consumers: butterhead, crisphead, green leaf, red leaf and romaine. The aim of the educational component of the study is to promote the production and consumption of Colorado "greens" through the distribution of pertinent health and food safety information to consumers, home-gardeners, and commercial producers using web-based fact sheets.

CHAPTER II

LITERATURE REVIEW

Lettuce classification, origin, and history.

Lettuce (*Lactuca sativa* L.) is an ancient vegetable crop thought to be native to the eastern Mediterranean basin (Rubatzky and Yamaguchi, 1997). Egyptian tomb paintings indicate lettuce many have been cultivated as early as 4500 BC. In 1543, Leonard Fuchs depicted a lettuce plant in his herbal, Kräuterbuch, under the name *Lactuca capitata* (Rätsch, 2005). Lettuce was considered an aphrodisiac by early Egyptians but Greeks thought it to be an anti-aphrodisiac due to its narcotic properties (Harlan, 1986).

Lettuce is an annual, dicotyledonous plant with sessile leaves spirally arranged in a dense rosette. Taxonomically, *Lactuca sativa* is classified in the sunflower or composite family (Asteraceae), the largest family of flowering plants containing about ten percent of all known angiosperm species (Zomlefer, 1994). Plants in this family are predominantly herbaceous and are known for several unique characteristics, including the production of novel secondary chemicals, their ability to thrive in inhospitable habitats, and formation of polymorphic flowers (Crosby, 1963). A number of unusual fatty acids are found in the seed oils of some composite species and the family is a rich source of powerful insecticides and industrial products, e.g., pyrethrum (*Chrysanthemum*) and rubber (guayule) (Heywood, Harborne, & Turner, 1977). *Echinacea* and other composites synthesize biologically active compounds with potential medical or nutritional benefits (Lewis and Elvin-Lewis, 2003).

One common family trait of plants in Asteraceae is the production, within stem laticifers, of milk-like latex which contains triterpenoid alcohols. The latex of *Lactuca* species exhibits soporific properties (Fennema, 1996) and has been utilized as a sedative (Rubatzky and Yamaguchi, 1997). The dried latex of domesticated lettuce has been used as a substitute for opium and marijuana and a lettuce extract, under the name Lettucene, has been sold as a hashish substitute (Rätsch, 2005).

There are over 20,000 species of Asteraceae which have been identified, but few have been cultivated and, including ornamentals, there are less than forty economically important species (Zomlefer, 1994). Sunflower seeds, endive, chicory, globe artichoke, Jerusalem artichoke, and lettuce are the principal foods in the composite family and safflower and sunflower seeds are used in vegetable oil production. Chamomile and *Echinacea* are used therapeutically, often as ingredients in herbal teas. Several modern vegetable crops were originally domesticated for their medicinal properties (Goldman, 2003).

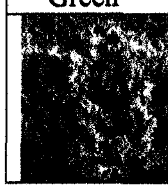
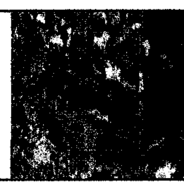
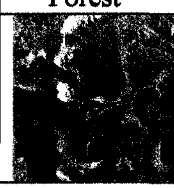
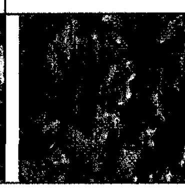
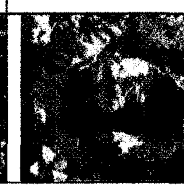
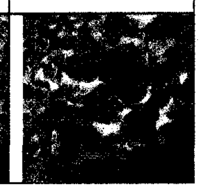
Lettuce is the only cultivated species in the genus *Lactuca*. Domestication of lettuce has resulted in dramatic changes in morphology, development, and physiology (Wien, 1997). In modern lettuce cultivars, the vegetative rosette has become exaggerated with a reduction in branching and a delay in flower stalk initiation. There has also been a reduction in spininess and bitter compounds (Harlan, 1986).

The taxonomic classification scheme of lettuce is shown in Table 2.1. All lettuce is categorized as the same species, *sativa*, but can differ in subspecies, and is designated the variety. A botanical variety, from the Latin word varietas, differs from the species plant, is abbreviated to var., and follows the genus and species name. Horticultural classification of different morphological types of lettuce includes butterhead, leaf, romaine and crisphead, which is further divided into iceberg and batavia subtypes (Ryder, 1999). Crisphead and butterhead lettuce are classified *Lactuca sativa* var. *capitata*, leaf lettuce is var. *crispa*, and romaine is var. *longifolia* (Rubatzky and Yamaguchi, 1997).

The term cultivar refers to cultivated variety, a particular plant that has arisen either naturally or through deliberate hybridization and can reproduce the same plant, vegetatively or by seed (McMahon et al., 2002). The term variety is sometimes used interchangeably for cultivar which can be confusing. The cultivar name follows the genus and species name and should be written in the language of the person who described it (McMahon, Kofranek, & Rubatzky, 2002). It is either written in single quotation marks or has cv. placed in front of the name, for example the cultivar 'Green Forest' is a romaine type of lettuce with the scientific name *Lactuca sativa* L. var. *longifolia.*

Crisphead cultivars have firm, closed heads and are known for resistance to mechanical damage and tolerance to long-distance shipping (Wien, 1997). The cultivar, 'Great Lakes', was released by the USDA and the Michigan Agricultural Experiment Station in 1941 and was the first true crisphead (Ryder, 1999). Crisphead lettuce now accounts for almost ten percent of the entire produce market and is known for its crisp texture and consistent mild flavor (Brown, 2004). Butterhead types of lettuce, also known as Boston or bibb, form loose heads with soft leaves, and are very susceptible to damage. Butterhead is sometimes called cabbage lettuce and has a mild taste and delicate, soft leaves. Leaf lettuce may be predominately green or red in color and has a texture similar to iceberg but does not form distinct heads. Romaine lettuce is also known as Cos, from the Greek island on which it originated (Rubatzky and Yamaguchi, 1997). Socrates was

Table 2.1. Taxonomic classification of lettuce cultivars.

Family: Asteraceace							
Genus: *Lactuca*							
Species: *sativa*							
Variety							
crispa		*longifolia*		*capitata*			
Type							
leaf		romaine		crisphead			butterhead
Subtype							
				batavia	batavia	iceberg	
Cultivar							
'Crisp and Green'	'Vulcan'	'Green Forest'	'Cimmaron'	'Nevada'	'Sierra'	'Crispino'	'Lockness'
							

Source: Rubatzky & Yamaguchi, 1997.
Photos: M. Bunning

rumored to have taken his lethal dose of hemlock with a romaine lettuce spoon (Voorhees, 1995). Romaine has more erect and elongated leaves which are darker toward the outside and lighter toward the interior. Romaine lettuce tends to have a more robust flavor and is less susceptible to damage than leaf or butterhead lettuce.

The genetic variation among lettuce cultivars influences many characteristics such as leaf size, texture, color, taste (Eskins et al., 1996), and chemical content (Liu et al., 2005). The major differences among lettuce cultivars are head formation, leaf shape, and pigmentation (Ryder, 1999). Head formation involves changes in leaf morphology and leaf orientation, and results from the accumulation of young leaves under the layer of leaves covering the growing point (Rubatzky and Yamaguchi, 1997). Continued expansion of the entrapped leaves increases head density. Head formation requires large individual leaves, a slow rate of stem elongation, short petioles and a high rate of leaf production (Wien, 1997). With romaine and leaf type lettuce, there is not progressive leaf shape changes and a true head is not formed. Plant breeding programs have been responsible for the introduction of lettuce cultivars with adaptations for specific locations and seasonal periods. In addition to this adaptability, other factors that influence cultivar selection are disease resistance, foliar characteristics, degree of heading and compaction, head size and shape, color, stem size, yield, shelf-life, and bolting resistance (McMahon, Kofranek, & Rubatzky, 2002).

Lettuce production and industry.

Important changes have occurred recently in the salad crop industry due to trends that encourage healthier eating habits (Ryder, 2002). Of all leafy vegetables, lettuce is by far the most widely consumed, higher per capita than all other types of greens combined.

In the U. S., per capita lettuce consumption in 2005 was reported to be 22.1 pounds for head lettuce, 8.3 pounds for romaine and 4.0 pounds for leaf lettuce (ERS/USDA, 2006). Lettuce is classified a staple crop because of its widespread and perennial consumption and consistently ranks as one of the top five vegetables in overall intake (Crosby, 1963; Vinson et al., 1998; Johnston et al., 2000). The 2005 per capita consumption of several kinds of leafy greens is illustrated in Figure 2.1.

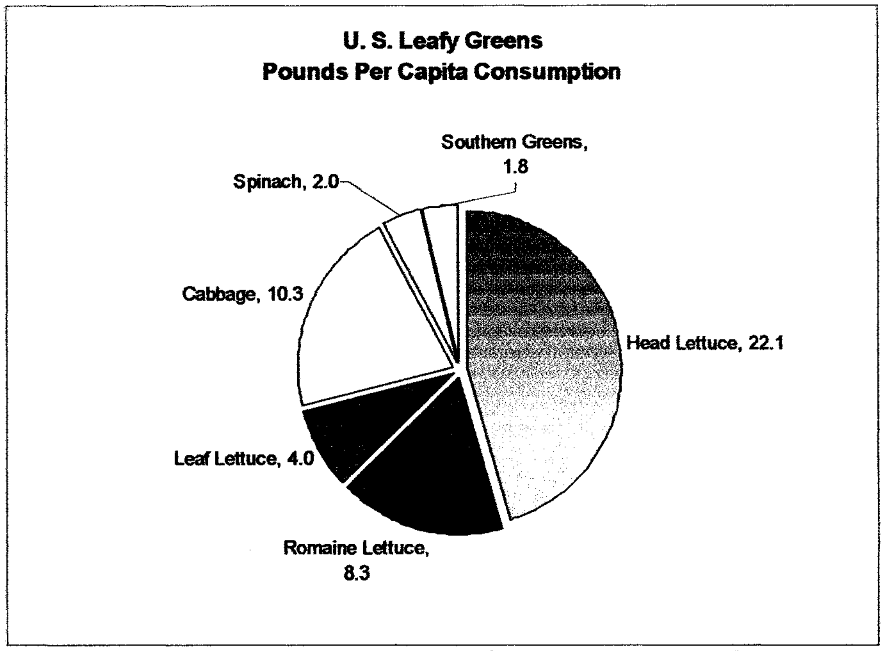


Figure 2.1. U. S. per capita consumption of common leafy greens in 2005. Source: Economic Research Service, U. S. D. A., 2006.

Lettuce is undoubtedly one of the most important vegetable crops in the U.S., with an annual farm value of over 1.9 billion dollars (ERS/USDA, 2006) and an export value in 2004 of over 275 million dollars. Lettuce is an important specialty crop in Colorado. According to the Colorado Department of Agriculture (2005), commercial head lettuce production in 2005 had a crop value of $8.1 million although the number of acres planted has decreased from 7000 acres in 1960 to 2200 acres in 2005. At 2005 prices, the value of the 1960 crop would have been worth over thirty million dollars. Leaf and romaine lettuce are also produced commercially in this state, although information on those crops grown in Colorado is not listed with the Economic Research Service (ERS). Numerous small farms in Colorado market their lettuce crops directly through farmers' markets, roadside stands, community supported agriculture (CSA) subscriptions, or to restaurants. A significant amount of lettuce is marketed through direct channels in the U.S. but those production values are not tracked. A comparison of U. S. head, romaine and leaf lettuce production is shown in Figure 2.2.

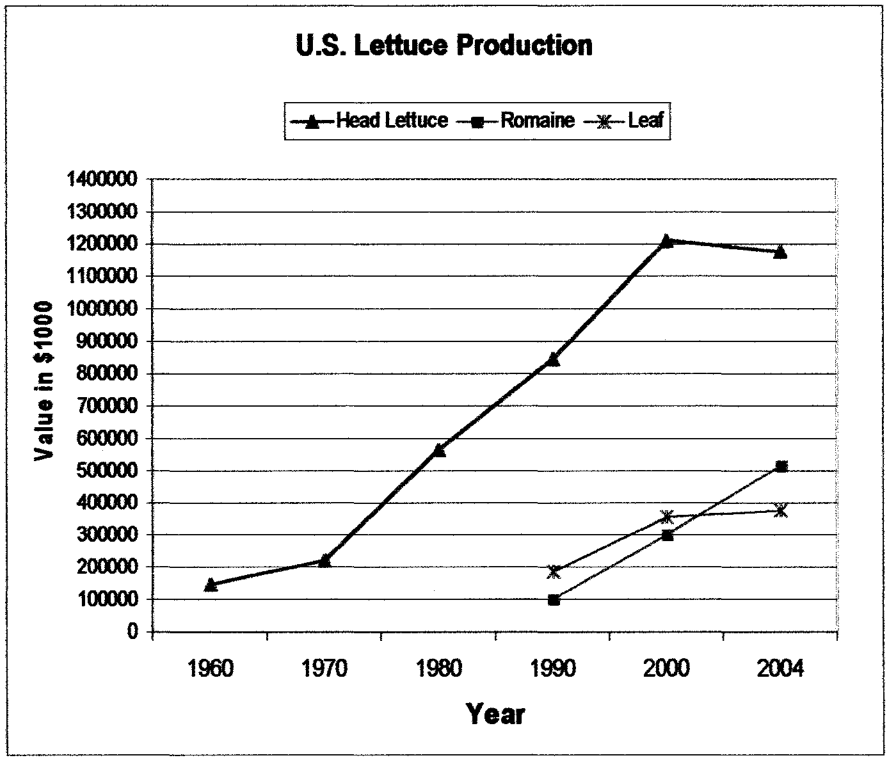


Figure 2.2. Production values for U. S. head, leaf, and romaine lettuce from 1960 to 2004. Source: Economic Research Service, U. S. D. A., 2006.

The designation, specialty crops, encompasses vegetables, fruits, turf, herbs, ornamentals, fiber, and nursery crops. These crops tend to be management intensive but provide alternatives to traditional agricultural crops and have a higher economic return. Specialty crops, like lettuce, generally have fewer problems with pests and pathogens than monocultures and increase the stability of farm economy.

The idea of considering health benefits when selecting produce is having an influence on the production of fresh produce (Raskin et al., 2002). The current concept of food quality commonly encompasses four categories: nutrient content, food safety, sensory factors, and the environmental impact of food production (Grimme and Dumontet, 2000). According to Grimme and Dumontet (2000), these criteria can best be fulfilled by food that is regionally and organically produced, seasonably marketed, and prepared without processing.

Plant leaves exhibit a rapid rate of respiration making lettuce a highly perishable food item (Fennema, 1996). The shelf-life of all lettuce is fairly short, even with refrigeration and careful handling, but postharvest treatment is especially critical for leaf and butterhead lettuce. Recent produce outbreaks, particularly those implicating *Escherichia coli* O157:H7, illustrate how important it is to educate growers and consumers about the potential for pathogen problems with fresh produce, including pathogens of animal origin (Sivapalasingam et al., 2004). Changes in production location and distribution mechanisms may be the best strategy for preventing future *E. coli* O157:H7 outbreaks in leafy greens (King, 2006). Point of origin and brand identification systems may be useful in improving food safety of fresh produce.

Lettuce, because of a relatively short production schedule, favorable edible-to-nonedible biomass ratio, nutrient content, and high photosynthetic rate throughout production, has been considered a good crop candidate for life support systems in space (Rivera, Battistelli et al., 2006) and could constitute a source of fresh food for astronauts during space missions (Knight and Mitchell, 1983). The extraction of antioxidant phenolics from various types of lettuce, to be used in the production of functional foods, is a viable possibility (Llorach et al., 2004).

Growth and development.

Leaf crops, like lettuce, are composed of tissues that are fundamentally different from the root and stem tissue of other vegetable crops. The primary daytime function of leaves is photosynthesis and function is closely associated with morphology. Leaves are flat, expanded organs with an epidermis, a well-developed cuticle, and stomata on the exterior and closely packed palisade cells loaded with chloroplasts in the interior (Fennema, 1996). Transpiration, the movement of water, and transportation, the movement of other solutes, take place through a vascular system of netlike veins. In lettuce, the major veins develop upward and outward from the base of the leaf and minor veins develop from the tip to the base which reflects the overall tip to base maturation pattern of the leaf (Raven et al., 1999).

Lettuce is considered to be a cool season crop because it grows optimally at temperatures below 30 °C but it is capable of growing under many different environmental conditions. At all growth stages, climatic factors influence the physiology and development of lettuce. Productivity of lettuce is directly related to incident light energy (Wien, 1997) and temperature is the main factor determining the growth rate of lettuce during emergence and early growing stages (Rubatzky and Yamaguchi, 1997). Vernalization, or exposure to low temperatures in early stages of development, can affect timing of seedstalk formation. The photoreversible properties of the plant pigment phytochrome were discovered in classic experiments using lettuce (Raven, Evert, & Eichhorn, 1999). Because of its high degree of sensitivity, lettuce is the standard crop for checking environmental conditions in plant growth chambers (Wien, 1997). Lettuce is normally a quantitative long-day plant that requires a critical length of light exposure to induce flowering (Raven, Evert, & Eichhorn, 1999), although many modern cultivars are day-neutral (Rubatzky and Yamaguchi, 1997). It undergoes dramatic morphological changes during flower stalk initiation, changing from a rosette of leaves to a tall plant with elongated internodes and terminal flowers, a process referred to as bolting.

Optimal temperature for lettuce growth is 18 °C, with a range from 7 °C to 24 °C (Wien, 1997) although a regime of 16 °C day and 13 °C night is reported to be the most advantageous during early growth and 20 °C days and 10 °C nights during later stages (Rubatzky and Yamaguchi, 1997). Temperatures above 30 °C (86° F) usually stunt lettuce growth, promote bolting and tipburn, and reportedly result in bitterness (Rubatzky and Yamaguchi, 1997). Bolting is a common problem in Colorado where summer temperatures often exceed 30°C. Production techniques and choice of bolt resistant cultivars can improve the chances of successful lettuce production throughout the growing season (Stonaker and Guenther, 2003). Maturation of lettuce can take from 60 to 120 days depending on cultivar and growing conditions (Ryder, 1999).

Besides their individual affects, the interactions of light and temperature factors also have a profound influence on the growth and development of lettuce. Although these mechanisms are not completely understood, growth hormones are thought to be involved and can be used to reverse many light and temperature effects (Wien, 1997). Light and temperature are also major determinants of leaf number and growth rate. These sensitivities to environmental factors can make lettuce a difficult crop to grow.

Seasonality and climatic stress.

Environmental stress is defined as a set of physical and chemical factors of the environment that are unfavorable to the growth of a plant species (Mano, 2002). Numerous abiotic (temperature, light, nutrients, water) factors of varying strengths affect plant growth and yield (Trewavas, 2005). Plants are normally sensitive to these signals and have developed responses that facilitate survival in changing environments. The reaction of a plant to stress or environmental change is usually in the direction of self-preservation and protection. Under field conditions, the performance of a crop depends on physiological mechanisms which allow plants to adapt and acclimate to fluctuating environmental conditions.

Radiant energy in the form of light is utilized by plants in two distinct ways: as a source of energy and as a source of information. Light information conveyed to plants can vary in quality, quantity, direction, and periodicity. Plants have developed specialized systems to process both the energy and the information (Bunning, 1943; Hart, 1988) and plants in the composite family appear to be especially responsive to light. Sunflowers exhibit phototropism, the tendency to move toward light, and *Lactuca serriola*, from which *Lactuca sativa* is believed to have been derived (Rubatzky and Yamaguchi, 1997), is known as the compass plant because it responds to sunlight by orienting leaves in a north-south configuration (Fitter and Hay, 2002). Light quality has been shown to influence the development of bitter taste in lettuce (Eskins, Warner, & Felker, 1996). Light is required for the formation of some phenolic compounds, like the flavonol glycosides, quercetin and kaempferol (Dumas et al., 2003). The higher light intensity that occurs in Colorado may create stresses in crops that shift the flux of metabolic processes and affect phytochemical synthesis (Langebartels et al., 2002; Perl-Treves and Perl, 2002).

The standard measure of solar radiation, given in values of energy per unit of area, provides information on how much of the sun's energy strikes a surface during a particular time period. Total langleys are units of radiant flux equal to one calorie per square centimeter (cal/cm^2). This measure of the total energy received in the ultraviolet, visible, and infrared wavelengths is a much wider band of wavelengths than plants can utilize but can be useful as an indicator of the amount of light available for plant growth (Hart, 1988).

In nature, changes in temperature are likely to occur more rapidly than other stress-causing factors. Plants are subject to diurnal temperature fluctuations as well as seasonal changes and must adapt efficiently to heat and cold stress to survive (Hirt and Shinozaki, 2004). Leafy vegetable crops, such as lettuce, have a high surface area to volume ratio and are subject to high transpiration losses and are clearly limited by excessive transpiration concurrent with exposure to high temperatures (Grierson, 2002).

The concept of growing degree days (GDD) is a useful concept for expressing heat units. Plant development is dependent on daily accumulation of heat and GDD can be used as a measure to estimate temperature effects on growth and development throughout the growing season. A certain amount of heat is required for plants to move to the next development stage and that value remains constant for a particular species. The minimum base temperature, or threshold below which development does not occur, has been determined to be 4.4°C for lettuce (Ryder, 1999). Daily GDD is calculated by subtracting the base temperature of lettuce (4.4°C) from the mean daily temperature (maximum + minimum/2). If the daily GDD value is a negative number, it is made equal to zero. The total of daily GDD values during a specified time period, generally thirty days before harvest, can be used as an accumulated heat index value.

The principles employed in plant breeding research take environmental conditions into consideration as well as the interaction of the genotype and the environment. A particular macro-environment may be characterized by the growing conditions of the location, a particular growing season or year, or may be the combination of location and year. Although it is beneficial to develop cultivars suited to local growing conditions, seasonal conditions are generally unpredictable. Due to genotype x environment interactions, it can be difficult to establish the contribution of plant breeding to crop improvement (Bos and Caligari, 1995). Vegetative growth of lettuce is typically responsive to changes in temperature so the development of lettuce cultivars adapted to specific temperature parameters would be valuable (Ryder, 1999).

