COMPARATIVE ANALYSIS OF ANTIOXIDANT PROPERTIES, QUALITY ATTRIBUTES AND YIELD OF ORGANICALLY AND CONVENTIONALLY GROWN MELONS (Cucumis melo L.) AND TOMATOES (Solanum lycopersicum L.)

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Spring 2008

COLORADO STATE UNIVERSITY

Department of Horticulture and Landscape Architecture

## THESIS

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### COMPARATIVE ANALYSIS OF ANTIOXIDANT PROPERTIES, QUALITY ATTRIBUTES AND YIELD OF ORGANICALLY AND CONVENTIONALLY GROWN MELONS (*Cucumis melo* L.) AND TOMATOES (*Solanum lycopersicum* L.)

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2008

#### COLORADO STATE UNIVERSITY

April 8, 2008

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY KAREN A. SALANDANAN ENTITLED COMPARATIVE ANALYSIS OF ANTIOXIDANT PROPERTIES, QUALITY ATTRIBUTES AND YIELD OF ORGANICALLY AND CONVENTIONALLY GROWN MELONS (*Cucumis melo* L.) AND TOMATOES (*Solanum lycopersicum* L.) BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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#### ABSTRACT OF THESIS

#### COMPARATIVE ANALYSIS OF ANTIOXIDANT PROPERTIES, QUALITY ATTRIBUTES AND YIELD OF ORGANICALLY AND CONVENTIONALLY GROWN MELONS (*Cucumis melo* L.) AND TOMATOES (*Solanum lycopersicum* L.)

Fresh fruits and vegetables including melons and tomatoes have gained considerable prominence in the American diet due to increasing awareness of their potential health and nutrition benefits. Consumers are starting to take a closer look not only at organoleptic qualities but also at nutritional value of the produce. This new trend of consumer preference for healthier food choices is one of the driving forces of the organic industry. Organically grown fruits and vegetables are perceived to be more nutritious than those that are produced under conventional production system. Comparison studies on selected fruit and vegetable crops have been carried out to examine this claim. However, verifiable results from well-designed experiments were very limited in many crops, including tomatoes and melons.

This research assessed the difference in the antioxidant properties, quality attributes, and yield of organically and conventionally grown tomatoes and melons. Nutritionally superior cultivars were also identified which could be beneficial for small and medium sized farmers. Ten commercial cultivars of tomatoes were grown in 2005 and 2006, under certified organic and conventional production systems at the Horticulture Field Research Center, Colorado State University, Colorado USA. Melon and tomato cultivars were analyzed for their ascorbic acid, total phenolic content, Trolox equivalent antioxidant capacity (TEAC), percent dry matter, soluble solids content, and yield.

A 3 