Lettuce is a true vegetable since the edible portions are leaves. The leaves are the major sites of photosynthesis and photorespiration and these processes produce a number of reactive oxygen species (ROS) including superoxide, hydrogen peroxide, hydroxyl radicals and singlet oxygen (Shahidi, 2003). Plant cells have developed a number of regulatory mechanisms to limit the accumulation of these molecules including the production of a variety of antioxidants. Stress caused by environmental factors may enhance the synthesis of antioxidant compounds. The series of interactions between photosynthesis and oxygen metabolism is highly complex. Oxygen molecules act as electron acceptor for photosynthesis and as a substrate in photorespiration. Photorespiration provides essential glycine for the synthesis of gluthione, contributing to the accrual of this antioxidant (Wien, 1997). Chloroplasts have embraced the potential for using oxygen for metabolism while limiting the deleterious effects of oxygen interactions. Exposure of plants to high intensity light can lead to an accumulation of SOD (superoxide dismutase) (Hirt and Shinozaki, 2004).

Improving plant resistance to stress may have the beneficial side effect of improving the nutritional quality of the human diet (Demmig-Adams and Adams, 2002). Studies looking at spinach and tomato have reported significant variation due to seasonality (Howard et al., 2002; Dumas, Dadomo, Di Lucca, & Grolier, 2003; Raffo et al., 2006). Few studies with lettuce have examined seasonality but Hertog and others (1992) found 3 to 5 times more flavonoid quantities in lettuce sampled in the summer compared to other seasons. Liu, et al. (2005) observed significantly higher antioxidant capacity in lettuce harvested late in the growing season ($P<0.05$), indicating that this characteristic may be affected by environmental conditions.

Chemical composition of lettuce.

Plants are complex systems with enzymatic, chemical, microbiological, and multiple defense mechanisms occurring simultaneously. The primary chemical synthesis pathways of plants are involved with photosynthesis, cellular respiration, growth, translocation, nutrient assimilation, and reproduction. Diverse metabolic pathways have been developed that branch from primary pathways and are not essential for survival. The enzyme, phenylalanine ammonia lyase (PAL), controls the diversion of carbon from primary metabolism into the production of phenylpropanoids and PAL activity is stimulated by red light and UV radiation (Hopkins and Hüner, 2004). The products of secondary metabolism play a role in attracting pollinators and providing protection from pathogens, predators, competitors, and stresses (Edwards, 1999). In plants, a relatively small number of precursors and types of reactions give rise to an enormous range of protective compounds but any given plant species only synthesizes a defined set of products (Edwards, 1999). Harvested vegetables contain a wide range of different chemical compounds and show great variation in composition (Fennema, 1996).

Lettuce, like other raw agricultural commodities, is not a stable, well-defined entity. The chemical composition of plants of the same species may vary considerably and be profoundly influenced by cultivar, developmental stage and degree of maturity, agronomic practices, moisture, soil composition, region, and climatic conditions (Wien, 1997); (Crosby, 1963; Lee and Chichester, 1974). Since lettuce is produced from seed, some variability can be expected among lettuce plants of the same cultivar and there may possibly be variation between leaves of the same lettuce plant. Several studies (Bilyk and Sapers, 1985; Crozier et al., 1997; DuPont et al., 2000; Hohl et al., 2001) have reported large variation in chemical composition between inner and outer leaves of lettuce. These differences are usually attributed to pigmentation and tissue dissimilarities.

Quantitative measurements of chemical components of lettuce are particularly important to study because lettuce is almost always consumed fresh; therefore its components are not subjected to the same degradative effects of processing and storage seen with many other vegetables (Hertog, Hollman, & Katan, 1992; Crozier, Lean, McDonald, & Black, 1997; Ninfali and Bacchiocca, 2003).

The primary chemical constituent in lettuce, like most produce, is water which generally accounts for 94, 95, and 96 percent of fresh romaine, crisphead, and butterhead lettuce, respectively (Crosby, 1963). The moisture content of lettuce represents not only water but all other easily volatilized substances. The maximum moisture content of leaf tissue is influenced by its structural and chemical characteristics as well as extrinsic factors like relative humidity (Fennema, 1996).

Latex is the milk-like substance produced in articulated laticifers of lettuce. Laticifers are the vessel-like series of cells that permeate various tissues of some plant species. Latex is composed of cytoplasm filled with globular vacuoles containing lytic enzymes, especially glycosidases and β-focosidase, which appear to play a role in the degradation of the cell wall that accompanies the formation of the laticifers (Giordani and Noat, 1988).

Terpenoids are the largest class of secondary metabolites; some, like gibberellins, act as plant hormones regulating physiological functions but others are involved in host defense and protective functions. Sesquiterpene lactones are terpenoids composed of three isoprene units and known for a variety of biological activities (de las Heras et al., 2003). The production of these compounds is a family characteristic and 430, out of nearly 500 sesquiterpene lactones identified, have been isolated from plants in the Asteraeace family (Heywood, Harborne, & Turner, 1977). Lactucin, identified from the genus *Lactuca*, is one of the major bitter principles and responsible for the sedative effects (Crosby, 1963). Another bitter compound in lettuce, lactucopicrin, has not been observed elsewhere in higher plants except in the related species, *Taraxacum officinale* (dandelion) (Crosby, 1963). Sesquiterpene lactones are responsible for the anti-inflammatory activity of a variety of medicinal plants.

Lutein, β-carotene, violaxanthin, and neoxanthin are the major carotenoids synthesized in green leaves (Calvo, 2005). In a review of lutein concentration in 74 fruits and vegetables, lutein was generally higher in green vegetables, like lettuce, (Calvo, 2005) and lutein content of lettuce ranged from .073 to 2.92 mg/100 g fresh weight depending on lettuce type. The carotenoid, lactucazanthin (ε,ε-carotene-3,3-diol) has been found only in lettuce (Kimura and Rodriquez-Amaya, 2003).

In most studies that have investigated the chemical composition of fresh produce, samples were collected for analysis from retail markets and consequently many factors, including cultivar, growing location and method, planting date, harvest date, environmental conditions, and post-harvest history, were unknown (Hertog, Hollman, & Katan, 1992; Cao et al., 1996; Crozier, Lean, McDonald, & Black, 1997; Vinson, Hao, Su, & Zubik, 1998; Chu et al., 2002; Wu et al., 2004). Research comparing the phytochemical content of produce has often reported conflicting results, in part because growing conditions were not parallel. Using uniform samples of known cultivars and controlling growing, harvest, and postharvest conditions assures product consistency and improves reliability of results.

Total Phenolic Content.

Phenolic compounds are ubiquitous in the plant kingdom and include a wide range of compounds with a broad spectrum of functions. Several thousand phenolic molecules have been identified from plant extracts and several hundred are found in edible plants (Manach et al., 2004). The phenolic content of produce can have a major impact on color and flavor (Fennema, 1996). Plant leaves usually contain some mixture of flavonol glycosides, hydroxycinnamate conjugates, condensed tannins, and anthocyanins. These phenolic compounds are localized in various parts of the plant based on the function they serve.

Plants can reduce the level of UV-B radiation that reaches mesophyll cells by producing a variety of light-absorbing compounds which accumulate in cuticle, cell wall and cytosol of epidermal cells (Langebartels, Schraudner, Heller, Ernst, & Sandermann, 2002). Pigments are synthesized in response to light in many plant species and provide an efficient screen because they absorb from 280 to 340 nm without diminishing the amount of photosynthetically active radiation (Wang et al., 1997). These pigments are usually flavonoids and include flavonols, flavones and anthocyanins. Quercetin and kaempferol are two of the major flavonols found in vegetables while luteolin and apigenin are important flavones. Although flavonols are commonly occurring in dietary plants their intake is generally low (Manach, Williamson, Morand, Scalbert, & Remesy, 2005). Several studies have reported the occurrence of quercetin and luteolin in *Lactuca sativa* (Rees and Harborne, 1984; Bilyk and Sapers, 1985; Crozier, Lean, McDonald, & Black, 1997).

The most colorful of the flavonoids are the anthocyanins, found in a wide array of plant families as flower, fruit, and leaf pigments. Anthocyanins are water-soluble glycosides and acyl-glycosides of anthocyanidins and, in living cells, are usually in solution within the vacuole (Strack and Wray, 1994; Lea and Leegood, 1999). Anthocyanins are multifunctional compounds that appear to be effective phytoprotectants in plants. They absorb solar radiation and scavenge ROS and their synthesis is influenced by a range of environmental stresses (Gould et al., 2002). Anthocyanins may protect cells from redox state alterations and reduce lipid peroxidation (Chalker-Scott, 1999). In the upper epidermis, anthocyanins can screen UV-B and, in the mesophyll, protect chloroplasts from photoinhibition. The predominant anthocyanin in lettuce is cyanidin 3-malonylglucoside(Gazula et al., 2005). A complementary gene pair determines the presence of anthocyanins and a multiple allelic system controls the pattern of red coloration (Gazula, Kleinhenz, Streeter, & Miller, 2005). Anthocyanin-rich vegetables, like red leaf lettuce, have shown more antioxidant activity than other vegetables (Hassimotto et al., 2005). Wu and Prior (2005) identified four anthocyanins in red leaf lettuce: cyanidin 3-glucoside, cyanidin 3-(6"-malonlyl)- glucoside, cyanidin 3-(6"-acetoyl)glucoside and cyanidin 3-(3"-malonoyl) glucoside. The localization of anthocyanins in leaf tissue may afford protection for the plant from a number of environmental stresses (Chalker-Scott, 1999). Red-pigmented types of lettuce appear to be better sources of nutrients and phytochemicals (Simon, 1997; Hassimotto, Genovese, & Lajolo, 2005). The variability in pigmentation between red and green leaf lettuce cultivars facilitates comparisons that may help in understanding the functions of anthocyanins.

The two major classes of phenolic compounds identified in lettuce are caffeic acid derivatives and flavonols (Romani et al., 2002). The main caffeic acid derivatives are monocaffeoyl tartaric acid, dicaffeoyltartaric acid (chicoric acid), 5-caffeoylquinic acid (chlorogenic acid) and 3, 5-di-O-caffeoylquinic acid (isoschlorogenic acid) and the main flavonols are quercetin derivatives. Qualitative and quantitative differences in these phenolic compounds have been observed among lettuce cultivars. Dupont and others (2000) identified quercetin conjugates in green leaf cultivars and cyanidin conjugates in red leaf cultivars. Nicolle et al. (2004) reported finding 10-fold more chlorogenic acid in a red leaf cultivar than in a green leaf cultivar as well as quantitative differences in dicaffeoyl tartaric acid among green leafed lettuce. Caldwell (2003) identified the major phenols in red leaf lettuce to be mono- and dicaffeoyl esters of tartaric and quinic acid and reported that red leaf lettuce appears to be sensitive to environmental factors. Over 50-fold differences in quercetin content have been reported among lettuce cultivars (Crozier, Lean, McDonald, & Black, 1997) with highest levels seen in a red-leafed cultivar. Hertog et al. (1992) found 15-fold differences in quercetin levels in lettuce due to seasonal influences, with highest levels observed in the summer. Bilyk and Saber (1985) reported highly variable amounts of quercetin in leaf and head lettuce and traces of kaempferol. It is interesting to note that in this 1985 study, it is suggested that levels of quercetin in lettuce could be reduced through breeding programs, if so desired.

Liu et al. (2005) assessed total phenolic content and antioxidant capacity of 25 cultivars of lettuce and found that phenolic levels between leaf lettuce cultivars varied more than 4-fold. Red pigmented cultivars possessed significantly higher ($P < 0.05$) levels of total phenolics and antioxidant activity as measured by $DPPH^{\bullet}$ radical scavenging. Among different types of lettuce, leaf lettuce and romaine exhibited significantly higher concentrations ($P < 0.05$) of total phenolics. Higher amounts of polyphenols have been reported in lettuce grown in open air compared to greenhouse lettuce (Romani et al., 2002).

A number of plant-based phenolic compounds are known to be bitter and therefore aversive to the consumer (Lesschaeve and Noble, 2005); yet many studies assessing phenolic content of various crops do not evaluate taste.

The Folin-Ciocalteu assay is a widely accepted spectrophotometric method based on a color reaction that is used to determine the quantity of total phenolic compounds in plant extracts (Singleton and Rossi, 1965; Spanos and Wrolstad, 1990) using a commercially available reagent. With reaction time and temperature being held constant, absorbance of the stable chromophore at 765nm is used to estimate phenolic content by derivation from a standard curve based upon gallic acid and expressed as milligrams per g of tissue weight. The presence of ascorbic acid in vegetable extracts can interfere with the Folin-Ciocalteu assay (Prior et al., 2005) but data can be adjusted for this interference if necessary.

Radical Scavenging Capacity.

Reactive oxygen species are constantly produced in chloroplasts, mitochondria and other plant cell organelles (Mano, 2002). During normal metabolic processes, molecules can lose an electron which creates unstable molecules which will attempt to remove electrons from other cell components in order to stabilize themselves (Willcox et al., 2004). Numerous stresses affect the production of damaging oxidative molecules in plant tissues, which is countered by a protective system of antioxidant compounds and enzymes (Foyer, 2002). An antioxidant is a substance that opposes oxidation or inhibits reactions promoted by oxygen or peroxides. Dietary antioxidants may decrease the adverse effects of reactive oxygen and nitrogen species on bioactive molecules such as DNA, proteins, and lipids via multiple mechanisms (Willcox, Ash, & Catignani, 2004).

The relationship between antioxidant status and health benefits is still poorly understood despite being the focus of numerous studies. Antioxidants may reduce the risks of life-threatening diseases like atherosclerosis and cancer by inhibiting reactive oxygen (ROS) or nitrogen species (RNS) formation, scavenging ROS/RNS or their precursors or deactivating pro-oxidant metal ions (Willcox, Ash, & Catignani, 2004). Natural antioxidants have been the focus of much recent scientific and public interest because of growing evidence that suggests they may inhibit oxidative damage that contributes to many chronic disease processes (Velioglu et al., 1998; Chu, Sun, Wu, & Liu, 2002). In particular, attention to the antioxidant activity of fruits and vegetables is increasing since epidemiological evidence has established their protective effect (Shahidi, 2003). Antioxidants may reduce the risks of life-threatening diseases by breaking the free radical chain mechanism, chelating pro-oxidant metal ions, scavenging free radicals, or stimulating the activity of antioxidative enzymes (Willcox, Ash, & Catignani, 2004). More than a 1000-fold difference in quantity has been reported among total antioxidants in dietary plants and members of the Asteraceae family are considered very good sources of antioxidants (Halvorsen et al., 2002).

Few studies have investigated the effect of environmental factors on radical scavenging capacity. Zhou and Yu (2004) reported correlations between solar radiation and radical scavenging capacity in wheat and Moore et al. (2006) investigated effects of environment on antioxidant properties in wheat bran. Liu and others (2005) observed significantly higher ($P < 0.05$) levels of $DPPH^{\bullet}$ radical scavenging in red-pigmented cultivars of lettuce and higher antioxidant capacity in lettuce harvested late in the growing season ($P<0.05$), indicating that this characteristic may be affected by environmental conditions.

Radical scavenging capacity can be assessed with several different assays. The overall goal of antioxidant assays is to predict biological effectiveness of antioxidants and that can be difficult to ascertain based on any single in vitro assay. With the ABTS (2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) method, a colorless chromagen is changed into a colored monocationic radical form ($ABTS^{\bullet+}$) by an oxidative agent and the addition of antioxidants results in a degree of decolorization relative to the antioxidant concentration (Miller and Rice-Evans, 1997). The measurement of relative activities of hydrogen-donating antioxidants is based on their ability to scavenge the radical cation $ABTS^{\bullet+}$ in comparison to standard amounts of the synthetic antioxidant Trolox (6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid), an analog of vitamin E (Miller and Rice-Evans, 1996). The use of trolox as a standard allows the results to be expressed as trolox equivalent antioxidant capacity, TEAC. The ABTS assay provides rapid and consistent estimates of radical scavenging capacity for hydrophilic antioxidants (Awika et al., 2003).

Vitamin C. L-Ascorbic acid (vitamin C), the most plentiful hydrophilic antioxidant in plant cells, is a 6-carbon lactone ring structure with 2,3-enediol. Vitamin C plays an important role in plant metabolism by acting as an antioxidant, an enzyme cofactor, and a participant in electron transport (Davey et al., 2002; Asard et al., 2004). Vitamin C is highly susceptible to oxidation in the presence of metal ions and oxidation is influenced by heat, light exposure, pH, oxygen concentration and water activity (Lee et al., 2003). Ascorbic acid is an important water-soluble vitamin in human nutrition and we must rely on plant sources of this essential vitamin because we lack the enzymes required for synthesis. The antioxidant mechanisms of ascorbic acid are based on quenching of singlet oxygen, hydrogen atom donation to lipid radicals, removal of molecular oxygen, scavenging aqueous radicals, and regeneration of vitamin E (Shils et al., 1999).

Nutritional content of lettuce.

The 2005 Dietary Guidelines suggest Americans consume 2 ½ to 6 ½ cups of fruits and vegetables daily, depending on calorie needs (DHHS/USDA, 2005). Consumption of a variety of fruits and vegetables daily is recommended and dark green leafy vegetables are mentioned specifically.

Leafy vegetables are well recognized sources of vitamins, minerals, and dietary fiber. The amounts of several common vitamins and nutrients listed in the USDA nutrient data base (2005) for various types of lettuce are shown in Table 2.2. Lettuce is a year-round source of vitamin A, vitamin C, beta-carotene, lutein, calcium and folate yet low in calories and sodium. The relative nutritional composition of foods can be estimated by an index of nutritional quality (INQ). The INQ is the percent of nutrient need provided by the food divided by the percent of caloric need provided from food (Sorenson et al., 1976). Fennema (1996) reported the INQ, based on seven major nutrients, to be about 10 times greater for lettuce, spinach, and broccoli than for whole milk, egg, or meat.

Fruits and vegetables contain compounds that have the potential to influence health beyond nutritional value (Goldman, 2003). Epidemiological evidence has consistently established the positive effects of botanical foods (Block et al., 1992) and nutrition education programs like the *National 5 A Day for Better Health Program* and the *DASH Eating Plan* encourage consumption of vegetables for their associated health benefits. A diet rich in fruits and vegetables appears to be protective against certain types of cancer, heart disease, gastrointestinal problems, and age-related eye diseases (Garewal, 1997; Lampe, 1999; Chu, Sun, Wu, & Liu, 2002). These effects seem to be related to antioxidative compounds and phenolic-based metabolites produced by plants (Lampe, 1999; Kris-Etherton et al., 2002; Morello, Shahidi, & Ho, 2002; McCann et al., 2003).

Table 2.2. Nutritional components of commonly available types of lettuce.

Nutrient*	**Units**	**Lettuce type**				
		Iceberg	*Green Leaf*	*Red Leaf*	*Romaine*	*Butterhead*
Vitamin A	IU	502	7405	7492	5807	3312
Vitamin C	mg	2.8	1.8	3.7	24	3.7
Vitamin K	mg	24	174	140	103	102
Folic Acid	mcg	29	38	36	136	73
Niacin	mg	0.12	0.38	0.32	0.31	0.36
Riboflavin	mg	0.03	0.08	0.08	0.07	0.06
Thiamin	mg	0.04	0.07	0.07	0.07	0.06
Calcium	mg	18	36	33	33	35
Iron	mg	0.41	0.86	1.2	0.97	1.24
Potassium	mg	141	194	187	247	238
Phosphorus	mg	20	29	28	30	33
Þ-carotene	mcg	229	4443	4495	3484	1987
Lutein + Zeaxanthin	mcg	277	1730	1724	2312	1223

*100g fresh weight/USDA National Nutrient Database for Standard Reference, Release 18 (Aug., 2005)

Phenolic compounds appear to contribute to the prevention of cardiovascular diseases, cancers, inflammation, neurodegenerative diseases and diabetes and roles other than antioxidation appear be involved (Finley, 2004; Heber, 2004; Halliwell et al., 2005; Manach, Williamson, Morand, Scalbert, & Remesy, 2005; Scalbert, Johnson, & Saltmarsh, 2005). Even substances like proanthocyanidins, which are poorly absorbed and therefore never reach plasma, may exert activity in the gastrointestinal tract. This can be important because the intestinal tract is frequently exposed to oxidizing agents and may be at risk for a variety of diseases, including cancer.

The best nutritional strategy for establishing a healthy diet may be to include a diverse variety of fruits and vegetables because of the multiplicity of agents with polyvalent characteristics. Antioxidants appear to interact in a network (Svilaas et al., 2004) and phytonutrients may act collectively and synergistically (Gopalan and Tamber, 2003; Liu, 2003). Integration of information regarding phenolics from different disciplines such as nutrition, food chemistry, and horticulture will be needed to identify the most effective phenolic compounds and to determine optimal dietary levels.

Of all food groups analyzed in a study that evaluated the relationship between fruit and vegetable intake and risk of major chronic disease, green leafy vegetable intake showed the strongest inverse association with major chronic diseases (Hung et al., 2004). Lettuce and other leafy vegetables are particularly good sources of nutrients and various bioactive compounds related to the physiological and defensive functions of leaves (Tarwadi and Agte, 2003). Botanical diversity of fruits and vegetables may play a role in determining the bioactivity of diets with high intake of produce (Thompson et al., 2006). Taxonomic families often have common biochemical characteristics (Fennema, 1996) and since lettuce is the only commonly consumed food from the plant family Asteraceae, it may provide phytonutrients not available from other foods.

Ingestion of fresh lettuce has been shown to increase plasma total antioxidant capacity (Serafini et al., 2002). When freeze-dried lettuce was substituted for twenty per cent of the dry matter in a daily diet fed to rats, antioxidant status improved and beneficial effects on lipid metabolism were observed (Nicolle, Cardinault et al., 2004). Lettuce is almost always served in a salad with other vegetables and fruits which is more nutritionally effective than individual foods (Su and Arab, 2006) (Ninfali et al., 2005) and dressings commonly served with salad may improve nutrient bioavailability (Brown et al., 2004).

Nutritional profiles vary considerably among types and cultivars of lettuce (Simonne, Simonne, Eitenmiller, & Coker, 2002) and with environmental factors which fluctuate widely throughout the long growing season of lettuce (Inzé and Van Montagu, 2002). The chemical profiles of plant leaves can be impacted by fluctuations in numerous biotic and abiotic signals (Trewavas, 2005, Krishna, 2004).

The practice of selecting cultivars based on yield or other factors has possibly resulted in decreases in nutritional composition of some foods (Davis et al., 2004). The emerging trend of choosing foods based on nutritional benefits (Heber and Bowerman, 2001; Pollard et al., 2002) is having an impact on agriculture (Shahidi, 2003) and factors like antioxidant and phenolic content are becoming recognized as desirable traits in fruit and vegetable crops (Velioglu, Mazza, Gao, & Oomah, 1998; Scalzo et al., 2005).

Vinson et al. (1998) reported the per capita phenolic contribution from lettuce to be 6.2 mg/day, placing lettuce 9th out of 23 vegetables tested. Chun and others (2005) reported the phenolic contribution from lettuce to be 9.8 mg per day, about one tenth of average intake, and for total flavonoids, head lettuce outranked broccoli, tomatoes, carrots and spinach.

Food safety considerations.

The 2005 Dietary Guidelines for Americans (DHHS/USDA, 2005) acknowledge the importance of food safety in promoting health. Safe production practices and food handling are particularly important with vegetables that are consumed raw, like lettuce. Not long ago, produce was considered relatively safe compared to meat, dairy and seafood but in light of recent outbreaks (Johnston et al., 2005), the food safety issues associated with leafy greens are receiving more attention. (Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). Increases in temperatures and humidity due to cellular respiration and wounds from the harvesting process can provide an ideal environment for growth of spoilage and pathogenic micro-organisms on lettuce. The increasing incidence of pathogens that can withstand refrigerated temperatures, along with changes in agriculture production, have increased the risk of food borne illness associated with salad crops (Johnston et al., 2005).

Sensory evaluation.

Sensory analysis of food is the scientific discipline used to evoke, measure, analyze and interpret reactions to food characteristics as they are perceived by the senses of sight, smell, taste, touch and hearing for the purposes of evaluating consumer products (Stone and Sidel, 1993). Human judges are used to measure sensory attributes in a conscious effort to identify and evaluate different sensations and components in a piece of food. Vision, olfaction gestation, tactile senses, kinesthetic senses, audition, and trigeminal sense (bitterness) are used in judging (Lawless and Heymann, 1999).

Favorable sensory attributes are essential for consumer acceptance of fresh fruits and vegetables (Pollard, Kirk, & Cade, 2002). The evaluation of sensory properties in fresh produce, especially leafy vegetables, presents a unique set of challenges including color variability, rapid perishability and product inhomogeneity. Five of the sensory characteristics of lettuce commonly evaluated in sensory analysis are bitterness, appearance, texture, flavor, and overall acceptability.

Bitterness. Bitterness is one of the four commonly accepted taste qualities but evaluating it can be tricky. The threshold for detecting bitterness is much lower than other tastes and may be an evolutionary adaptation to prevent the ingestion of poisonous substances (Drewnowski and Gomez-Carneros, 2000). Humans are able to detect bitterness at a much higher acuity level (1 part in 2,000,000) than sweetness (1 part in 200) although individual bitterness perception varies considerably (Drewnowski, 1997; Roy, 1997). Bitter taste is associated with a great number of structurally diverse compounds and is usually aversive although a slight bitter taste can contribute to the palatability of some foods (Hofmann et al., 2004). Bitterness can be detected at the back of the tongue, back of the hard palate and the pharynx but the intensity of bitter taste in many foods is strongest after swallowing. The sensation of bitterness requires the simultaneous participation of a number of different papillae and the binding of bitter compounds to receptor membranes can be relatively slow. (Crosby, 1963). The oral response time to bitterness is generally slower than the other common tastes and aftertaste may be quite long. The complexity of bitterness response makes studying this taste quality difficult.

Although bitter substances are widely distributed in the Asteraeace family, only extracts of *Artemisia absinthium* (wormwood) and *Cnicus benedictus* are used as bitter substances in the pharmalogical and food industries. The bitter characteristics of wormwood have been known since biblical times (Revelation, Chapter 8, v 10-11). *Artemisia* supplies the chief flavoring ingredient of the liqueur known as absinthe and is used therapeutically for its carminative, anti-microbial, and anthelmintic properties (Heywood, Harborne, & Turner, 1977).

Excessive bitterness is generally associated with consumer rejection (Drewnowski and Gomez-Carneros, 2000). In a study that examined bitter and sweet flavors as mediators of vegetable preference and intake, bitterness was considered a deterrent to vegetable consumption (Dinehart et al., 2006). Bitterness is an important flavor characteristic of lettuce and variation in degree of bitterness has been reported among lettuce cultivars (Simonne, Simonne, Eitenmiller, & Coker, 2002). It is generally accepted that this results from higher levels of certain phytochemicals, particularly the sesquiterpene lactone compounds, lactucin and lactucopricrin (Price et al., 1990). The glycoside of lactucin was found to be the compound with the highest correlation ($r = 0.800$) with bitterness in lettuce.

Appearance. Outward appearance is one of most important attributes evaluated by the consumer in the selection of vegetables (Shewfelt, 1990). It is especially important for lettuce since it is the primary attribute used by consumers in making the decision to purchase (Allende et al., 2004; Piagentini et al., 2005). Visual imperfections, both type and quantity, can influence acceptance by consumers. Due to the variations in leaf pigmentation and patterns associated with chlorophyll and anthocyanin content, color is a key element of appearance in lettuce and important to crop quality (Kleinhenz et al., 2003). Lettuce leaf pigment concentrations may be reduced at higher growing temperatures (Gazula, Kleinhenz, Streeter, & Miller, 2005) and could impact the acceptability of lettuce appearance.

Flavor. Perception of flavor involves a number of factors and has a major role in determining the acceptability of foods. In foods that are consumed raw, like lettuce, flavor is determined by substances that are either naturally present or formed immediately by chemical reaction (Carpenter et al., 2000). Lettuce is well-known for its mild flavor (Labensky, Hause, & Labensky, 2003) and the key components of lettuce flavor are believed to be sweetness and bitterness (Delaquis et al., 2000).

Texture. The texture of fresh vegetables is primarily determined by cell wall structure and turgor pressure (Waldron et al., 2003). Texture is a critical feature of produce because consumers associate crispness and crunchiness with freshness and wholesomeness (Allende, Aguayo, & Artes, 2004). Fresh lettuce is expected to have a crispy consistency despite having a composition that is approximately 95 percent water. The perception of a crunchy texture includes sounds, fracture characteristics, density, and geometry (Fillion and Kilcast, 2002). Crunchy is more universally used to describe produce and is defined as being associated with a hard, dense texture that fractures without prior deformation, producing a low-pitch sound that is repeated over several chews (Waldron, Parker, & Smith, 2003).

Overall acceptability. Overall acceptability may be thought of as the sum total of physical characteristics embodied in the product (Fenwick, 1996). The testing of product acceptability is different from other sensory applications because it is not analytical but is based on consumer judgment (Carpenter, Lyon, & Hasdell, 2000). It is not generally considered appropriate to recruit and train special assessors for measuring consumer acceptability because training is likely to introduce bias (Meilgaard et al., 1999). Evaluating consumer acceptability requires a group of respondents representative of the target population of product users.

Trends in fresh produce consumption are strongly influenced by consumer perception (Barrios and Costell, 2004). Many factors, including product characteristics and quality perception, influence the consumer's decision to purchase (Waldron, Parker, & Smith, 2003), so it is important that produce meets the standard expectations of the consumer.

Consumer trends in produce selection.

Despite government initiatives and consumer education programs addressing fruit and vegetable consumption, most U.S. consumers are not hitting the target amounts of these foods (Guenther et al., 2006). Consumer attitudes toward fresh produce selection are driven by many factors including quality, safety and health considerations. Intrinsic properties, like flavor and nutrition, and extrinsic qualities, like price, affect the consumer's purchasing assessment (Frewer et al., 1998). Long-term quality characteristics, such as health benefits, and environmentally friendly/sustainable food production methods are becoming more important (Waldron, Parker, & Smith, 2003). The consumer is faced with a confusing array of information, from science-based to faddish. One of the purposes of nutrition education is to develop a communication network to inform consumers so their decisions can be based on the best scientific information available. Horizontal integration of research areas that encompass safety, healthiness, freshness, taste, texture, nutritional value, and appearance of fresh produce involve multiple disciplines (Fenwick, 1996) and contribute to information that can be provided to the consumer and used in quality assessment. Although consumers are interested in improving health through dietary means, they are generally not willing to compromise on taste (Verbeke, 2006). Pollard and others (2002) recommend customizing nutrition education programs that address vegetable intake based on gender, level of fresh produce consumption, and on health behavior models. Results from an intervention study that encouraged an increase in fruit and vegetable consumption showed that people making their own food choices can adhere to advice concerning increased intake (John et al., 2002).

Consumers are interested in foods that are satisfying, pleasurable, and are healthy as well (Fenwick, 1996). Recent government programs reflect the importance of the role of diet in relation to health. The consumer preference for healthy foods may help to maintain general well-being and provide protection against some of the major diseases of our time, such as cardiovascular disease and cancer.

The combination of cultivar selection and responsiveness to climatic conditions can create opportunities for production of fruits and vegetables with improved antioxidant properties (Kalt, 2005).

Statistical evaluation.

Designing a study that has both controlled and uncontrolled elements is very challenging. Blocking is a technique used to limit the effects of variation among experimental units. A randomized blocks design is an experimental devise for estimating and comparing treatment means in blocks consisting of homogeneous experimental units

(Ott and Longnecker, 2001). The treatment effects are generally considered fixed because the treatments chosen for the experiment, in this study cultivars, are the only ones to which inference is to be made and block effects are usually considered random because the blocks are only a small subset of the larger set of blocks over which inference about treatment means is to be made; in this study the blocks are the lettuce plots. The data from a randomized blocks design include fixed effects for the treatment contributions and random effects for the block contributions, making it a mixed model (Littell et al., 2002). Proc mixed is a SAS (Statistical Analysis Systems Inc., Cary, N. C.) procedure, based on likelihood, which is used to analyze randomized blocks design data.

R. A. Fisher developed a procedure for making pairwise comparisons among a set of population means called Fisher's least significant difference (LSD) (Ott and Longnecker, 2001). The alpha level of Fisher's LSD is valid for a given comparison only if used for independent or preplanned comparisons so it is recommended to apply only after the F test for treatments has been shown to be significant (Ott and Longnecker, 2001). This revised method is referred to as Fisher's protected LSD.

Correlation values measure the strength of the linear relation between two variables. The stronger the correlation, the better one variable, x, predicts the value of another variable, y. Correlation coefficients can be used to represent the correlation between different variables.

OBJECTIVES:

- To assess the effect of cultivar differences and regional weather conditions on total phenolic content and antioxidant activity of eight selected cultivars of lettuce (*Lactuca sativa* L.) grown at six different times during two growing seasons.
- To evaluate the effect of seasonal variation in growing conditions and cultivar differences on bitterness, flavor, appearance, texture, and overall acceptability of five cultivars of lettuce.
- To examine relationships between total phenolic content, radical scavenging capacity, sensory properties, and growing conditions of selected lettuce cultivars.
- To develop an outreach plan to provide information to producers and consumers regarding nutritional attributes of Colorado-grown lettuce through web-based fact sheets and news articles.

CHAPTER III

EFFECTS OF SEASONAL VARIATION ON SENSORY PROPERTIES AND TOTAL PHENOLIC CONTENT OF FIVE LETTUCE CULTIVARS

ABSTRACT

Sensory properties of lettuce (*Lactuca sativa* L.) may vary in response to environmental factors which fluctuate throughout the growing season. Bitterness is generally thought to increase in lettuce grown at higher temperatures and may vary with phenolic content. This study evaluated sensory properties and total phenolic content of five lettuce cultivars harvested early, mid-way, and late in the growing season. Thirty panelists rated bitterness, appearance, flavor, texture, and overall acceptability of 'Crisp and Green' (green leaf), 'Crispino' (crisphead), 'Green Forest' (romaine), 'Lochness' (butterhead), and 'Vulcan' (red leaf) lettuce. There was considerable variation in sensory ratings among the 5 cultivars ($P < 0.005$) but few differences within cultivars across the growing season. 'Crispino' received higher scores ($P < 0.01$) for flavor, texture, and overall acceptability and was rated less bitter ($P < 0.05$) than other cultivars. Total phenolic content varied significantly ($P < 0.001$) among cultivars with 'Vulcan' exhibiting the highest levels. Mean scores of all attributes remained within the acceptable range, indicating that marketable lettuce can be grown in this region during summer months.

Introduction

Substantial changes have occurred in the salad crop industry in the last decade, including increased awareness of the nutrient and antioxidant content of leafy vegetables (Ryder, 2002). Recent updates in dietary recommendations (DHHS/USDA, 2005; AHA, 2006) reflect the accumulation of research associating health benefits with the consumption of fruits and vegetables. Lettuce, the most important salad crop (van Wyk, 2005), is classified as a staple because of its widespread and year-round intake (Ryder, 1999). Per capita lettuce consumption in 2005 was reported to be 22.1 pounds per year for head lettuce, 8.3 pounds for romaine and 4.0 pounds for leaf lettuce (ERS/USDA, 2006). Stevens (1974) ranked the relative nutritional value of 39 crops based on nutrient composition and annual production; lettuce ranked 26th in nutritional value but 4th in relative contribution to nutrition due to it's high frequency of consumption. The contribution lettuce currently makes to nutrient intake is very likely higher since the calculations for rank were based on 1970 statistics when total per capita consumption was reported to be 22.4 pounds of head lettuce per year and only the nutrient values of head lettuce were included. In recent years more diverse and colorful types of lettuce have gained favor with consumers (Allende, Aguayo, & Artes, 2004; Zind, 2005). Darker lettuces such as romaine and green or red leaf lettuce are better sources of several nutrients than head lettuce, including vitamin A, niacin, riboflavin, thiamine, calcium, iron, potassium, manganese, selenium, and beta-carotene (USDA, 2005).

Lettuce is an important specialty crop in Colorado and regional environmental conditions, particularly higher altitude and light intensity, may create stresses that shift the pattern of phytochemical synthesis and could influence the organoleptic properties of this crop. Bitterness, an important flavor characteristic of lettuce, is generally thought to increase with higher growing season temperatures (Simonne, Simonne, Eitenmiller, & Coker, 2002) and longer days (Rubatzky and Yamaguchi, 1997). Light quality has also been shown to influence the development of bitter taste in lettuce (Eskins, Warner, & Felker, 1996).

Favorable sensory attributes are essential for consumer acceptance of fresh produce (Pollard, Kirk, & Cade, 2002) and excessive bitterness is usually associated with consumer rejection (Drewnowski and Gomez-Carneros, 2000). In a study that examined bitter and sweet flavors as mediators of vegetable preference and intake, bitterness was considered a deterrent to vegetable consumption (Dinehart, Hayes, Bartoshuk, Lanier, & Duffy, 2006). Significant variation in degree of bitterness has been reported among lettuce cultivars (Simonne, Simonne, Eitenmiller, & Coker, 2002) and may be attributed to differing quantities of various bitter compounds including the sesquiterpene lactones, lactucin and lactucopricrin (Price, Dupont, Shepherd, Chan, & Fenwick, 1990).

A number of plant-based phenolic compounds are known to be bitter and therefore aversive to the consumer (Lesschaeve and Noble, 2005); yet many studies assessing phenolic content of various crops do not evaluate taste. Qualitative and quantitative differences in phenolic content have been observed between lettuce cultivars in several studies but the impact of variations in phenolic compounds on bitterness is unknown. Dupont and others (2000) identified quercetin conjugates in green leaf cultivars and cyanidin conjugates in red leaf cultivars. Nicolle et al. (2004) reported finding 10-fold more chlorogenic acid in a red leaf cultivar than in a green leaf cultivar as well as quantitative differences in dicaffeoyl tartaric acid among green leafed lettuce. Caldwell (2003) identified the major phenolics in red leaf lettuce to be mono- and dicaffeoyl esters of tartaric and quinic acid and reported that red leaf lettuce appears to be sensitive to environmental factors. Over 50-fold differences in quercetin content have been reported among lettuce cultivars (Crozier, Lean, McDonald, & Black, 1997). Bilyk and Saber (1985) also reported highly variable amounts of quercetin in leaf and head lettuce but only traces of kaempferol. The carotenoid, lactucazanthin (ϵ,ϵ-carotene-3,3-diol) has been found only in lettuce (Kimura and Rodriguez-Amaya, 2003). Liu and others (2005) reported that variations in phenolic content of lettuce were influenced by cultivar, type and pigmentation with red leaf cultivars exhibiting the highest levels and concluded that the effects of growing conditions on phenolic content of lettuce needs further investigation.

The evaluation of sensory and chemical properties in fresh produce, especially leafy vegetables, presents a unique set of challenges, including color variability, rapid perishability and product inhomogeneity. Considerable differences in composition may exist between samples of the same lettuce cultivars which may be attributed to amount of tissue pigmentation (Crozier, Lean, McDonald, & Black, 1997) or tissue variability between outer and inner leaves (Bilyk and Sapers, 1985; Hohl, Neubert, Pforte, Schonof, & Bohm, 2001). Using uniform samples of known cultivars and controlling growing, harvest, and postharvest conditions improves product consistency, which is essential for analytical evaluation.

The genetic composition of lettuce is an important determinate of characteristics such as leaf size, texture, color, taste (Eskins, Warner, & Felker, 1996), and phenolic content (Liu et al., 2005). Hundreds of genotypes of lettuce are currently available so identification of specific cultivars with acceptable taste and visual attributes that grow well in higher altitudes would be beneficial. Few studies have examined the effects of genetics and seasonality on sensory or chemical properties of lettuce. The goal of this study was to assess the effects of seasonal variation in regional growing conditions on sensory properties of five selected cultivars of lettuce and to investigate possible correlations between bitterness, total phenolic content, and three environmental indexes (growing degree days, radiation, measured in total Langleys, and number of days with temperatures exceeding 30° C in the thirty days preceding harvest). The five selected cultivars have exhibited promising production characteristics and represent the types most commonly available to consumers: butterhead, crisphead, green leaf, red leaf and romaine.

Materials and Methods

2.1 Plant material and growth conditions.

Five lettuce cultivars were selected for evaluation from a group of 25 previously screened for bolting resistance (Stonaker and Guenther, 2003) and total phenolic content (Liu, 2004a). 'Crisp and Green' (green leaf), 'Crispino' (crisphead), 'Vulcan' (red leaf), and 'Green Forest' (romaine) seeds were purchased from Johnny's Selected Seeds (Albion, ME) and 'Lochness' (butterhead) seeds were purchased from Vilmorin (La Ménitré, France). 'Crispino' is classified as an iceberg type of lettuce but forms a looser, less dense head than the typical head lettuce sold in retail markets. Multiple plantings of all cultivars were grown by the Specialty Crops Division of the Colorado State University Horticulture and Landscape Architecture Department in the spring and summer of 2004. The organic production methods conformed to requirements set by USDA for organic certification (add ref). Field grown plants were spaced at 8-inch intervals except for romaine transplants which were spaced 10 inches apart. Moisture levels were maintained using drip irrigation with municipal water. The lettuce cultivars were harvested at market maturity in June, July and August, 2004.

2.2. Environmental Indexes.

The metabolic processes of lettuce are particularly sensitive to temperature so maximum and minimum temperatures and total langleys, a measure of solar radiation, were monitored to determine possible correlations with sensory quality and chemical assay data. Weather data was collected from an online site that tracks data at a weather station located within 100 meters of the lettuce plot. Growing degree days (GDD) and number of days above 30 °C (86 °F) during the thirty days prior to harvest were used as measures of heat exposure. Plant development is dependent on daily accumulation of heat and GDD are used as a heat accumulation index to estimate temperature effects on growth and development during the growing season (Grierson, 2002). The amount of heat required for plants to move to the next development stage remains constant for a particular species. The minimum base temperature, or threshold below which development does not occur, has been determined to be 4.4 °C for lettuce. Daily GDD was calculated by subtracting the base temperature of lettuce (4.4 °C) from the mean daily temperature (maximum + minimum/2) (McMaster and Wilhelm, 1997). In calculating GDD, if the daily median temperature was less than the base, it was set equal to the base temperature. The daily GDD was totaled over the thirty days preceding harvest to give an accumulated heat index value.

Solar radiation data, measured with an Epply pyranometer, provides information on the amount of energy striking a surface during a particular time period. Total langleys are units of radiant flux equal to one calorie per square centimeter (cal/cm^2). This measure of the total energy received in the ultraviolet, visible and infrared wavelengths is a much wider band of wavelengths than plants can utilize but can be useful as an indicator of the amount of light available for plant growth (Hart, 1988).

2.3 Sensory Analyses.

The CSU Human Research Committee reviewed and approved the study protocol. Freshly prepared lettuce samples were evaluated by a consumer panel of 30, recruited from university students, faculty and staff members. Prospective panelists completed questionnaires concerning time availability, lettuce preferences and consumption habits. Training was limited to evaluation of bitterness. The 21 female and 9 male panelists were trained to recognize bitterness by tasting 5 solutions of wormwood (*Artemisia absinthium* L.) extract (Herbal Remedies, Casper, WY) with increasing levels of bitterness, from 0 to 100 ul/ml. Lettuce and wormwood are classified in the same taxonomic subfamily, Lactuceae, within the family, Asteraceae, and contain similar bitter substances (Wagner, 1977). On the wormwood extract label it is recommended that pregnant or lactating females not use this product and that warning was included on the consent form.

In preparation for sensory evaluation, outer, damaged or discolored leaves were removed and the lettuce was thoroughly rinsed with tap water and patted-dry with paper towels. Four bite-size pieces (2 cm x 2 cm) from inner and outer leaves were placed in snack-size zippered-sealed bags labeled with three-digit code numbers which corresponded to labeled score sheets. The order of presentation of the five cultivars was completely randomized. Water and unsalted crackers were provided for cleansing the palate between samples.

Samples were evaluated individually by panel members mid-morning on the day following harvesting. Two entire leaves of each cultivar were used for appearance evaluation. Panelists indicated their perception of flavor, appearance, texture, and overall acceptability on a 168-mm unstructured line positioned directly above 7 categories ranging from very unacceptable to very acceptable. Bitterness was evaluated using a similar 7-category line scale, labeled from extremely mild to extremely bitter. For computation and analysis, the distance in millimeters from the left end (0.00) to the marked line was measured and values were converted to a 100-point scale.

It is generally accepted that a trained panel should not be used for assessment of product acceptability because panelists would no longer behave as naïve consumers (Meilgaard, Civille, & Carr, 1999; Carpenter, Lyon, & Hasdell, 2000). The sensory panelists in this study received training in identification of various concentrations of a bitter compound in order to familiarize them with intensity of bitterness (Poli et al., 2002) but were not trained in any other sensory elements. Bitterness intensity was analyzed separately from other attributes. Since acceptability of lettuce from harvests throughout the summer months was being evaluated, the same panel was used for all three evaluations.

Bitter intensity has traditionally been measured using scalar techniques (Thorngate, 1997). The use of a linear scale allowed panelists freedom to use intermediate points along the scale for perceived intensity of attributes, as opposed to being limited to discrete options (Lawless and Heymann, 1999). Linear rating scales have been used for sensory evaluation in lettuce (Delaquis, Stewart, Cliff, Toivonen, & Moyls, 2000) and by consumers for evaluation of bitterness in beer (Einstein, 1976).

2.4. Chemical Analyses.

From each planting of the five cultivars, 35 g wedge-shaped samples, that included inner and outer leaves, were collected from three heads of lettuce and placed in a Genesis Freeze-Drier (Virtis Inc. Gardiner, NY) at – 40 °C, - 10 °C, +18 °C, +28 °C for 24 hours each. The dried samples were ground into powder using an Osterizer10 Speed Blender (Oster Inc., Boca Raton, FL) and sieved with a No. 20 Tyler sieve (WS Tyler Inc., Mentor, OH). The powdered samples were stored in 25 ml plastic centrifuge tubes sealed with a screw cap to prevent uptake of moisture and stored at -20 °C. Four hundred mg of each sample was extracted in 10 mL of 80% acetone, vortexed, and rotated in the dark for 2 hours. After centrifugation, aliquots were transferred and vacuum-centrifuged. Dried samples were stored at -80 °C.

The Folin-Ciocalteu assay, a widely accepted spectrophotometric method based on a color reaction (Singleton and Rossi, 1965; Spanos and Wrolstad, 1990), was used to quantify total phenolic content of dried lettuce tissue extracts using a commercially available reagent. Reaction time and temperature were held constant and absorbance of the stable chromophore at 765nm was used to estimate phenolic content by derivation from a standard curve based upon gallic acid and expressed as milligrams per g of dry tissue weight.

The presence of ascorbic acid in vegetable extracts can interfere with the Folin-Ciocalteu assay (Prior, Wu, & Schaich, 2005). Extracted samples were analyzed for Vitamin C content after vacuum-centrifugation to verify the absence of Vitamin C prior to the Folin-Ciocalteu assay. A 5% w/v aqueous solution of metaphosphoric acid containing 1% w/v dithiothreitol was added to extracted samples which were centrifuged and filtered through a 0.45 mm nylon syringe filter, prior to injection onto an Inertsil 4C HPLC (high performance liquid chromatography) column and run with a phosphoric acid/methanol gradient (Esparza-Rivera et al., 2006).

Chemicals. Folin-Ciocalteu reagent was obtained from Fluka Biochemica (Buchs, Switzerland), gallic acid and ascorbic acid were purchased from Sigma and Aldrich (St. Louis, MO), dithiothreitol from Research Products International (Mount Prospect, IL), and HPLC-grade methanol from EMD (San Diego, CA).

2.5. Statistical Analyses.

Mixed model analysis of variance was performed with panelist and panelist x harvest treated as random effects and harvest, cultivar, and harvest x cultivar as fixed effects. Statistical analysis of the data was performed using SAS Proc Mixed (Statistical Analysis Systems Inc., Cary, N. C., v. 9.1) with Fisher's protected least significant difference test and Pearson correlation analysis ($P < 0.05$).

Results

3.1. Seasonal variation

Variations in temperature and radiation during the three growing periods are shown in Table 3.1. The daily median temperature always exceeded 4.4 °C, the base temperature of lettuce. The June growing period had the lowest average minimum and maximum temperatures and the widest variation while July had the highest average minimum and maximum temperatures. There was considerable variation in total phenolic content (TPC) between lettuce harvested across the three harvest dates but differences among the five cultivars were not consistent (Figure 3.1). The only significant differences within cultivars by harvest dates were seen in the August harvest, when TPC was higher ($P < 0.005$) in 'Vulcan' (red leaf) and lower ($P < 0.05$) in 'Crispino' (crisphead). Correlations were measured between total phenolic content, bitterness and three environmental indexes (growing degree days (GDD), radiation (total langleys), and number of days with temperatures exceeding 30 °C (86 °F), but no significant correlations were observed ($P > 0.05$).

3.2. Sensory panel

The sensory panel consisted of Colorado State University faculty (8), staff (12) and students (8). Fifty-three per cent were above the age of 40 and 70% were female. According to pre-evaluation questionnaires, the majority of panelists consumed lettuce three or more times per week (Table 3.2). Romaine was the lettuce type most preferred while romaine and mixed greens were the kinds most frequently purchased.

3.3. Sensory Attributes

3.3.1. Appearance

For each of the 5 cultivars, appearance ratings did not vary ($P > 0.05$) by harvest month (Table 3.3). Mean appearance scores of the five cultivars, averaged over the three harvest times, varied significantly ($P < 0.0001$) and ranged from 78.6 for 'Lochness' (butterhead) to 88.3 for 'Vulcan' (red leaf) (Table 3.4). The ratings for 'Vulcan' and 'Crisp and Green' (green leaf) lettuce were in the "very acceptable" range and were significantly higher ($P < 0.05$) than the ratings for Green Forest (romaine), 'Crispino' (crisphead) and 'Lochness' (butterhead), which were classified in the "acceptable" range by the sensory panel.

3.3.2. Flavor

Within the 5 cultivars, flavor ratings did not vary by harvest month except for 'Green Forest' (romaine). For this cultivar, lettuce harvested in July scored significantly higher ($P< 0.05$) in flavor acceptability than lettuce harvested in June (Table 3.3). Across cultivars, the overall mean flavor rating for 'Crispino' (crisphead) was 79.6, which was significantly higher ($P < 0.01$) than the other four cultivars (Table 3.4). Still mean flavor scores for all cultivars were in the "acceptable" range; except for 'Green Forest' which was classified "somewhat acceptable".

3.3.3 Texture

Texture ratings did not vary ($P > 0.05$) by harvest month for any of the 5 cultivars (Table 3.3). Mean texture acceptability scores for the three harvest dates varied significantly ($P = .0006$) across cultivars from 72.7 for 'Green Forest' (romaine) to 81.4 for 'Crispino' (crisphead) (Table 3.4) but all were in the "acceptable" range.

3.3.4. Overall Acceptability

'Crisp and Green' (green leaf) lettuce harvested in August scored higher ($P <$ 0.05) in overall acceptability than 'Crisp and Green' lettuce harvested in July but otherwise there were no significant differences in overall acceptability due to harvest date (Table 3.3). Similar to the flavor results, 'Crispino' (crisphead) scored higher ($P < 0.02$) than the other cultivars in overall acceptability (Table 3.4). Mean overall acceptability scores ranged from 69.5 for 'Lochness' (butterhead) to 79.6 for 'Crispino'. The rating for

'Lochness' placed it in the "somewhat acceptable" category while the ratings for the other four cultivars were in the "acceptable" range.

3.3.5. Bitterness

In this evaluation, higher ratings for bitterness equate to higher levels of bitterness. Three of the five cultivars displayed significant differences in bitterness score by harvest date (Table 3.5). The bitterness scores of 'Crispino' (crisphead) lettuce decreased ($P < 0.05$) during the course of the growing season while 'Lochness' (butterhead) increased ($P < .05$) and 'Crisp and Green' (green leaf) lettuce exhibited higher scores ($P < 0.05$) in July than August. In contrast, 'Vulcan' (red leaf) and 'Green Forest' (romaine) did not vary ($P > 0.05$) in bitterness ratings across the three harvest dates. Across cultivars, mean bitterness score (all harvest dates) was significantly lower ($P < 0.05$) for 'Crispino' than for the other 4 cultivars evaluated (Table 3.5).

3.4. Total phenolic content

Analytical evaluations revealed considerable variation in total phenolic content among the five cultivars but variations within cultivars across the growing season were only seen for 'Vulcan' and 'Crispino' (Figure 3.1). Total phenolic content was significantly higher ($P < 0.005$) in 'Vulcan' (red leaf) and lower ($P < 0.05$) in 'Crispino' (crisphead) harvested in August compared to June and July harvests. Mean total phenolic content of the five cultivars averaged across harvest dates, varied significantly ($P < 0.0001$) from 15.1 mg GAE/g in 'Crispino' (crisphead) to 48.2 mg GAE/g in 'Vulcan' (red leaf). Increases in phenolic content did not parallel bitterness scores (Figure 3.2) and for all cultivars, correlations between phenolic content and bitterness score were not significant ($P > 0.05$).

Discussion

Favorable sensory attributes are essential for consumer acceptance of fresh produce since appearance is the first indicator consumers use in predicting quality and taste impacts repeat purchases (Pollard, Kirk, & Cade, 2002). Selection of lettuce cultivars has usually been based on shelf-life, transportability, insect resistance, and yield (Ryder, 1999) rather than sensory or nutritional attributes. Sensory and phytochemical profiles may vary considerably among cultivars and with environmental factors which fluctuate widely throughout growing seasons, particularly in Colorado. From germination to flower stalk initiation, the interactions of light and temperature have profound affects on the growth and development of lettuce (Wien, 1997) and variations in these climatic factors may also influence organoleptic properties.

Lettuce is considered a cool-season crop but is capable of growing under a wide variety of environmental conditions. Optimal temperatures for lettuce growth are reported to be 18 °C to 25 °C during the day and 10 °C to 15 °C at night (Ryder, 1999). The five cultivars chosen for this study reached maturity within 70 days although maximum and minimum temperatures during the three growing periods were often outside these ranges.

Significant cultivar differences were observed for all sensory attributes ($P \leq 0.003$) and total phenolic content ($P < 0.0001$) indicating genetic composition plays a major role in sensory and chemical properties of these lettuce cultivars. 'Crispino', an iceberg subtype of crisphead lettuce, is leafier and less dense than commercially grown iceberg lettuce. It was rated highest in flavor, texture and overall acceptability and lowest in bitterness at all harvest dates (Table 3.4 and Table 3.5) although iceberg lettuce received the lowest preference ranking in the pretest questionnaire (Table 3.2). 'Vulcan' (red leaf) and ‘Crisp and Green’ (green leaf) lettuce cultivars scored highest in appearance. ‘Vulcan’ exhibited the highest level of total phenolic content of all cultivars from all harvests (Figure 3.1). No significant correlations were observed in these cultivars between total phenolic content and the environmental indexes.

Appearance is particularly important for lettuce since it is the primary attribute consumers use in making the decision to purchase (Allende, Aguayo, & Artes, 2004). Visual imperfections, both type and quantity, can influence acceptance by consumers. Due to the variations in leaf pigmentation and patterns, color is also a key element of appearance in lettuce. Lettuce leaf pigment concentrations may be reduced at higher growing temperatures (Gazula, Kleinhenz, Streeter, & Miller, 2005).

Perception of flavor involves a number of factors and has a major role in determining the acceptability of foods. In foods that are consumed raw, like lettuce, flavor is due to substances that are either naturally present or formed immediately by chemical reaction (Carpenter, Lyon, & Hasdell, 2000). The key components of lettuce flavor are believed to be sweetness and bitterness (Delaquis, Stewart, Cliff, Toivonen, & Moyls, 2000).

Texture of fresh vegetables is primarily determined by cell wall structure and turgor pressure (Waldron, Parker, & Smith, 2003) and is particularly important in raw produce because consumers associate it with freshness and wholesomeness (Allende, Aguayo, & Artes, 2004). Texture is a critical feature because fresh lettuce is expected to have a crispy, crunchy texture despite having a composition that is approximately 95 percent water. Perception of a crunchy texture is a complex concept which includes sounds, fracture characteristics, density, and geometry (Fillion and Kilcast, 2002).

The study of bitter taste can be problematic: the threshold for detecting bitterness is much lower than other tastes, bitterness acuity is highly variable, and bitterness is associated with a great number of structurally diverse compounds (Thorngate, 1997). Although bitterness is thought to increase with higher growing season temperatures, only 'Crisp and Green' (green leaf) was classified Somewhat Bitter by the panel and rated significantly higher in bitterness in the July growing period which had the highest average temperatures. Increased phenolic content could be attributed to anthocyanin pigments that are not usually associated with bitter taste in vegetables. Anthocyanin content of potato cultivars can be significantly higher without resulting in bitterness (Stushnoff, Külen et al., 2006).

Trends in fresh produce consumption are strongly influenced by consumer perception (Barrios and Costell, 2004). Many factors, including product characteristics and quality perception, influence the consumer's decision to purchase (Waldron, Parker, & Smith, 2003), so it is important that produce meets the standard expectation of the consumer. All samples in this study were scored at the higher end of the acceptability scale, suggesting that lettuce quality was not negatively impacted by environmental variations.

From the sensory analysis, scores in appearance and texture did not vary significantly between early, mid-season and late harvest dates and, with only two exceptions, flavor and overall acceptability did not vary significantly (Table 3.3). There was more variability observed with bitterness ratings from different harvest times although it was dependent upon cultivar (Table 3.5). The mean bitterness scores decreased for 'Crispino' (crisphead) and 'Crisp and Green' (green leaf) in later harvest dates but increased with 'Lochness' (butterhead).

Conclusions

Sensory panel and analytical evaluations of these five cultivars demonstrated that genetic constitution had a greater impact on sensory qualities and total phenolic content than seasonal variation. Appearance, flavor, texture, overall acceptability, degree of bitterness, and total phenolic content varied significantly ($P < 0.005$) among lettuce cultivars of different types and pigmentation patterns but, with two exceptions, did not vary within cultivars across the growing season. Sensory characteristics of fresh lettuce are important variables to producers because of their impact on consumer acceptance. Although there was significant variation in sensory ratings among the five cultivars, mean scores of all attributes remained within the acceptable range indicating that various types of lettuce with acceptable sensory qualities can be grown in this region during summer months. Red and green leaf lettuce cultivars received the highest scores for appearance but 'Crispino', an iceberg type of crisphead lettuce, was rated highest in flavor, texture, and overall acceptability and lowest in bitterness. 'Vulcan', a red leaf cultivar, exhibited the highest levels ($P < 0.001$) of total phenolic content. Evaluation of sensory properties and quantification of phenolic content in lettuce can be used to identify specific cultivars that exhibit superior attributes and may improve market competitiveness of various types of lettuce.

Table 3.1. Environmental data for the lettuce plots recorded over the 30 days preceding each harvest.

Harvest		**Temperature (°C)**				**GDD**[1]	**# Days**[2] **> 30°C**	**Solar Radiation** **Total Langleys**	
		Maximum		**Minimum**					
#	*Date*	*Mean*	*Range*	*Mean*	*Range*	*Sum*		*Mean*	*Sum*
1	6/29/2004	23.2	11.4 - 34.7	8.2	1.7 - 14.2	339.5	2	343.8	10313
2	7/27/2004	28.2	14.8 - 36.3	11.6	7.5 - 17.0	464.4	13	482.7	14482
3	8/29/2004	26.5	17.2 - 34.4	9.3	4.6 - 14.5	404.7	6	486.0	14581

[1]Heat accumulation indicator: Growing Degree Day (GDD) = ((Tmax + Tmin) ÷ 2) – 4.4° C
[2]Heat stress indicator: # of days with maximum temperatures exceeding 30° C

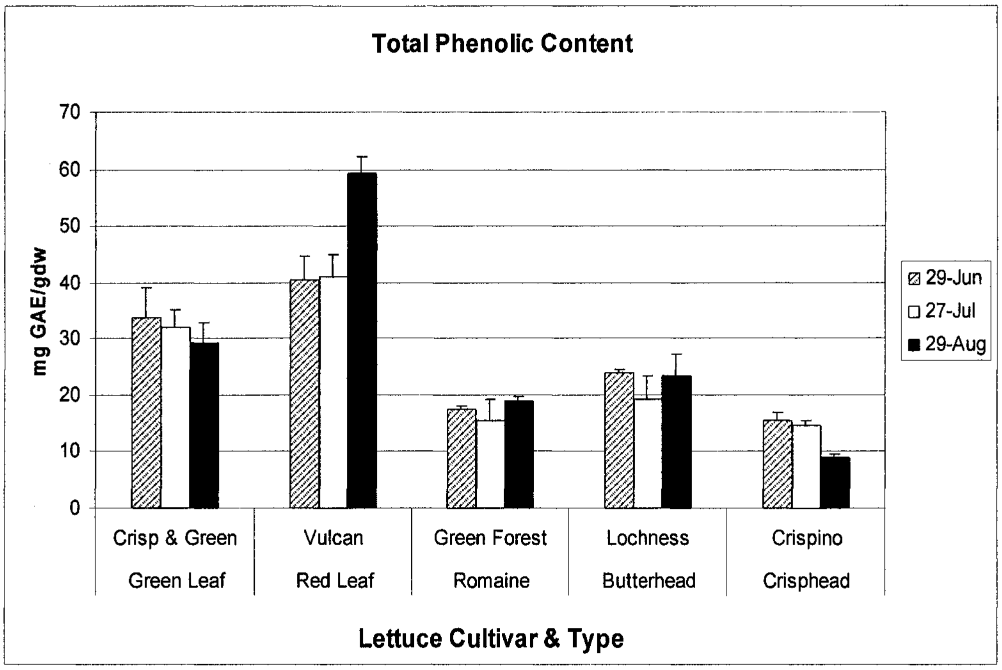


Figure 3.1. Total phenolic content of five cultivars of lettuce, measured in mg of gallic acid equivalents per gram dry weight, grown early, mid-way, and late in the growing season.

Table 3.2. Lettuce purchase habits and preferences of consumers participating in sensory evaluations (n = 30; 21 females, 9 males).

1. Approximately how often do you eat lettuce?	%
Daily	3.3
5 times per week	6. 7
3 times per week	50.0
1 time per week	26.7
Less than once per week	13.3
2. Do you have a lettuce preference?	
No	8.1
Butterhead	8.1
Green Leaf	16.2
Iceberg	10.8
Red Leaf	10.8
Romaine	46.0
3. What type of lettuce do you usually purchase?	
Butterhead	0
Green Leaf	7.9
Iceberg	5.3
Mixed greens	31.6
Red Leaf	7.9
Romaine	31.6
Various types	15.7

Table 3.3. Mean rating for sensory quality and acceptability of 5 lettuce cultivars.

Attribute	Cultivar	Type	Harvest Month		
Appearance			**June**	**July**	**August**
	Crispino	Crisphead	81.5[1] ± 2.5 a	82.6 ± 2.6a	78.3 ± 2.7a
	Crisp and Green	Green Leaf	85.2 ± 2.5 a	90.0 ± 2.6a	88.0 ± 2.7a
	Vulcan	Red Leaf	88.1 ± 2.6 a	86.4 ± 2.6a	90.5 ± 2.7a
	Green Forest	Romaine	79.6 ± 2.5 a	82.4 ± 2.6a	77.7 ± 2.7a
	Lochness	Butterhead	76.3 ± 2.5 a	80.2 ± 2.6a	79.4 ±2.7a
Flavor					
	Crispino	Crisphead	79.2 ± 3.4a	78.0 ± 3.4a	81.5 ± 3.8a
	Crisp and Green	Green Leaf	74.5 ± 3.3a	67.4 ± 3.4a	76.8 ± 3.8a
	Vulcan	Red Leaf	70.2 ± 3.4a	75.8 ± 3.3a	68.7 ± 3.8a
	Green Forest	Romaine	**64.9 ± 3.4b**	**77.1 ± 3.4a**	**70.5 ± 3.8ab**
	Lochness	Butterhead	72.3 ± 3.3a	70.4 ± 3.3a	72.6 ± 3.8a
Texture					
	Crispino	Crisphead	82.3 ± 2.9a	78.1 ± 2.9a	83.9 ± 3.1a
	Crisp and Green	Green Leaf	79.1 ± 2.8a	75.6 ± 2.9a	78.9 ± 3.1a
	Vulcan	Red Leaf	72.4 ± 2.8a	75.7 ± 2.8a	73.4 ± 3.1a
	Green Forest	Romaine	72.0 ± 2.9a	76.6 ± 2.9a	69.5 ± 3.1a
	Lochness	Butterhead	73.9 ± 2.8a	76.2± 2.9a	75.2 ± 3.1a
O. Acceptability					
	Crispino	Crisphead	75.9 ± 3.4a	82.6 ± 3.4a	80.3 ± 3.8a
	Crisp and Green	Green Leaf	**71.5 ± 3.3ab**	**68.9 ± 3.4b**	**79.5 ± 3.8a**
	Vulcan	Red Leaf	72.0 ± 3.3a	75.7 ± 3.3a	72.3 ± 3.8a
	Green Forest	Romaine	68.6 ± 3.4a	77.4 ± 3.5a	68.9 ± 3.8a
	Lochness	Butterhead	69.9 ± 3.3a	68.9 ± 3.4a	69.9 ± 3.8a

[1]Data expressed as means ± SEM. Values were measured on a 168-mm line and converted to a 100- point scale, 0-14.6 = Very Unacceptable, 14.7-29.0 = Unacceptable, 29.1-43.4 = Somewhat Unacceptable, 43.5-57.8 = Neither Acceptable nor Unacceptable, 56.9-71.2 = Somewhat Acceptable, 71.3-85.6 = Acceptable, 85.7-100 = Very Acceptable.

Means within rows followed by different letters designate significant differences ($P < 0.05$).

Table 3.4. Ratings for sensory quality and acceptability of 5 lettuce cultivars[1].

Sensory Attribute	**Cultivar**					**P value**
	Crispino Crisphead	*Crisp and Green* Green Leaf	*Vulcan* Red Leaf	*Green Forest* Romaine	*Lochness* Butterhead	
Appearance	80.8 ± 1.7b	87.7 ± 1.8a	88.3 ± 1.7a	79.9 ± 1.7b	78.6 ± 1.7b	<0.0001
Flavor	79.6 ± 2.2a	72.9 ± 2.2b	71.6 ± 2.2b	70.9 ± 2.2b	71.7 ± 2.2b	0.0030
Texture	81.4 ± 1.8a	77.8 ± 1.8ab	73.8 ± 1.8bc	72.7 ± 1.8c	75.1 ± 1.8bc	0.0006
Overall Acceptability	79.6 ± 1.7a	73.3 ± 1.7b	73.4 ± 1.7b	71.7 ± 1.7b	69.5 ± 1.7b	0.0013

[1]Data expressed as means ± SEM. Values were measured on a 168-mm line and converted to a 100- point scale, 0-14.6 = Very Unacceptable, 14.7-29.0 = Unacceptable, 29.1-43.4 = Somewhat Unacceptable, 43.5 -57.8 = Neither Acceptable nor Unacceptable, 56.9-71.2 = Somewhat Acceptable, 71.3-85.6 = Acceptable, 85.7-100 = Very Acceptable.

Means within rows followed by different letters designate significant differences (P < 0.05).

P value = calculated probability value, Pr < F.

Table 3.5. Bitterness ratings of 5 cultivars of lettuce grown early, mid-way, and late in the growing season[1].

		Bitterness Rating			
Cultivar	**Type**	**June**	**July**	**August**	**Mean Score**
Crispino	Crisphead	46.0 ± 4.0b	39.6 ± 4.0ab	29.7 ± 4.3a	38.5 ± 2.8[1]
Crisp and Green	Green Leaf	50.0 ± 3.9ab	58.8 ± 4.0b	44.8 ± 4.3a	51.2 ± 2.8[2]
Vulcan	Red Leaf	50.2 ± 3.9a	47.8 ± 3.9a	52.9 ± 4.3a	50.3 ± 2.7[2]
Green Forest	Romaine	48.3 ± 4.0a	40.9 ± 4.0a	47.5 ± 4.3a	45.6 ± 2.8[2]
Lochness	Butterhead	44.4 ± 3.9a	49.6 ± 4.0ab	56.1 ± 4.3b	50.1 ± 2.8[2]

[1]Data expressed as means ± SEM. Scored on a 168-mm categorical line scale and converted to a 100-point scale, 0-14.6 = Extremely Mild, 14.7-29.0 = Very Mild, 29.1-43.4 = Somewhat Mild, 43.5-57.8 = Neither Bitter nor Mild, 56.9-71.2 = Somewhat Bitter, 71.3-85.6 = Very Bitter, 85.7-100 = Extremely Bitter.
Means within rows followed by different letters designate significant differences ($P < 0.05$).
Means within mean score column followed by different numbers designate significant differences ($P < 0.05$).

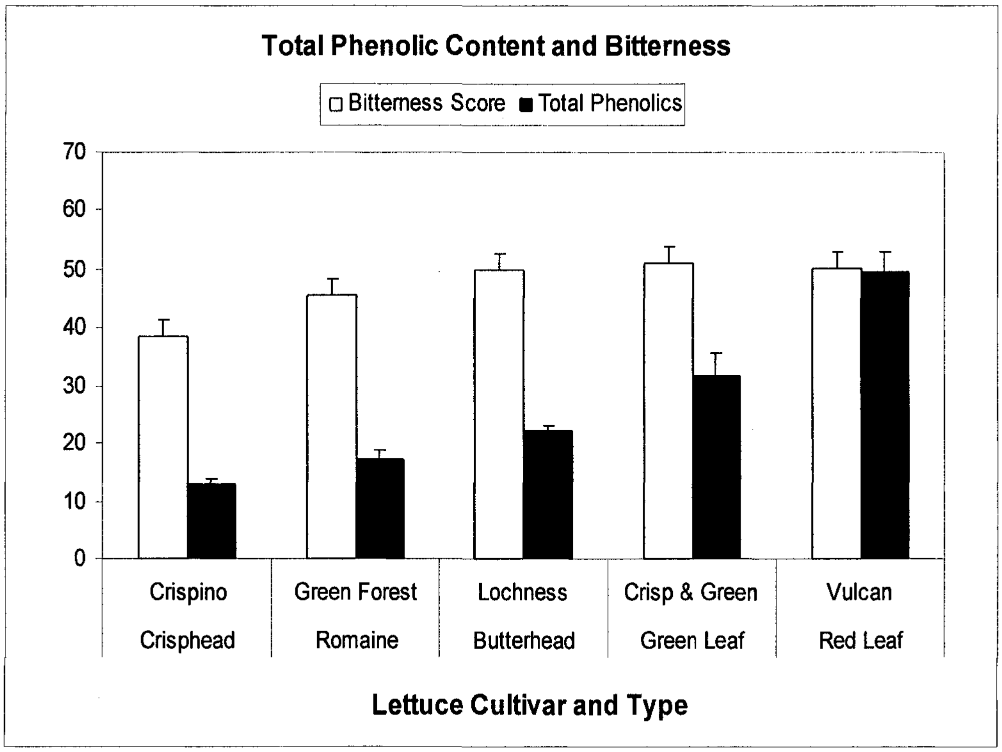


Figure 3.2. Total phenolic content and bitterness scores of five lettuce cultivars. Bitterness scores were evaluated on a 168-mm line and converted to a 100-point scale. Total phenolic values were expressed as mg gallic acid equivalents (GAE) per g dry weight lettuce. Error bars indicate SEM (standard error of the mean).

CHAPTER IV

GENETIC AND SEASONAL VARIATION OF TOTAL PHENOLIC CONTENT AND RADICAL SCAVENGING CAPACITY IN LETTUCE (*LACTUCA SATIVA* L.)

ABSTRACT

Antioxidative properties of different types of lettuce (*Lactuca sativa* L.) may vary in response to environmental factors that can fluctuate widely throughout the growing season. Eight lettuce cultivars harvested early, mid-way, and late during two growing seasons were assessed for total phenolic content (TPC) and radical scavenging capacity. 'Cimmaron' (red romaine), 'Crisp and Green' (green leaf), 'Crispino' (iceberg), 'Green Forest' (green romaine), 'Lochness' (butterhead), 'Nevada' (green batavia), 'Sierra' (red batavia) and 'Vulcan' (red leaf) cultivars were grown in Colorado using organic production methods. Significant variation ($P < 0.001$) existed among the eight cultivars in both TPC and radical scavenging capacity measured by an ABTS assay, but consistent trends were not attributable to seasonality. Total phenolics varied from 13.1 mg gallic acid equivalents/gram dry weight in 'Crispino' to 48.2 mg GAE/gdw in 'Vulcan'. Radical scavenging capacity ranged from 160.3 μmole TEAC/100 grams fresh weight for 'Crispino' to 653.8 μmole TEAC/100gfw for 'Cimmaron'. Leaf lettuce had the highest phenolic levels among the four types and red-pigmented cultivars exhibited the highest levels of TPC and radical scavenging capacity.

Introduction

The association between botanical foods and health benefits continues to grow stronger (Lampe, 1999; Thompson et al., 2006) and appears to be related to numerous antioxidative compounds (Willcox, Ash, & Catignani, 2004) and phenolic-based metabolites produced by plants (Kris-Etherton et al., 2002; Morello, Shahidi, & Ho, 2002; McCann, Freudenheim, Marshall, & Graham, 2003). Dietary antioxidants obtained from fruits and vegetables may inhibit the oxidative damage that contributes to many chronic disease processes (Mathers, 2006). Health-promoting benefits associated with polyphenolic plant compounds, like prevention of cardiovascular diseases and cancer, have also been gaining recognition (Manach, Williamson, Morand, Scalbert, & Remesy, 2005; Scalbert, Johnson, & Saltmarsh, 2005). The chemical composition of different food crops varies considerably and may be profoundly influenced by genetics, agronomic practices, region, developmental stage, and climatic conditions (Lee and Chichester, 1974; Wien, 1997).

Leafy vegetables are particularly good sources of bioactive compounds since, in addition to being principal photosynthetic sites, leaves are accrual areas for various phytochemicals with antioxidant, light-filtering, antimicrobial, antiherbivorial, and other defensive properties (Bidlack, 1998; Tarwadi and Agte, 2003). Lettuce (*Lactuca sativa* L.) is the most important of all salad crops (van Wyk, 2005) and a year-round source of Vitamin A, Vitamin C, beta-carotene, lutein, calcium, folate, and fiber (USDA Nutrient Data Base, 2005).With the increasing availability of bagged salads, consumption of colorful and diverse types of lettuce is increasing (Ryder, 1999). Nutritional profiles may vary considerably among lettuce cultivars (Simonne, Simonne, Eitenmiller, & Coker, 2002) and as a result of environmental factors, which fluctuate widely throughout the long growing season of lettuce (Inzé and Van Montagu, 2002).

Quantitative measurements of nutritional and chemical components of lettuce are particularly relevant because lettuce is generally consumed fresh and the constituents are not subjected to degradative processing and storage effects seen with many other vegetables (Crozier, Lean, McDonald, & Black, 1997; Ninfali and Bacchiocca, 2003). In the U. S., per capita lettuce consumption in 2005 was reported to be 22.1 pounds for head lettuce, 8.3 pounds for romaine and 4.0 pounds for leaf lettuce (ERS/USDA, 2006). Stevens (1974) ranked relative nutritional value of 39 crops based on nutrient composition and annual production. Head lettuce ranked 4th in relative contribution to nutrition when per capita consumption for all lettuce was reported to be 22.4 pounds. Considering the current per capita consumption rate of more than 34 pounds of lettuce per year that includes intake of leaf and romaine types (ERS/USDA, 2005), lettuce consumption likely adds more to current nutrient intake. Substituting 20 percent of dry matter in diets with freeze-dried lettuce improved antioxidant status in rats and demonstrated beneficial effects on lipid metabolism (Nicolle, Cardinault et al., 2004). Lettuce is almost always served in a salad with other vegetables and fruits which is more nutritionally effective than individual foods (Ninfali, Mea, Giorgini, Rocchi, & Bacchiocca, 2005; Su and Arab, 2006) and dressings commonly served with salad may improve nutrient bioavailability (Brown et al., 2004).

Lettuce is an important specialty crop in Colorado and regional environmental conditions, higher altitude and light intensity in particular, may create stresses that shift the directions of metabolic processes and affect phytochemical synthesis (Inzé and Van Montagu, 2002; Hirt and Shinozaki, 2004). Bolting, or flower stalk initiation, in lettuce is a common problem for producers in areas where summer temperatures exceed 30° C (86° F). Production techniques and choice of bolt resistant cultivars can improve the chances of successful lettuce production throughout the growing season (Stonaker and Guenther, 2003).

Vegetables high in anthocyanins, like red leaf lettuce, have shown more antioxidant activity than other vegetables measured by inhibition of liposome peroxidation and co-oxidation of linoleic acid and b-carotene (Hassimotto, Genovese, & Lajolo, 2005). Liu (2004a) assessed total phenolic content (TPC) and antioxidant capacity of 25 cultivars of lettuce and found a 4-fold difference in phenolic levels between cultivars and significantly higher ($P < 0.05$) levels of DPPH$^{\bullet}$ radical scavenging in red-pigmented cultivars. Caldwell (2003) identified the major sources of antioxidant activity in red leaf lettuce to be mono- and dicaffeoyl esters of tartaric and quinic acid. Nicolle et al. (2004) found quantitative differences in phenolic profiles among six cultivars of lettuce with red leaf lettuce possessing two-fold more dicaffeoyl tartaric acid and 10-fold more chlorogenic acid than butterhead or batavia lettuce.

Qualitative and quantitative differences in flavonoid content have been observed among lettuce cultivars. Dupont et al. (2000) identified quercetin conjugates in green leaf cultivars and cyanidin conjugates in red leaf cultivars. Over 50-fold differences in quercetin content have been reported among lettuce cultivars (Bilyk and Sapers, 1985;

Crozier, Lean, McDonald, & Black, 1997). The carotenoid, lactucazanthin (є,є-carotene-3,3-diol), has only been identified in lettuce (Kimura and Rodriguez-Amaya, 2003).

The concentration of phenolic compounds in red leaf lettuce appears to be sensitive to environmental factors (Caldwell, 2003). Liu and others (2005) observed significantly higher antioxidant capacity in lettuce harvested late in the growing season ($P < 0.05$), indicating that this characteristic may be affected by environmental conditions. Carotenoid levels in lettuce have also been shown to vary with harvest date (Nicolle, Carnat *et al.*, 2004).

Variations in phenolic content and antioxidant capacity of lettuce appear to be influenced by cultivar, type, pigmentation, and growing conditions and warrant further investigation. Differences observed between samples of the same lettuce cultivars may be attributed to amount of tissue pigmentation (Crozier, Lean, McDonald, & Black, 1997) or tissue variability between outer and inner leaves (Bilyk and Sapers, 1985; DuPont, Mondin, Williamson, & Price, 2000; Wu et al., 2004). Using uniform samples of known cultivars and controlling growing, harvest, and postharvest conditions improves product consistency, which is essential for chemical evaluation.

The objective of this study was to quantify TPC and radical scavenging capacity in eight selected cultivars of lettuce grown at six different times over two growing seasons and to investigate possible correlations between TPC, radical scavenging capacity, and selected environmental factors. The eight chosen cultivars have exhibited promising production characteristics and represent four lettuce types and two color patterns: red leaf, green leaf, red romaine, green romaine, red batavia, green batavia, butterhead, and crisphead. TPC and antioxidant capacity were correlated with three environmental indexes (growing degree days, radiation, measured as total langleys, and number of days with temperatures exceeding 30°C (86°F) in the thirty days prior to harvest).

Materials and methods

Plant selection and preparation. The cultivars chosen for this study were selected from a group of 25 cultivars screened for favorable bolting properties (Stonaker and Guenther, 2003) and total phenolic content (Liu, 2004b). 'Crispino', an iceberg cultivar, was included as a reference cultivar because crisphead is the most commonly consumed lettuce. 'Crisp and Green' (green leaf), 'Crispino' (crisphead), 'Vulcan (red leaf), and 'Green Forest' (romaine) seeds were purchased from Johnny's Selected Seeds (Albion, ME); 'Lochness' (butterhead), 'Nevada' (green batavia), and 'Sierra' (red batavia) seeds were purchased from Vilmorin (La Ménitré, France); and 'Cimmaron' (red romaine) seeds were obtained from Rocky Mountain Seed Company (Denver, CO). Multiple plantings of all cultivars were grown by the Specialty Crops Division of the Colorado State University Horticulture and Landscape Architecture Department in the spring and summer of 2003 and 2004. The lettuce was initially sown in a greenhouse at the Plant Environmental Research Center into 72-cell trays. After 30 days, the lettuce was transplanted to the field in raised double rows. All cultivars were hand planted 8 inches apart except romaine cultivars, which were planted 10 inches apart. Organic production methods conformed to requirements set by USDA for organic certification (USDA/NOP) and moisture levels were maintained using drip irrigation. Triplicate heads of lettuce were collected from the field and transported directly to a CSU laboratory for processing. To include inner and outer leaf tissue, a 6-centimeter wide wedge-shaped sample was cut and weighed. Samples were placed in a Genesis Freeze-Drier (Virtis Inc. Gardiner, NY) at – 40 °C, - 10 °C, +18 °C, +28 °C for 24 hours each. The dried samples were ground into powder using an Osterizer10 Speed Blender (Oster Inc., Boca Raton, FL) and sieved with a No. 20 Tyler sieve (WS Tyler Inc., Mentor, OH). The powdered samples were stored in 25 ml plastic centrifuge tubes sealed with a screw cap to prevent uptake of moisture and stored at -20 °C. Four hundred mg of each sample was extracted in 10 mL of 80% acetone, vortexed, and rotated in the dark for 2 hours. After centrifugation, aliquots were transferred and vacuum-centrifuged. Dried samples were stored at -80° C. Prior to total phenolic and ABTS assays, dried samples were reconstituted in 80 percent acetone.

Chemical Analyses. *Chemicals.* Folin-Ciocalteu reagent was obtained from Fluka Biochemica (Buchs, Switzerland), gallic acid and ascorbic acid were obtained from Sigma and Aldrich (St. Louis, MO), dithiothreitol from Research Products International (Mount Prospect, IL), and HPLC-grade methanol, trolox, and 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid (ABTS) from EMD (San Diego, CA).

Total Phenolic Content Assay. The Folin-Ciocalteu assay, a widely accepted spectrophotometric method based on a color reaction (Singleton and Rossi, 1965 ; Spanos and Wrolstad, 1990), was used to quantify total phenolic content of dried lettuce tissue extracts using a commercially available reagent. Reaction time and temperature were held constant and absorbance of the stable chromophore at 765nm was used to estimate phenolic content by derivation from a standard curve based upon gallic acid and expressed as milligrams per g of dry tissue weight.

The presence of ascorbic acid in vegetable extracts can interfere with the Folin-Ciocalteu assay (Prior, Wu, & Schaich, 2005). Extracted samples were analyzed for Vitamin C content after vacuum-centrifugation to verify the absence of Vitamin C prior to the Folin-Ciocalteu assay. A 5% w/v aqueous solution of metaphosphoric acid containing 1% w/v dithiothreitol was added to extracted samples which were centrifuged and filtered through a 0.45 mm nylon syringe filter, prior to injection onto an Inertsil 4C HPLC (high performance liquid chromatography) column and run with a phosphoric acid/methanol gradient (Esparza-Rivera, Stone, Stushnoff, Pilon-Smits, & Kendall, 2006).

TEAC Assay. The antioxidant activity of lettuce samples was measured using a microplate modification (Stushnoff, Kulen et al., 2006) of the ABTS (2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) decolorization assay (Miller and Rice-Evans, 1997). This method measures the capacity of an antioxidant in the reduction of an oxidant, which changes color when reduced, with degree of decolorization correlated to the antioxidant concentration of the sample (Huang et al., 2005). The radical $ABTS^{\bullet +}$ was generated by chemical reduction with manganese dioxide and absorbances were measured at 734nm. The measurement of relative activities of hydrogen-donating antioxidants was based on their ability to scavenge the radical cation $ABTS^{\bullet +}$ in comparison to standard amounts of the synthetic antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), an analog of vitamin E. Results were expressed as trolox equivalent antioxidant capacity, TEAC (Sanchez-Moreno, 2002).

Vitamin C Assay. Lyophilized lettuce tissue was extracted in 5% w/v aqueous solution of metaphosphoric acid containing 1% w/v dithiothreitol, centrifuged and filtered through a

0.45 mm nylon syringe filter, prior to injection onto an Inertsil 4C HPLC (Hewlett Packard Model 1050 Series, Palo Alto, Ca.) column and run with a phosphoric acid/methanol gradient (Rivera, Stone et al., 2006). Ascorbic acid, naturally occurring in lettuce, is known to interfere with the Folin-Ciocalteu assay (Prior, Wu, & Schaich, 2005) so extracted lettuce tissue samples were analyzed for Vitamin C content with high performance liquid chromatography (HPLC) to verify that it was not present in significant amounts after vacuum-centrifugation.

Environmental Data Analysis. Daily maximum and minimum temperatures and total langleys, a measure of solar radiation, were monitored to appraise possible correlations with chemical assay data. Daily readings of temperature and radiation values were collected from an online site recorded from a weather station located within 100 meters of the lettuce plot.

Growing degree days (GDD) and number of days above 30° C (86° F) during the thirty days preceding harvest were used as measures of heat exposure. Plant development is dependent on daily accumulation of heat and GDD are used as a heat accumulation index to estimate temperature effects on growth and development during the growing season (Grierson, 2002). The amount of heat required for plants to move to the next development stage remains constant for a particular species. The minimum base temperature, or threshold below which development does not occur, has been determined to be 4.4° C for lettuce (Raven, Evert, & Eichhorn, 1999). Daily GDD was calculated by subtracting the base temperature of lettuce (4.4 °C) from the mean daily temperature (maximum + minimum/2) (McMaster and Wilhelm, 1997) and daily GDD was totaled over the thirty days preceding harvest to give an accumulated heat index value.

A Epply pyranometer was used to measure solar radiation and provided information on amount of energy striking a surface during a particular time period. Total langleys are units of radiant flux equal to one calorie per square centimeter (cal/cm^2). This measure of the total energy received in the ultraviolet, visible and infrared wavelengths is a much wider band of wavelengths than plants can utilize but can be useful as an indicator of the amount of light available for plant growth (Hart, 1988).

Statistical Analyses. Mixed model analysis of variance was performed with harvest and harvest x cultivar treated as random effects and cultivar and type as fixed effects. Statistical analysis of the data was performed using SAS Proc Mixed (Statistical Analysis Systems Inc., Cary, N. C., ver. 9.1) with Fisher's protected least significant difference test and Pearson correlation analysis ($P < 0.05$).

Results

Effects of cultivar on Total Phenolic Content and TEAC. Analytical evaluations revealed considerable variation ($P < 0.0001$) in total phenolic content among the eight cultivars (Table 4.1). There was over a 6-fold difference in TPC, measured by gallic acid equivalents/gram dry weight, among the different harvests of the eight cultivars ranging from 9.1GAE/gdw for 'Crispino' (crisphead), harvested late season, to 59.5 GAE/gdw for 'Vulcan' (red leaf), harvested late season. Mean total phenolic content of the cultivars, averaged across all six growing periods, differed significantly ($P < 0.0001$) from 15.1 mg GAE/g in 'Crispino' (crisphead) to 48.2 mg GAE/g in 'Vulcan' (red leaf) lettuce, followed by 41.1 mg in 'Cimmaron' (red romaine) (Table 4.2).

When the eight cultivars were grouped by lettuce type, variations in TPC between the four types were significant ($P < 0.0001$) (Table 4.2). Leaf lettuce, with 38.1 mg

GAE/g, was significantly higher ($P < 0.05$) than romaine which was higher ($P < 0.05$) than butterhead and the batavia subtype which were ($p < 0.05$) higher than the crisphead subtype. Two of the three red-pigmented cultivars, 'Vulcan' and 'Cimmaron', were significantly higher ($P < 0.05$) in TPC than the green-pigmented cultivars within the same type but the same difference was not seen with the batavia cultivars.

There was almost a 10-fold difference ($P < 0.0001$) in radical scavenging capacity, measured by µmole TEAC/ 100gfw, among the different harvests of the eight cultivars (Figure 4.2) ranging from 99.3 µmole TEAC/ 100gfw for 'Crispino' (crisphead) harvested late season to 978.9 µmole TEAC/ 100gfw for 'Cimmaron' (red romaine), harvested late season (Table 4.4). Mean radical scavenging capacity of the cultivars, averaged across the six growing periods, varied significantly ($P<.0001$), ranging from 157.6 µmole TEAC/ 100gfw for 'Crispino' to 670.1 µmole TEAC/ 100gfw for Vulcan (Table 4.2). Throughout the six harvest times, 'Cimmaron' and 'Vulcan' (red leaf) were consistently higher in antioxidant activity than other cultivars. All three of the red-pigmented cultivars had higher radical scavenging capacity than their green-pigmented counterparts ($P < 0.05$). When grouped by type, differences in TEAC levels were significant ($P < 0.05$) (Table 4.2) with leaf and romaine types having higher ($P< 0.05$) levels than batavia and crisphead types.

Seasonal variation. Environmental conditions fluctuated considerably between the growing periods of each year and between early, mid and late-season growing periods of both years (Table 4.4). Temperatures were unusually high during mid-summer of 2003. The mean daily GDD of the 30 days preceding the mid-season harvest of 2003 was 82% higher than that of the late-season harvest in the same year and 20% higher than the mid-season harvest of 2004. Maximum temperatures exceeded 30° C on 77 per cent of the days in the 30 days preceding the mid-season harvest in 2003. High amounts of rainfall early in the 2004 growing season resulted in reduced radiation levels recorded in the field plots. Radiation during the early-season harvest of the first year was 63% higher than the early-season harvest of the second year.

Effects of seasonal variation on Total Phenolics and TEAC. Variations in total phenolic content (TPC) throughout the growing season were not consistent among cultivars (Figure 4.1). Total phenolic values did vary significantly ($P < 0.05$) with harvest date for 5 of the 8 cultivars: 'Lochness' (butterhead), 'Vulcan (red leaf), 'Nevada' (batavia), 'Sierra' (red batavia), and 'Crispino' (crisphead) (Table 4.1). In 2003, the total phenolic content of four cultivars was higher in the July harvest, which corresponded to higher growing season temperatures. However, the same trend in total phenolic content was not observed in 2004, likely because mid-season temperatures were lower. With the exception of a significantly higher level ($P < 0.05$) of TPC for 'Vulcan' in the late season harvest and 'Crispino' in the early season harvest, 6 of the 8 cultivars did not differ significantly in TPC between growing periods in 2004. In 2004, TEAC values did not vary with harvest date for any of the cultivars but in 2003, when temperature variations were higher, 'Nevada', 'Sierra' and 'Lockness' had higher TEAC values for the mid-season harvest (Table 4.3). 'Green Forest' harvested in late season was significantly higher than early season harvest. Across harvest dates, TEAC values were highest for 'Vulcan' (red leaf) and 'Cimmaron' (red romaine) followed by 'Crisp and Green' (green leaf) (Table 4.2). By type, leaf and romaine were higher than other cultivars ($P < 0.001$) (Table 4.2).

Vitamin C. Vitamin C levels were determined from one growing period in both years and ranged from 6.8 mg/100 grams fresh weight in 'Nevada' (green batavia) to over 16 mg/100 gfw in 'Green Forest' (green romaine) but did not vary significantly by cultivar ($P = 0.089$) or type ($P = 0.0831$) of lettuce.

Correlations between antioxidant assays and environmental indexes. Correlations between TPC and TEAC values were significant for four of the eight cultivars: 'Nevada' (green batavia) ($P = 0.002$), 'Vulcan' (red leaf) ($P = 0.009$), 'Lochness' (butterhead) ($P = 0.022$), and 'Sierra' (red batavia) ($P = 0.03$) (Table 4.2). No significant correlations were observed between the three environmental indexes and the TPC or the TEAC assay.

Discussion

The emerging trend of choosing foods based on nutritional benefits (Heber and Bowerman, 2001; Pollard, Kirk, & Cade, 2002; Gopalan and Tamber, 2003) is having a significant impact on agriculture (Morello, Shahidi, & Ho, 2002) and factors like antioxidant and phenolic content are becoming recognized as advantageous traits in specialty crops (Velioglu, Mazza, Gao, & Oomah, 1998). Genetic characteristics associated with individual cultivars appear to have a profound influence on phytochemical content (Kalt, 2005; Ninfali, Mea, Giorgini, Rocchi, & Bacchiocca, 2005).

Several kinds of green and red-pigmented lettuce are commonly available to consumers. Genotype has been shown to affect nutrient content (USDA Nutrient Data Base, 2005) and appears to influence phenolic and antioxidant properties of lettuce although information on types and cultivars is limited. Fluctuations in environmental factors throughout the long growing season of lettuce may also alter the production of phenolic compounds and affect antioxidant activity. Liu and others (2005) reported that variations in phenolic content of lettuce were influenced by cultivar, type and pigmentation with red leaf cultivars exhibiting the highest levels and concluded that the effects of growing conditions on phenolic content of lettuce needs further investigation.

Numerous biotic and abiotic stresses effect the production of reactive oxygen species in plant tissues which is countered by a protective system of antioxidant compounds and enzymes (Fowden et al., 1993). Pathological and physiological stresses may vary throughout the long growing season of lettuce, which in the present study extended from May to October.

It was shown that cultivar significantly influenced TPC and radical scavenging capacity in lettuce indicating genetic composition plays an important role in chemical properties of this crop. Differences between various lettuce types and pigmentation patterns were also significant ($P < 0.05$). There was a large variation among the commonly available types of lettuce cultivars. Red pigmented cultivars of leaf and romaine lettuce types exhibited appreciably higher levels of TPC and ABTS+• radical scavenging capacity which matches results seen with DPPH$^{\bullet}$ radical scavenging in lettuce reported by Liu et al. (2005). With 4 of the 8 cultivars, TPC correlated significantly with antioxidant capacity, which suggests variations in antioxidant mechanisms among cultivars.

Climatic conditions associated with various harvest dates appeared to affect TPC and antioxidant capacity but patterns varied between cultivars and growing seasons. A significantly higher level ($P = < 0.05$) of TPC was observed for 'Vulcan' (red leaf) in the late season harvest, a trend which was also observed by Liu et al. (2005).

In conclusion, variations associated with cultivar were much greater than differences attributable to growing season and red-pigmented cultivars exhibited the highest levels of TPC and radical scavenging capacity. Selection of lettuce cultivars has usually been based on shelf-life, transportability, and yield rather than nutritional traits. The assessment of genetic variation on nutritional profiles in cultivars of lettuce may be useful in identifying specific cultivars that exhibit superior antioxidative properties and could highlight potential salutary benefits and improve market competitiveness of Colorado-grown lettuce.

Table 4.1. Total phenolic content of eight lettuce cultivars grown at six times during two years.

Cultivar	**Type**	**Harvest Date**					
		7/7/03	8/18/03	10/14/03	6/29/04	7/27/04	8/29/04
Crispino	crisphead				**23.8 ± 1.3a**	14.6 ± 1.0ab	9.1 ± 0.4b
Crispy Green	green leaf	23.8 ± 5.4a	29.8 ± 1.6a	18.8 ± 2.5a	33.6 ± 5.6a	32.1 ± 2.9a	29.3 ± 3.6a
Vulcan	red leaf	44.6 ± 3.6b	**59.5 ± 3.7a**	44.2 ± 0.5b	40.5 ± 4.1b	41.1 ± 3.8b	**59.4 ± 2.8a**
Green Forest	romaine	18.3 ± 2.5a	23.7 ± 4.5a	20.5 ± 1.5a	17.6 ± 0.4a	15.5 ± 3.7a	18.8 ± 0.8a
Cimmaron	red romaine	33.0 ± 4.2a	38.5 ± 1.2a	38.7 ± 4.8a	33.9 ± 4.2a	51.1 ± 1.2a	51.2 ± 4.8a
Nevada	green batavia	9.9 ± 0.7c	**32.0 ± 5.9a**	23.4 ± 3.5ab	22.0 ± 1.8ab	22.3 ± 3.6ab	20.0 ± 2.4bc
Sierra	red batavia	18.9 ± 4.9ab	**29.9 ± 4.5a**	16.5 ± 2.6b	24.6 ± 0.9ab	23.1 ± 3.5ab	27.9 ± 0.3ab
Lochness	butterhead	12.9 ± 3.6b	**26.9 ± 3.5a**	21.7 ± 4.1ab	23.2 ± 3.6ab	18.6 ± 4.2ab	22.9 ± 3.7ab

Data expressed as mg gallic acid equivalents (GAE) per g dry weight lettuce (means ± SEM).
Means within rows followed by different letters designate significant differences ($P < 0.05$).

Table 4.2. Total phenolics, TEAC values, and correlations of eight lettuce types and cultivars from six harvests.

Type	**Leaf**		**Romaine**		**Butterhead**	**Crisphead**		
Subtype						**Batavia**		**Iceberg**
TPC	38.1 ± 2.3a		30.1 ± 2.3b		21.0 ± 3.0c	22.6 ± 2.3c		15.1 ± 3.6d
TEAC	525.3 ± 40.9a		502.4 ± 41.2a		262.2 ± 50.1c	317.4 ± 40.9b		157.7 ± 68.2d
Pigmentation	Green	Red	Green	Red	Green	Green	Red	Green
Cultivar	**Crisp & Green**	**Vulcan**	**Green Forest**	**Cimmaron**	**Lochness**	**Nevada**	**Sierra**	**Crispino**
TPC	27.9 ± 2.6c	48.2 ± 2.6a	19.1 ± 2.6de	41.1 ± 2.6b	21.0 ± 2.6de	21.6 ± 2.6de	23.5 ± 2.7cd	15.1 ± 3.6e
TEAC	380.7 ± 49.7b	670.1 ± 49.7a	361.7 ± 49.7c	652.2 ± 50.2a	262.2 ± 49.7d	298.3 ± 49.7d	336.6 ± 49.7c	157.6 ± 67.2e
TPC x TEAC Correlation								
Pearson	0.733	0.923	0.430	0.783	0.876	0.965	0.856	0.956
Prob > r	0.097	0.009	0.394	0.065	0.022	0.002	0.030	0.189

Total phenolic contents were measured in mg of gallic acid equivalents per gram dry weight.
TEAC values were measured as μmole TEAC/ 100gfw.
Data are means from 3 replications x 6 harvests ± SEM. Means within rows followed by different letters designate significant differences($P<0.05$).
Correlations values are Pearson correlation coefficients and probability of a greater r value under HO: Rho = 0.

Table 4.3. TEAC values of eight lettuce cultivars grown at six times during two years.

Cultivar	**Type**	**Harvest Date**					
		7/7/03	8/18/03	10/14/03	6/29/04	7/27/04	8/29/04
Crispino	crisphead				220.0 ± 13.3a	177.2 ± 11.7a	104.8 ± 3.5b
Crisp & Green	green leaf	334.6 ± 51.1bc	354.6 ± 18.2bc	204.7 ± 27.7c	378.1 ± 36.4abc	481.1 ± 30.5ab	530.9 ± 19.9a
Vulcan	red leaf	657.6 ± 20.3a	837.3 ± 125.2a	608.0 ± 44.6a	525.7 ± 29.9a	632.9 ± 23.2a	758.6 ± 36.5a
Green Forest	romaine	292.2 ± 33.8b	367.8 ± 17.5ab	444.8 ± 8.9a	343.7 ± 9.3b	336.7 ± 20.5ab	385.0 ± 3.3ab
Cimmaron	red romaine	535.0 ± 22.7b	538.6 ± 10.5b	675.0 ± 47.7ab	528.6 ± 40.9b	666.6 ± 71.0ab	978.9 ± 67.1a
Nevada	gr. batavia	99.26 ± 7.9c	475.4 ± 45.0a	275.5 ± 17.7bc	300.8 ± 19.6ab	322.1 ± 53.6ab	316.6 ± 30.0ab
Sierra	red batavia	283.7 ± 29.5bc	628.2 ± 53.2a	169.3 ± 31.0c	305.6 ± 29.5bc	237.1 ± 5.0bc	395.5 ± 8.1b
Lochness	butterhead	126.3 ± 34.8b	419.5 ± 36.5a	273.3 ± 40.8ab	244.5 ± 14.9b	262.4 ± 31.0ab	247.0 ± 22.1b

Data expressed as means ± SEM µmole TEAC/ 100gfw.

Means within rows followed by different letters designate significant differences ($P < 0.05$).

Table 4.4. Environmental data for the lettuce plots recorded over the 30 days preceding each harvest.

Harvest		**Temperature (°C)**				**GDD**[1]	**# Days**[2] **≥ 30°C**	**Solar Radiation** **Total Langleys**	
		Maximum		**Minimum**					
#	*Date*	*Mean*	*Range*	*Mean*	*Range*	*Sum*		*Mean*	*Sum*
1	7/7/2003	27.0	18.9 - 35.3	10.1	2.2 - 14.5	410.7	8	559.8	16795
2	8/17/2003	31.1	26.6 - 37.3	13.3	9.3 - 17.5	535.9	23	548.0	16441
3	10/13/2003	22.6	10.3 – 31.2	1.8	7.3 - -3.2	220.7	2	414.2	12425
4	6/29/2004	23.2	11.4 - 34.7	8.2	1.7 - 14.2	331.8	2	343.8	10313
5	7/27/2004	28.2	14.8 - 36.3	11.6	7.5 - 17.0	447.1	13	482.7	14482
6	8/29/2004	26.5	17.2 - 34.4	9.3	4.6 - 14.5	381.8	6	486.0	14581

[1]Heat accumulation indicator = 30 day summation of GDD (Growing Degree Day)
GDD) = ((Tmax + Tmin) ÷ 2) – 4.4°C (lettuce base temperature)
[2]Heat stress indicator = number of days with maximum temperatures exceeding 30°C

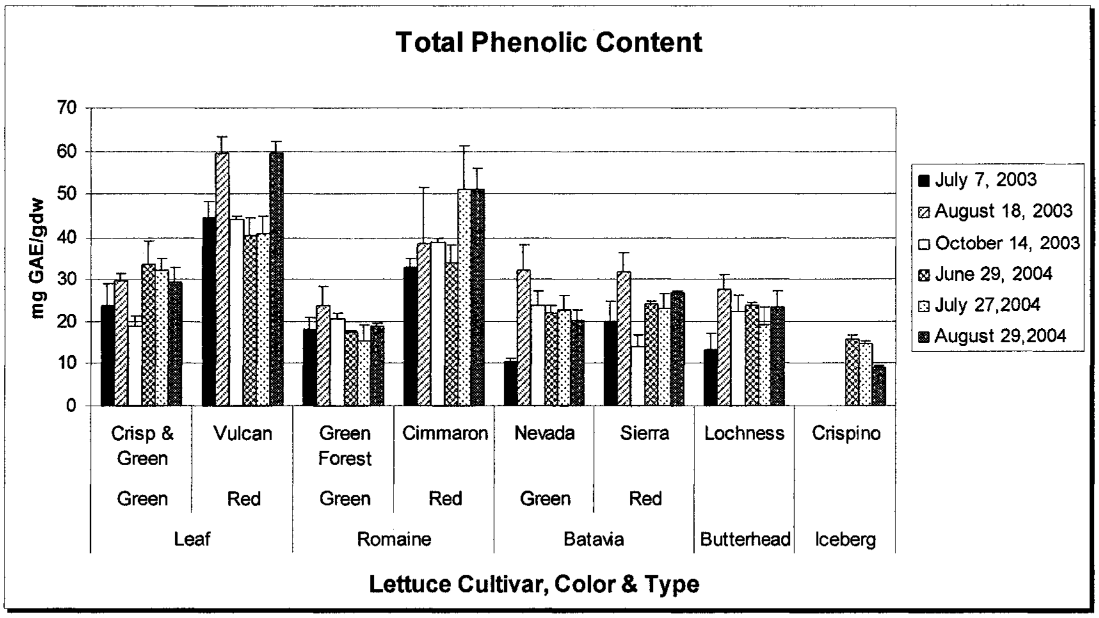


Figure 4.1. Total phenolic content (mg GAE/GDW) of eight lettuce cultivars grown at six different times over two years. Data are expressed as means ± SEM.

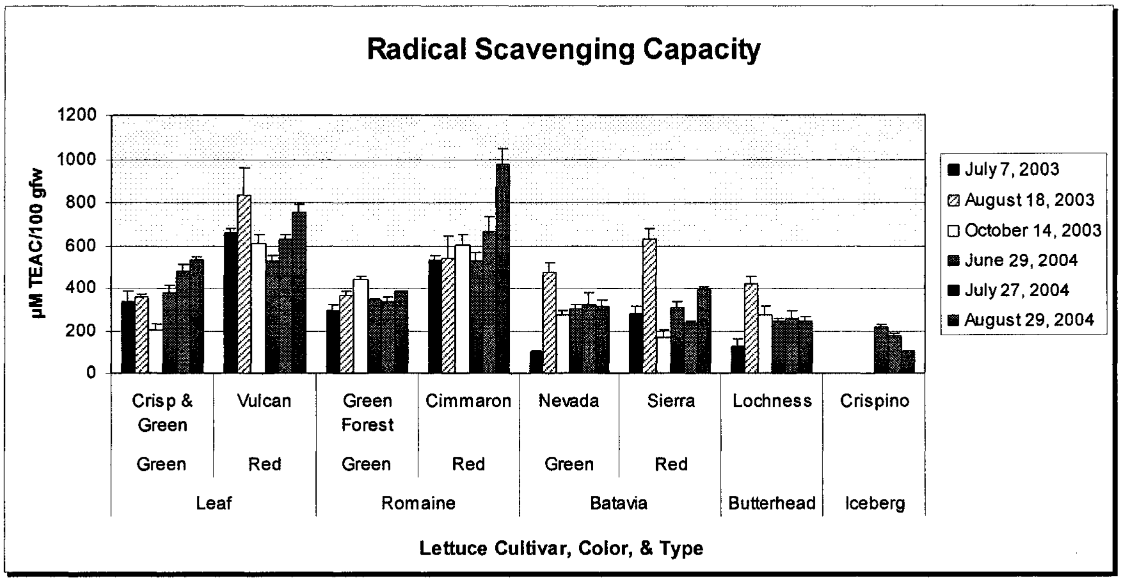


Figure 4.2. Radical scavenging capacity of eight lettuce cultivars expressed as TEAC (trolox equivalent antioxidant capacity) (µMTrolox/100 gfw). Data are means from 3 heads of lettuce and 9 replicate cuvette analyses.

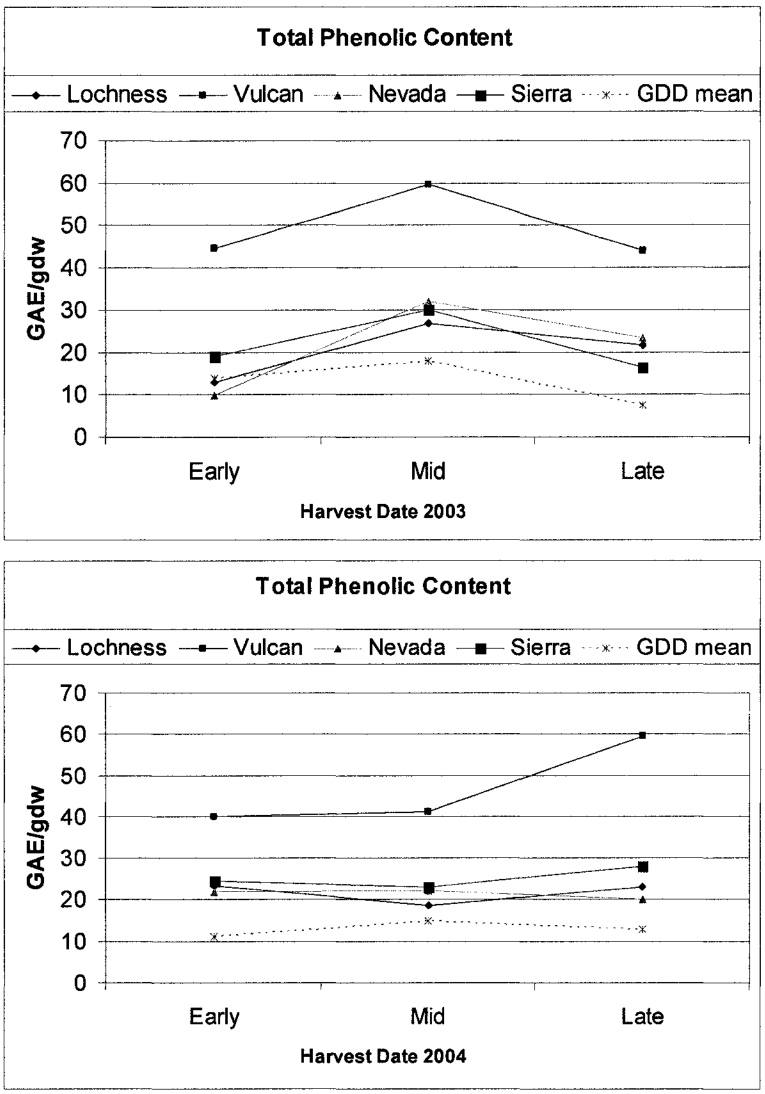


Figure 4.3. Variation in total phenolic content (gallic acid equivalents per gram dry weight) of 4 lettuce cultivars harvested early, mid-way, and late season over 2 years. GDD = growing degree days.

CHAPTER V

GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

General conclusions

1. Sensory panel evaluations demonstrated that cultivar had a greater impact on sensory qualities in lettuce than seasonal variation. Appearance, flavor, texture, overall acceptability, degree of bitterness, and total phenolic content varied significantly among lettuce cultivars but trends attributable to growing season were not consistent.

2. Although there was variation in sensory ratings among the five cultivars, mean scores of all attributes remained within the acceptable range indicating that various types of lettuce with acceptable sensory qualities can be grown in this region during summer months.

3. Variations in levels of total phenolic content and radical scavenging capacity associated with cultivar were much greater than differences attributable to growing season.

4. Red-pigmented cultivars of leaf and romaine types had significantly higher levels of total phenolic content and radical scavenging capacity.

5. Evaluation of sensory properties and quantification of antioxidant content in lettuce can be used to identify specific cultivars that exhibit superior attributes and may improve market competitiveness of various cultivars and types of Colorado-grown lettuce.

Recommendations for further studies

1. Information about agronomic practices used in produce production is becoming increasingly important to consumers and more research is needed to evaluate nutritional differences related to growing method. A comparison of organic and conventional growing methods on sensory and antioxidative properties of different types and cultivars of lettuce that grow well in this region would be very useful. It would be particularly beneficial to have information about the sensory properties and consumer acceptability of the batavia cultivar, 'Nevada', since it has shown promising production characteristics but it was not included in the present sensory study. More information on vitamin C levels and other measures of antioxidant capacity would also be useful in providing a more complete picture of nutritional properties.
2. A follow-up study that incorporates microbiological and pesticide residue testing in comparisons between locally grown lettuce and commercially available lettuce would also be interesting and could provide some very useful information. The results of this type of study might offer a competitive edge to local producers and would be of interest to consumers that are trying to follow healthier diets. This study could include the investigation of a series of post-harvest sanitizing methods that might be utilized in the development of guidelines for local producers that sell their produce through direct markets. The construction of a website for distribution of information about nutritional and production characteristics of various cultivars and food safety recommendations for small farm produce producers could prove to be very valuable to Colorado producers.

CHAPTER VI

REFERENCES

AHA. (2006). *Diet and Lifestyle Recommendations*: American Heart Association.

Allende, A., Aguayo, E., & Artes, F. (2004). Microbial and sensory quality of commercial fresh processed red lettuce throughout the production chain and shelf life. *International Journal of Food Microbiology, 91*(2), 109-117.

Asard, H., May, J., & Smirnoff, N. (2004). *Vitamin C : function and biochemistry in animals and plants*. New York

Awika, J. M., Rooney, L. W., Wu, X. L., Prior, R. L., & Cisneros-Zevallos, L. (2003). Screening methods to measure antioxidant activity of sorghum (Sorghum bicolor) and sorghum products. *Journal of Agricultural and Food Chemistry, 51*(23), 6657-6662.

Barrios, E., & Costell, E. (2004). Review: Use of Methods of Research into Consumers' Opinions and Attitudes in Food Research. *Food Science and Technology International, 10*(6), 359-371.

Bidlack, W. R. (1998). *Phytochemicals : a new paradigm*. Lancaster: Technomic Pub. Co.

Bilyk, A., & Sapers, G. M. (1985). Distribution of Quercetin and Kaempferol in Lettuce, Kale, Chive, Garlic Chive, Leek, Horseradish, Red Radish, and Red Cabbage Tissues. *Journal of Agricultural and Food Chemistry, 33*(2), 226-228.

Birt, D. F. (2006). Phytochemicals and cancer prevention: From epidemiolgy to mechanism of action. *Journal of the American Dietetic Association, 106*(1), 20-21.

Block, G., Patterson, B., & Subar, A. (1992). Fruit, Vegetables, and Cancer Prevention - a Review of the Epidemiologic Evidence. *Nutrition and Cancer-an International Journal, 18*(1), 1-29.

Bos, I., & Caligari, P. D. S. (1995). *Selection methods in plant breeding*. London ; New York: Chapman & Hall.

Brown, A. (2004). *Understanding food : principles and preparation*. Belmont, Ca.: Wadsworth/Thomson Learning.

Brown, M. J., Ferruzzi, M. G., Nguyen, M. L., Cooper, D. A., Eldridge, A. L., Schwartz, S. J., et al. (2004). Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. *American Journal of Clinical Nutrition, 80*(2), 396-403.

Bunning, E. (1943). The adoptation of plants to yearly and daily periodic changes of the environment. *Naturwissenschaften, 31*, 493-499.

Caldwell, C. R. (2003). Alkylperoxyl radical scavenging activity of red leaf lettuce (Lactuca sativa L.) phenolics. *Journal of Agricultural and Food Chemistry, 51*(16), 4589-4595.

Calvo, M. M. (2005). Lutein: A valuable ingredient of fruit and vegetables. *Critical Reviews in Food Science and Nutrition, 45*(7-8), 671-696.

Cao, G. H., Sofic, E., & Prior, R. L. (1996). Antioxidant capacity of tea and common vegetables. *Journal of Agricultural and Food Chemistry, 44*(11), 3426-3431.

Carpenter, R. P., Lyon, D. H., & Hasdell, T. A. (2000). *Guidelines for sensory analysis in food product development and quality control* (2nd / ed.). Gaithersburg, Md.: Aspen Publishers.

Chalker-Scott, L. (1999). Environmental significance of anthocyanins in plant stress responses. *Photochemistry and Photobiology, 70*(1), 1-9.

Chu, Y. F., Sun, J., Wu, X. Z., & Liu, R. H. (2002). Antioxidant and anti proliferative activities of common vegetables. *Journal of Agricultural and Food Chemistry, 50*(23), 6910-6916.

Chun, O. K., Kim, D. O., Smith, N., Schroeder, D., Han, J. T., & Lee, C. Y. (2005). Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *Journal of the Science of Food and Agriculture, 85*(10), 1715-1724.

ColoradoDeptAgric. (2005). State of Colorado Department of Agriculture, accessed at: http://www.ag.state.co.us/.

Crosby, D. G. (1963). Organic Constituents of Food .1. Lettuce. *Journal of Food Science, 28*(3), 347-&.

Crozier, A., Lean, M. E. J., McDonald, M. S., & Black, C. (1997). Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *Journal of Agricultural and Food Chemistry, 45*(3), 590-595.

Davey, M., Van Montagu, M., & Inze, D. (2002). Ascorbate Metabolism. In D. Inzé & M. Montagu (Eds.), *Oxidative Stress in Plants* (pp. 271-296). New York: Taylor & Francis.

Davis, D. R., Epp, M. D., & Riordan, H. D. (2004). Changes in USDA food composition data for 43 garden crops, 1950 to 1999. *Journal of the American College of Nutrition, 23*(6), 669-682.

de las Heras, B., Rodriguez, B., Bosca, L., & Villar, A. M. (2003). Terpenoids: Sources, structure elucidation and therapeutic potential in inflammation. *Current Topics in Medicinal Chemistry, 3*(2), 171-185.

Delaquis, P. J., Stewart, S., Cliff, M., Toivonen, P. M., & Moyls, A. L. (2000). Sensory quality of ready-to-eat lettuce washed in warm, chlorinated water. *Journal of Food Quality, 23*(6), 553-563.

Demmig-Adams, B., & Adams, W. W. (2002). Antioxidants in photosynthesis and human nutrition. *Science, 298*(5601), 2149-2153.

DHHS/USDA. (2005). *Dietary Guidelines for Americans*: Department of Health and Human Services and U. S. Department of Agriculture.

Dinehart, M. E., Hayes, J. E., Bartoshuk, L. M., Lanier, S. L., & Duffy, V. B. (2006). Bitter taste markers explain variability in vegetable sweetness, bitterness, and intake. *Physiology & Behavior, 87*(2), 304-313.

Drewnowski, A. (1997). Taste preferences and food intake. *Annual Review of Nutrition, 17*, 237-253.

Drewnowski, A., & Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: a review. *American Journal of Clinical Nutrition, 72*(6), 1424-1435.

Dumas, Y., Dadomo, M., Di Lucca, G., & Grolier, P. (2003). Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *Journal of the Science of Food and Agriculture, 83*(5), 369-382.

DuPont, M. S., Mondin, Z., Williamson, G., & Price, K. R. (2000). Effect of variety, processing, and storage on the flavonoid glycoside content and composition of lettuce and endive. *Journal of Agricultural and Food Chemistry, 48*(9), 3957-3964.

Edwards, R. (1999). Secondary Metabolism. In P. J. Lea & R. C. Leegood (Eds.), *Plant Biochemistry* (pp. 193-218). New York: Wiley.

Einstein, M. A. (1976). Use of Linear Rating Scales for Evaluation of Beer Flavor by Consumers. *Journal of Food Science, 41*(2), 383-385.

ERS/USDA. (2006). Economic Research Service, United States Department of Agriculture.

Eskins, K., Warner, K., & Felker, F. C. (1996). Light quality during early seedling development influences the morphology and bitter taste intensity of mature lettuce (Lactuca sativa) leaves. *Journal of Plant Physiology, 147*(6), 709-713.

Esparza-Rivera, J. R., Stone, M. B., Stushnoff, C., Pilon-Smits, E., & Kendall, P. A. (2006). Effects of ascorbic acid applied by two hydrocooling methods on physical and chemical properties of green leaf lettuce stored at 5 degrees C. *Journal of Food Science, 71*(3), S270-S276.

Fennema, O. R. (1996). *Food chemistry* (3rd ed.). New York: Marcel Dekker.

Fenwick, G. R. (1996). *Agri-food quality : an interdisciplinary approach.* Cambridge: Royal Society of Chemistry.

Fillion, L., & Kilcast, D. (2002). Consumer perception of crispness and crunchiness in fruits and vegetables. *Food Quality and Preference, 13*(1), 23-29.

Finley, J. W. (2004). Phenolic antioxidants and prevention of chronic inflammation. *Food Technology, 58*(11).

Fitter, A., & Hay, R. K. M. (2002). *Environmental physiology of plants* (3rd ed.). San Diego, Calif.: Academic Press.

Fowden, L., Mansfield, T. A., & Stoddart, J. (1993). *Plant adaptation to environmental stress* (1st ed.). London ; New York: Chapman & Hall.

Foyer, C. (2002). The Contribution of Photosynthetic Oxygen Metabolism to Oxidative Stress in Plants. In D. Inzé (Ed.), *Oxidative Stress in Plants* (pp. 333-368). London: Taylor & Francis.

Frewer, L., Miles, S., Howard, C., & Shepherd, R. (1998). Public Understanding of Food Science and Consumer Acceptance of Novel Products. In M. Hagg, R. Ahvenainen, A. Evers & K. Tiilikkala (Eds.), *Agri-Food Quality II* (pp. 31-34). Cambridge: Royal Society of Chemistry.

Garewal, H. S. (1997). *Antioxidants and disease prevention.* Boca Raton: CRC Press.

Gazula, A., Kleinhenz, M. D., Streeter, J. G., & Miller, A. R. (2005). Temperature and cultivar effects on anthocyanin and chlorophyll b concentrations in three related Lollo Rosso lettuce cultivars. *Hortscience, 40*(6), 1731-1733.

Giordani, R., & Noat, G. (1988). Isolation, Molecular-Properties and Kinetic-Studies of a Strict Beta-Fucosidase from Lactuca-Sativa Latex - Its Possible Role in the Cell-Wall Degradation of Articulated Laticifers. *European Journal of Biochemistry, 175*(3), 619-625.

Goldman, I. L. (2003). Recognition of fruit and vegetables as healthful: Vitamins and phytonutrients (vol 13, pg 252, 2003). *Horttechnology, 13*(3), 404-404.

Gopalan, C., & Tamber, B. (2003). Food-Based Approaches to Prevent and Control Micronutrient Malnutrition: Scientific Evidence and Policy Implications. In A. P. a. G. Simopoulos, C. (Ed.), *Plants in Human Health and Nutrition Policy* (pp. 76-131). Basel: Karger.

Gould, K. S., Neill, S. O., & Vogelmann, T. C. (2002). A unified explanation for anthocyanins in leaves? *Advances in Botanical Research, Vol 37, 37*, 167-192.

Grierson, W. (2002). Role of Temperature in the Physiology of Crop Plants: Pre- and Postharvest. In M. Pessarakli (Ed.), *Handbook of Plant and Crop Physiologyv* (pp. 13-33). New York: Mrcel Dekker, Inc.

Grimme, L. H., & Dumontet, S. (2000). *Food quality, nutrition, and health : 5th Heidelberg Nutrition Forum : proceedings of the ECBA - symposium and workshop, February 27-March 1, 1998 in Heidelberg, Germany.* Berlin ; New York: Springer.

Guenther, P. M., Dodd, K. W., Reedy, J., & Krebs-Smith, S. M. (2006). Most Americans eat much less than recommended amounts of fruits and vegetables. *Journal of the American Dietetic Association, 106*(9), 1371-1379.

Halliwell, B., Rafter, J., & Jenner, A. (2005). Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not? *American Journal of Clinical Nutrition, 81*(1), 268s-276s.

Halvorsen, B. L., Holte, K., Myhrstad, M. C. W., Barikmo, I., Hvattum, E., Remberg, S. F., et al. (2002). A systematic screening of total antioxidants in dietary plants. *Journal of Nutrition, 132*(3), 461-471.

Harlan, J. (1986). Lettuce and the Sycamore - Sex and Romance in Ancient-Egypt. *Economic Botany, 40*(1), 4-15.

Hart, J. W. (1988). *Light and plant growth.* London ; Boston: Unwin Hyman.

Hassimotto, N. M. A., Genovese, M. I., & Lajolo, F. M. (2005). Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *Journal of Agricultural and Food Chemistry, 53*(8), 2928-2935.

Heber, D. (2004). Phytochemicals beyond antioxidation. *Journal of Nutrition, 134*(11), 3175s-3176s.

Heber, D., & Bowerman, S. (2001). Applying science to changing dietary patterns. *Journal of Nutrition, 131*(11), 3078s-3081s.

Hertog, M. G. L., Hollman, P. C. H., & Katan, M. B. (1992). Content of Potentially Anticarcinogenic Flavonoids of 28 Vegetables and 9 Fruits Commonly Consumed in the Netherlands. *Journal of Agricultural and Food Chemistry, 40*(12), 2379-2383.

Heywood, V., Harborne, J., & Turner, B. (Eds.). (1977). *The Biology and Chemistry of the Compositae*. London: Academic Press.

Hirt, H., & Shinozaki, K. (2004). *Plant responses to abiotic stress*. Berlin ; New York: Springer.

Hofmann, T. F., Ho, C.-T., & Pickenhagen, W. (2004). *Challenges in taste chemistry and biology*. Washington, DC: American Chemical Society, Division of Agricultural and Food Chemistry.

Hohl, U., Neubert, B., Pforte, H., Schonof, I., & Bohm, H. (2001). Flavonoid concentrations in the inner leaves of head lettuce genotypes. *European Food Research and Technology, 213*(3), 205-211.

Hopkins, W. G., & Hüner, N. P. A. (2004). *Introduction to plant physiology* (3rd ed.). Hoboken, NJ: J. Wiley.

Howard, L. R., Pandjaitan, N., Morelock, T., & Gil, M. I. (2002). Antioxidant capacity and phenolic content of spinach as affected by genetics and growing season. *Journal of Agricultural and Food Chemistry, 50*(21), 5891-5896.

Huang, D. J., Ou, B. X., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*(6), 1841-1856.

Hung, H. C., Joshipura, K. J., Jiang, R., Hu, F. B., Hunter, D., Smith-Warner, S. A., et al. (2004). Fruit and vegetable intake and risk of major chronic disease. *Journal of the National Cancer Institute, 96*(21), 1577-1584.

Inzé, D., & Van Montagu, M. (2002). *Oxidative stress in plants*. London: Taylor & Francis.

John, J. H., Ziebland, S., Yudkin, P., Roe, L. S., Neil, H. A. W., Grp, O. F. V. S., et al. (2002). Effects of fruit and vegetable consumption on plasma antioxidant concentrations and blood pressure: a randomised controlled trial. *Lancet, 359*(9322), 1969-1974.

Johnston, Jaykus, L. A., Moll, D., Martinez, M. C., Anciso, J., Mora, B., et al. (2005). A field study of the microbiological quality of fresh produce. *Journal of Food Protection, 68*(9), 1840-1847.

Johnston, C. S., Taylor, C. A., & Hampl, J. S. (2000). More Americans are eating "5 a day" but intakes of dark green and cruciferous vegetables remain low. *Journal of Nutrition, 130*(12), 3063-3067.

Kalt, W. (2005). Effects of production and processing factors on major fruit and vegetable antioxidants. *Journal of Food Science, 70*(1), R11-R19.

Kimura, M., & Rodriguez-Amaya, D. B. (2003). Carotenoid composition of hydroponic leafy vegetables. *Journal of Agricultural and Food Chemistry, 51*(9), 2603-2607.

King, L. (2006). *The Convergence of Human and Animal Health.* Paper presented at the Zoonotic Disease Colloquium Fort Collins, CO

Kleinhenz, M. D., French, D. G., Gazula, A., & Scheerens, J. C. (2003). Variety, shading, and growth stage effects on pigment concentrations in lettuce grown under contrasting temperature regimens. *Horttechnology, 13*(4), 677-683.

Knight, S., & Mitchell, C. (1983). Stimulation of Lettuce Productivity by Manipulation of Diural Temperature and LIght. *HortScience, 18*(4), 462-463.

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., et al. (2002). Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *American Journal of Medicine, 113*, 71-88.

Labensky, S. R., Hause, A. M., & Labensky, S. (2003). *On cooking : techniques from expert chefs* (3rd ed.). Upper Saddle River, N.J.: Prentice Hall.

Lampe, J. W. (1999). Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. *Am J Clin Nutr, 70*(3 Suppl), 475S-490S.

Langebartels, C., Schraudner, M., Heller, W., Ernst, D., & Sandermann, H. (2002). Oxidative Stress and Defense Reactions in Plants Exposed to Air Pollutants and

UV-B Radiation. In D. a. M. Inzé, M. (Ed.), *Oxidative Stress in Plants* (pp. 105-135). London: Taylor & Francis.
Lawless, H. T., & Heymann, H. (1999). *Sensory evaluation of food : principles and practices*. Gaithersburg, MD: Aspen Publishers.
Lea, P. J., & Leegood, R. C. (1999). *Plant biochemistry and molecular biology* (2nd ed.). Chichester ; New York: John Wiley.
Lee, J., Koo, N., & Min, D. (2003). Reactive Oxygen Species, Aging, and Antioxidative Nutraceuticals. *Comprehensive Reviews in Food Science and Food Safety, 3*, 21-33.
Lee, T., & Chichester. (1974). The Influence of Harvest Time on Nutritional Value. In P. W. a. N. Selvey (Ed.), *Nutritional Qualities of Fresh Fruits and Vegetables*. Mount Kisco, NY: Futura.
Lesschaeve, I., & Noble, A. C. (2005). Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. *American Journal of Clinical Nutrition, 81*(1), 330S-335S.
Lewis, W. H., & Elvin-Lewis, M. P. F. (2003). *Medical botany : plants affecting human health* (2nd ed.). Hoboken, N.J.: J. Wiley.
Littell, R. C., Stroup, W. W., & Freund, R. J. (2002). *SAS for linear models* (4th ed.). Cary, NC, USA: SAS Institute.
Liu, R. H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *American Journal of Clinical Nutrition, 78*(3), 517s-520s.
Liu, X. (2004). *Total phenolic content and DPPH radical scavenging ability of lettuce (Lactuca sativa L.) grown in Colorado.* Unpublished Thesis (M.S.), Colorado State University, 2004.
Liu, X., Ardo, S., Bunning, M., Parry, J., Zhou, K., Stushnoff, C., et al. (2005). Total Phenolic Content and DPPH• Radical Scavenging Ability of Lettuce (Lactuca sativa L.) Grown in Colorado. *Food Science and Technology, (in press).*
Llorach, R., Tomas-Barberan, F. A., & Ferreres, F. (2004). Lettuce and chicory byproducts as a source of antioxidant phenolic extracts. *Journal of Agricultural and Food Chemistry, 52*(16), 5109-5116.
Manach, C., Morand, C., Gil-Izquierdo, A., Bouteloup-Demange, C., & Remesy, C. (2003). Bioavailability in humans of the flavanones hesperidin and narirutin after the ingestion of two doses of orange juice. *European Journal of Clinical Nutrition, 57*(2), 235-242.
Manach, C., Scalbert, A., Morand, C., Remesy, C., & Jimenez, L. (2004). Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition, 79*(5), 727-747.
Manach, C., Williamson, G., Morand, C., Scalbert, A., & Remesy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *American Journal of Clinical Nutrition, 81*(1), 230s-242s.
Mano, J. i. (2002). Early Events in Environmental Stresses in Plants-Induction Mechanisms of Oxidative Stress. In D. Inzé (Ed.), *Oxidative Stress in Plants* (pp. 217-245). London: Taylor & Francis.
Mathers, J. C. (2006). Plant foods for human health: research challenges. *Proceedings of the Nutrition Society, 65*(2), 198-203.

McCann, S. E., Freudenheim, J. L., Marshall, J. R., & Graham, S. (2003). Risk of human ovarian cancer is related to dietary intake of selected nutrients, phytochemicals and food groups. *Journal of Nutrition, 133*(6), 1937-1942.

McMahon, M., Kofranek, A. M., & Rubatzky, V. E. (2002). *Hartmann's plant science : growth, development, and utilization of cultivated plants* (3rd ed.). Upper Saddle River, N.J.: Prentice Hall.

McMaster, G. S., & Wilhelm, W. W. (1997). Growing degree-days: one equation, two interpretations. *Agricultural and Forest Meteorology, 87*(4), 291-300.

Meilgaard, M., Civille, G. V., & Carr, B. T. (1999). *Sensory evaluation techniques* (3rd ed.). Boca Raton, Fla.: CRC Press.

Miller, N. J., & Rice-Evans, C. A. (1996). Spectrophotometric determination of antioxidant activity. *Redox Report, 2*(3), 161-171.

Miller, N. J., & Rice-Evans, C. A. (1997). Factors influencing the antioxidant activity determined by the ABTS(center dot+) radical cation assay. *Free Radical Research, 26*(3), 195-199.

Moore, J., Liu, J. G., Zhou, K. Q., & Yu, L. L. (2006). Effects of genotype and environment on the antioxidant properties of hard winter wheat bran. *Journal of Agricultural and Food Chemistry, 54*(15), 5313-5322.

Morello, M. J., Shahidi, F., & Ho, C.-T. (2002). *Free radicals in food : chemistry, nutrition, and health effects*. Washington, DC: American Chemical Society : Distributed by Oxford University Press.

Nicolle, C., Cardinault, N., Gueux, E., Jaffrelo, L., Rock, E., Mazur, A., et al. (2004). Health effect of vegetable-based diet: lettuce consumption improves cholesterol metabolism and antioxidant status in the rat. *Clinical Nutrition, 23*(4), 605-614.

Nicolle, C., Carnat, A., Fraisse, D., Lamaison, J. L., Rock, E., Michel, H., et al. (2004). Characterisation and variation of antioxidant micronutrients in lettuce (Lactuca sativa folium). *Journal of the Science of Food and Agriculture, 84*(15), 2061-2069.

Ninfali, P., & Bacchiocca, M. (2003). Polyphenols and antioxidant capacity of vegetables under fresh and frozen conditions. *Journal of Agricultural and Food Chemistry, 51*(8), 2222-2226.

Ninfali, P., Mea, G., Giorgini, S., Rocchi, M., & Bacchiocca, M. (2005). Antioxidant capacity of vegetables, spices and dressings relevant to nutrition. *British Journal of Nutrition, 93*(2), 257-266.

Ott, L., & Longnecker, M. (2001). *An introduction to statistical methods and data analysis* (5th ed.). Pacific Grove, CA: Duxbury.

Perl-Treves, R., & Perl, A. (2002). Oxidative Stress: An Introduction. In D. a. M. Inzé, M. (Ed.), *Oxidative Stress in Plants* (pp. 1-32). London: Taylor & Francis.

Piagentini, A. M., Mendez, J. C., Guemes, D. R., & Pirovani, M. E. (2005). Modeling changes of sensory attributes for individual and mixed fresh-cut leafy vegetables. *Postharvest Biology and Technology, 38*(3), 202-212.

Poli, F., Sacchetti, G., Tosi, B., Fogagnolo, M., Chillemi, G., Lazzarin, R., et al. (2002). Variation in the content of the main guaianolides and sugars in Cichorium intybus var. "Rosso di Chioggia" selections during cultivation. *Food Chemistry, 76*(2), 139-147.

Pollard, J., Kirk, S. F. L., & Cade, J. E. (2002). Factors affecting food choice in relation to fruit and vegetable intake: a review. *Nutrition Research Reviews, 15*(2), 373-387.

Price, K. R., Dupont, M. S., Shepherd, R., Chan, H. W. S., & Fenwick, G. R. (1990). Relationship between the Chemical and Sensory Properties of Exotic Salad Crops - Colored Lettuce (Lactuca-Sativa) and Chicory (Cichorium-Intybus). *Journal of the Science of Food and Agriculture, 53*(2), 185-192.

Prior, R. L., Wu, X. L., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*(10), 4290-4302.

Raffo, A., La Malfa, G., Fogliano, V., Malani, G., & Quaglia, G. (2006). Seasonal variations in antioxidant components of cherry tomatoes (Lycopersicon esculentum cv. Naomi F1). *Journal of Food Composition and Analysis, 19*(1), 11-19.

Raskin, I., Ribnicky, D. M., Komarnytsky, S., Ilic, N., Poulev, A., Borisjuk, N., et al. (2002). Plants and human health in the twenty-first century. *Trends in Biotechnology, 20*(12), 522-531.

Rätsch, C. (2005). *The Encyclopedia of Psychoactive Plants : Ethnopharmacology and its Applications* (1st U.S. ed.). Rochester, Vt.: Park Street Press.

Raven, P. H., Evert, R. F., & Eichhorn, S. E. (1999). *Biology of plants* (6th ed.). New York: W.H. Freeman : Worth Publishers.

Rees, S., & Harborne, J. (1984). Flavonoids and Other Phenolics of Cichorium and Related Members of the Lactuceae (Compositae). *Botanical Journal of the Linnean Society, 89*(4), 313-319.

Rivera, C. M., Battistelli, A., Moscatello, S., Proietti, S., Rouphael, Y., Cardarelli, M., et al. (2006). Influence of simulated microgravity on growth, yield, and quality of leafy vegetables: Lettuce and rocket. *European Journal of Horticultural Science, 71*(1), 45-51.

Rivera, J. R. E., Stone, M. B., Stushnoff, C., Pilon-Smits, E., & Kendall, P. A. (2006). Effects of ascorbic acid applied by two hydrocooling methods on physical and chemical properties of green leaf lettuce stored at 5 degrees C. *Journal of Food Science, 71*(3), S270-S276.

Romani, A., Pinelli, P., Galardi, C., Sani, G., Cimato, A., & Heimler, D. (2002). Polyphenols in greenhouse and open-air-grown lettuce. *Food Chemistry, 79*(3), 337-342.

Roy, G. M. (1997). *Modifying bitterness : mechanism, ingredients, and applications.* Lancaster, Pa.: Technomic Pub. Co.

Rubatzky, V. E., & Yamaguchi, M. (1997). *World vegetables : principles, production, and nutritive values* (2nd ed.). New York Chapman & Hall.

Ryder, E. J. (1999). *Lettuce, endive, and chicory.* New York, NY: CABI Pub.

Ryder, E. J. (2002). The New Salad Crop Revolution. In J. J. a. A. Whipkey (Ed.), *Trends in New Crops and New Uses.* Alexandria, VA: ASHS Press.

Sanchez-Moreno, C. (2002). Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science and Technology International, 8*(3), 121-137.

Scalbert, A., Johnson, I. T., & Saltmarsh, M. (2005). Polyphenols: antioxidants and beyond. *American Journal of Clinical Nutrition, 81*(1), 215S-217S.
Scalzo, J., Politi, A., Pellegrini, N., Mezzetti, B., & Battino, M. (2005). Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition, 21*(2), 207-213.
Serafini, M., Bugianesi, R., Salucci, M., Azzini, E., Raguzzini, A., & Maiani, G. (2002). Effect of acute ingestion of fresh and stored-lettuce (Lactuca sativa) on plasma total antioxidant capacity and antioxidant levels in human subjects. *British Journal of Nutrition, 88*(6), 615-623.
Shahidi, F. (2003). *Food factors for health promotion and disease prevention.* Washington, DC: American Chemical Society : Distributed by Oxford University Press.
Shewfelt, R. (1990). Quality of Fruits and Vegetables. *Food Technology, 44*(6), 99-106.
Shils, M. E., Olson, J. A., Shike, M., & Ross, A. (1999). *Modern nutrition in health and disease* (9th ed.). Philadelphia: Lea & Febiger.
Simon, P. W. (1997). Plant pigments for color and nutrition. *Hortscience, 32*(1), 12-13.
Simonne, A., Simonne, E., Eitenmiller, R., & Coker, C. H. (2002). Bitterness and composition of lettuce varieties grown in the southeastern United States. *Horttechnology, 12*(4), 721-726.
Singleton, V. L., & Rossi, J. A. J. (1965). Colorimetry of total phenolics with phosphmolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic., 16*, 144-158.
Sivapalasingam, S., Friedman, C. R., Cohen, L., & Tauxe, R. V. (2004). Fresh produce: A growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *Journal of Food Protection, 67*(10), 2342-2353.
Sorenson, A. W., Wyse, B. W., Wittwer, A. J., & Hansen, R. G. (1976). Index of Nutritional Quality for a Balanced Diet - New Help for an Old Problem. *Journal of the American Dietetic Association, 68*(3), 236-242.
Spanos, G. A., & Wrolstad, R. E. (1990). Influence of Processing and Storage on the Phenolic Composition of Thompson Seedless Grape Juice. *Journal of Agricultural and Food Chemistry, 38*(7), 1565-1571.
Stevens, M. A. (1974). Varietal Influence on Nutritional Value. In P. White & N. Selvey (Eds.), *Nutritional Qualities of Fresh Fruits and Vegetables*. Mount Kisco, NY: Futura.
Stonaker, F., & Guenther, D. (2003). The lettuce bolting resistance project.
Stone, H., & Sidel, J. L. (1993). *Sensory evaluation practices* (2d ed.). San Diego: Academic Press.
Strack, D., & Wray, V. (1994). The anthocyanins. In J. Harborne (Ed.), *The Flavoids*. New York: Chapman & Hall.
Stushnoff, C., Külen, O., Wilson, P., & Holm, D. (2006). *Some Antioxidant Properties of Purple Potato Cultivars and Selections.* . Paper presented at the PAA/Solanaceae, Genomics Meets Biodiversity, Madison, Wisconsin, USA.
Stushnoff, C., Kulen, O., Wilson, P. E., Joyce, N. I., Holm, D. G., & Yohn, A. (2006). *Some Antioxidant Properties of Pigmented Potato Cultivar and Selections.*
Su, L. J., & Arab, L. (2006). Salad and Raw Vegetable Consumption and Nutritional Status in the Adult US Population: Results from the Third National Health and

Nutrition Examination Survey. *Journal of the American Dietetic Association, 106*(9), 1394-1404.
Svilaas, A., Sakhi, A. K., Andersen, L. F., Svilaas, T., Strom, E. C., Jacobs, D. R., et al. (2004). Intakes of antioxidants in coffee, wine, and vegetables are correlated with plasma carotenoids in humans. *Journal of Nutrition, 134*(3), 562-567.
Tarwadi, K., & Agte, V. (2003). Potential of commonly consumed green leafy vegetables for their antioxidant capacity and its linkage with the micronutrient profile. *International Journal of Food Sciences and Nutrition, 54*(6), 417-425.
Thompson, H. J., Heimendinger, J., Diker, A., O'Neill, C., Haegele, A., Meinecke, B., et al. (2006). Dietary botanical diversity affects the reduction of oxidative biomarkers in women due to high vegetable and fruit intake. *Journal of Nutrition, 136*(8), 2207-2212.
Thorngate, J. H. (1997). Factors Affecting the Perception of Bitterness: A Review. In G. Roy (Ed.), *Modifying Bitterness*. Lancaster, Pennsylvania: Technomic Publishing Company.
Trewavas, A. (2005). Green plants as intelligent organisms. *Trends in Plant Science, 10*(9), 413-419.
USDA. (2005). *United States Department of Agriculture National Nutrient Database for Standard Reference, Release 18*: (accessed September, 2006).
van Wyk, B.-E. (2005). *Food Plants of the World*. Portland, Oregon: Timber Press.
Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry, 46*(10), 4113-4117.
Verbeke, W. (2006). Functional foods: Consumer willingness to compromise on taste for health? *Food Quality and Preference, 17*(1-2), 126-131.
Vinson, J. A., Hao, Y., Su, X. H., & Zubik, L. (1998). Phenol antioxidant quantity and quality in foods: Vegetables. *Journal of Agricultural and Food Chemistry, 46*(9), 3630-3634.
Voorhees, D. (1995). *Why Does Popcorn Pop?* New York: MJF Books.
Wagner, H. (1977). Pharmaceutical and economic uses of the Compositae. In V. Heywood, J. Harborne and B. Turner (Ed.), *The Biology and Chemistry of the Compositae*
San Francisco: Academic Press.
Waldron, K., Parker, M., & Smith, A. (2003). Plant Cell Walls and Food Quality. *Comprehensive Reviews in Food Science and Food Safety, 2*, 101-119.
Wang, W.-c., Gorsuch, J. W., & Hughes, J. S. (1997). *Plants for environmental studies*. Boca Raton: CRC Press.
Wien, H. C. (1997). *The physiology of vegetable crops*. Oxford ; New York: CAB International.
Willcox, J. K., Ash, S. L., & Catignani, G. L. (2004). Antioxidants and prevention of chronic disease. *Critical Reviews in Food Science and Nutrition, 44*(4), 275-295.
Wu, X. L., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry, 52*(12), 4026-4037.

Wu, X. L., & Prior, R. L. (2005). Identification and characterization of anthocyanins by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry in common foods in the United States: Vegetables, nuts, and grains. *Journal of Agricultural and Food Chemistry, 53*(8), 3101-3113.

Zhou, K. Q., & Yu, L. L. (2004). Antioxidant properties of bran extracts from Trego wheat grown at different locations. *Journal of Agricultural and Food Chemistry, 52*(5), 1112-1117.

Zind, T. (2005, November). Foodservice Trends: Salads and Salad Dressings. *Prepared Foods,* 55-60.

Zomlefer, W. B. (1994). *Guide to flowering plant families*. Chapel Hill: University of North Carolina Press.

APPENDIX

STATISTICAL PROCEDURES

PROC MIXED COMMANDS

```
proc mixed; class harvest cultivar;
model TP = cultivar; random harvest harvest*cultivar; lsmeans cultivar/diff; run;
```

PROC MIXED calls the procedure.
The CLASS statement specifies that HARVEST and CULTIVAR are classification variables as opposed to continuous variables.
The MODEL statement is an equation whose left-hand side contains the name of the response variable to be analyzed, in this case total phenolic content. The right-hand side of the MODEL statement contains a list of the fixed-effect variables, in this case the variable cultivar.
The RANDOM statement contains a list of the random effects, in this case harvest.

The Tests of Fixed Effects generated from the proc mixed procedure is like an abbreviated ANOVA table showing a line of computations for each term in the model statement, in this example, cultivar. Included is an F-test for testing the null hypothesis, Ho: $\mu_1 = \mu_2 = \mu_3$.

The basic Proc Mixed computations are based on likelihood principles, but many of the statistical computations are the same as those obtained from analysis of variance methods for a balanced data set (Littell, Stroup, & Freund, 2002).

STATISTICAL TABLES

Table 1. Statistical Analysis of Appearance Scores

Class Level Information

Class	Levels	Values
Harvest	3	1 2 3
Panelist	30	
Cultivar	5	Crispino Crisp and Green Green Forest Lochness Vulcan

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Harvest	2	38	0.86	0.4319
Cultivar	4	262	12.02	<.0001
Harvest*Cultivar	8	262	0.88	0.5313

Cultivar	Mean	Std. Error
Crispino	80.8	1.72
Crisp and Green	87.7	1.79
Green Forest	79.9	1.72
Lochness	78.6	1.72
Vulcan	88.3	1.72

Table 2. Statistical Analysis of Flavor Scores

Class Level Information

Class	Levels	Values
Harvest	3	1 2 3
Panelist	30	
Cultivar	5	Crispino Crisp and Green Green Forest Lochness Vulcan

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Harvest	2	38	0.24	0.79
Cultivar	4	262	4.11	0.003
Harvest*Cultivar	8	262	2.08	0.038

Cultivar	Mean	Std. Error
Crispino	79.6	2.17
Crisp and Green	72.9	2.17
Green Forest	70.9	2.17
Lochness	71.7	2.17
Vulcan	71.6	2.17

Table 3. Statistical Analysis of Texture Scores

Class Level Information

Class	Levels	Values
Harvest	3	1 2 3
Panelist	30	
Cultivar	5	Crispino Crisp and Green Green Forest Lochness Vulcan

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Harvest	2	38	0.7	0.5041
Cultivar	4	261	4.61	0.0013
Harvest*Cultivar	8	261	1.56	0.1373

Cultivar	Mean	Std. Error
Crispino	81.4	1.83
Crisp and Green	77.8	1.83
Green Forest	72.7	1.83
Lochness	75.1	1.83
Vulcan	73.8	1.82

Table 4. Statistical Analysis of Overall Acceptability Scores

Class Level Information

Class	**Levels**	**Values**
Harvest	3	1 2 3
Panelist	30	
Cultivar	5	Crispino Crisp and Green Green Forest Lochness Vulcan

Type 3 Tests of Fixed Effects

Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Harvest	2	37	0.86	0.4319
Cultivar	4	258	12.02	<.0001
Harvest*Cultivar	8	258	0.88	0.5313

Cultivar	**Mean**	**Std. Error**
Crispino	79.6	1.72
Crisp and Green	73.3	1.72
Green Forest	71.7	1.72
Lochness	69.5	1.72
Vulcan	73.3	1.74

Table 5. Statistical Analysis of Bitterness Scores

Class Level Information

Class	Levels	Values
Harvest	3	1 2 3
Panelist	30	
Cultivar	5	Crispino Crisp and Green Green Forest Lochness Vulcan

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F	
Harvest	2	38	0.22	0.8063	
Cultivar	4	262	6.48	<.0001	
Harvest*Cultivar	8	262	3.2	0.0018	
Crispino Harvest 1 & Crispino Harvest 3					0.0023
Crisp and Green Harvest 2 & Crisp and Green Harvest 3					0.0085
Lochness Harvest 1 & Lochness Harvest 2					0.0268

Cultivar	Mean	Std. Error
Crispino	38.5	2.8
Crisp and Green	51.2	2.8
Green Forest	45.6	2.8
Lochness	50.1	2.8
Vulcan	50.3	2.7

Table 6. Total Phenolic Content of Lettuce Cultivars

Class Level Information

Class	Levels	Values
Harvest	6	1 2 3 4 5 6
Cultivar	8	Cimmaron Crisp and Green Crispino Green Forest Lockness Nevada Sierra Vulcan

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Cultivar	7	32	23.55	<.0001

Cultivar	Mean	Std. Error
Cimmaron	41.06	2.64
Crisp and Green	27.9	2.64
Crispino	15.09	3.56
Green Forest	19.07	2.64
Lockness	21.04	2.64
Nevada	21.6	2.64
Sierra	23.49	2.68
Vulcan	48.2	2.64

Table 7. Total Phenolic Content of Lettuce Types

Class Level Information

Class	Levels	Values
Harvest	6	1 2 3 4 5 6
Type/subtype	5	Batavia Butterhead Crisphead Leaf Romaine

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type/subtype	4	17	13.79	<.0001

Type	Mean	Std. Error
Batavia	22.54	2.29
Butterhead	21.04	2.95
Crisphead	15.21	4.06
Leaf	38.05	2.27
Romaine	30.06	2.27

Table 8. Total Phenolic Content of Lettuce Cultivars by Harvest

Variable: Total Phenolic Content
Alpha: 0.05

Cultivar	Mean	LSD	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Crispino	15.83	9.95	Harvest	2	329.72	164.86	6.65	0.0301
Crisp and Greei	27.9	15.1	Harvest	5	469.93	93.99	1.31	0.325
Cimmaron	41.06	22.42	Harvest	5	998.62	199.72	1.26	0.3431
Green Forest	19.07	8.32	Harvest	5	118.96	23.79	1.09	0.415
Lochness	21.04	10.78	Harvest	5	348.16	69.63	1.9	0.1687
Nevada	21.6	10.64	Harvest	5	752.95	150.59	4.21	0.0193
Sierra	23.74	12.6	Harvest	5	376.58	75.32	1.66	0.2245
Vulcan	48.2	10.26	Harvest	5	1175.67	235.13	7.07	0.0027

Table 9. Antioxidant Capacity of Lettuce Cultivars

Class Level Information

Class	Levels	Values
Harvest	6	1 2 3 4 5 6
Cultivar	8	Cimmaron Crisp and Green Crispino Green Forest Lockness Nevada Sierra Vulcan

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Cultivar	7	32	15.15	<.0001

Cultivar	Mean	Std. Error
Cimmaron	652.17	50.25
Crisp and Green	380.66	49.67
Crispino	157.64	67.21
Green Forest	361.72	49.67
Lockness	262.17	49.67
Nevada	298.29	49.67
Sierra	336.56	49.67
Vulcan	670.01	49.67

Table 10. Antioxidant Capacity of Lettuce Types

Class Level Information

Class	Levels	Values
Harvest	6	1 2 3 4 5 6
Type/subtype	5	Batavia Butterhead Crisphead Leaf Romaine

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type/subtype	4	17	13.52	<.0001

Type	Mean	Std. Error
Batavia	317.43	40.9
Butterhead	262.17	50.08
Crisphead	157.74	68.15
Leaf	525.33	40.9
Romaine	502.43	41.2

Table 11. Antioxidant Capacity of Lettuce Cultivars by Harvest

Variable: TEAC
Alpha: 0.05

Cultivar	Mean	LSD	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Crispino	167.34	62.5	Harvest	2	20371.4	10185.7	10.41	0.0112
Crisp and Greei	380.66	173.72	Harvest	5	199245	39849	4.18	0.0197
Cimmaron	652.54	374.75	Harvest	5	447521	89504.3	2.23	0.1244
Green Forest	361.72	98.52	Harvest	5	39821.8	7964.36	2.6	0.0816
Lochness	262.17	167.17	Harvest	5	131722	26344.4	2.98	0.0561
Nevada	298.29	176.41	Harvest	5	217263	43452.6	4.42	0.0163
Sierra	336.56	163.31	Harvest	5	390446	78089.3	9.27	0.0008
Vulcan	670.01	314.65	Harvest	5	186138	37227.7	1.19	0.3702

Panelist # ______
Time ________

Bitterness evaluation

Bitterness may be desirable or undesirable in food flavors, and because of genetic differences, individuals vary in their ability to perceive certain bitter substances.

You are being asked to taste four solutions of increasing bitterness. Water and crackers are provided to cleanse your palate. Start with solution #1, the weakest. Hold in your mouth for at least 10 seconds, you don't have to swallow. Wait at least 10 seconds before tasting the next solution. The first solution has a bitterness score of 0 (see table). Please repeat with the other three solutions, cleansing your palate between tasting solutions.

Solution:	#1	#2	#3	#4
	Neither bitter nor mild	Slightly bitter	Bitter	Very bitter
Bitterness score:	0	25	50	100

Please taste solution Q and assign a bitterness score between 0 and 100.

Solution Q
Bitterness score: ________

Comments:
____
____
____
____

Thanks for your time and participation.

COLORADO STATE UNIVERSITY
INFORMED CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

TITLE OF PROJECT: Sensory Evaluation of Colorado Grown Summer Lettuce

NAME OF PRINCIPAL INVESTIGATOR: Patricia A. Kendall, Ph.D., R.D.

NAME OF CO-INVESTIGATOR: Marisa Bunning, M. S.

CONTACT NAME & PHONE NUMBER FOR QUESTIONS/PROBLEMS: Marisa Bunning, 970-491-3060

SPONSOR OF PROJECT: Colorado AES

PURPOSE OF THE RESEARCH:
This study involves the sensory evaluation of five varieties of lettuce grown at the CSU Horticulture Research Center during different time periods in 2004. This study is designed to determine the acceptability of lettuce grown in Colorado during various times through the summer months.

PROCEDURES/METHODS TO BE USED:
You will taste solutions containing varying amount of a bitter substance. The solutions will be prepared in a food laboratory in the Department of Food Science and Human Nutrition. You will be told the bitterness rating of the samples and ask to evaluate an unlabeled bitter solution. The sample testing will not take more than 30 minutes. You will not be videotaped or audiotaped during any tastings.

RISKS INHERENT IN THE PROCEDURES:
There are no known risks involved in this research. It is not possible to identify all potential risks in an experimental procedure, but the researcher(s) have taken reasonable safeguards to minimize any known and potential, but unknown, risks. Pregnant or lactating females may not participate.

BENEFITS:
You will be able to taste and consume bitter solutions of varying strength. You will receive fruit juice beverages at the completion of the tasting session. In addition, you will further research to determine acceptability of Colorado-grown lettuce.

CONFIDENTIALITY:
Strict confidentiality of information will be maintained by recording data using sequential numbers to identify panelists. Resulting data will be reported in research materials in aggregate. Only the investigators and necessary personnel (graduate student) will have access to the individual sensory evaluation sheets.

LIABILITY:
The Colorado Governmental Immunity Act determines and may limit Colorado State University's legal responsibility if an injury happens because of this study. Claims against the University must be filed within 180 days of the injury.

Questions about subjects' rights may be directed to Celia S. Walker at (970) 491-1563.

Page 1 of 2 Subject initials _______ Date _______

PARTICIPATION:
Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled.

Your signature acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing 2 pages.

____
Participant name (printed)

_________________________________ __________________
Participant signature Date

_________________________________ __________________
Witness to signature (project staff) Date

PARENTAL SIGNATURE FOR MINOR

As parent or guardian you authorize ________________________ (print name) to become a participant for the described research. The nature and general purpose of the project have been satisfactorily explained to you by _____________________ and you are satisfied that proper precautions will be observed.

____
Minor's date of birth

____
Parent/Guardian name (printed)

_________________________________ __________________
Parent/Guardian signature Date

Sample # _____
Panelist # _____
Date _________

Lettuce Sensory Evaluation

Please complete scoring for this sample using the attributes listed below. Make a vertical pencil mark to indicate your opinion of the sample along the given line. Please eat the entire sample and cleanse palate with water and crackers between samples.

Appearance:

Very Unacceptable	Unacceptable	Somewhat Unacceptable	Neither Acceptable nor Unacceptable	Somewhat Acceptable	Acceptable	Very Acceptable

Flavor:

Very Unacceptable	Unacceptable	Somewhat Unacceptable	Neither Acceptable nor Unacceptable	Somewhat Acceptable	Acceptable	Very Acceptable

Texture:

Very Unacceptable	Unacceptable	Somewhat Unacceptable	Neither Acceptable nor Unacceptable	Somewhat Acceptable	Acceptable	Very Acceptable

Overall Acceptability:

Very Unacceptable	Unacceptable	Somewhat Unacceptable	Neither Acceptable nor Unacceptable	Somewhat Acceptable	Acceptable	Very Acceptable

Bitterness:

Extremely Mild	Very Mild	Somewhat Mild	Neither Bitter nor Mild	Somewhat Bitter	Very Bitter	Extremely Bitter

Comments:

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Thanks for your time and participation.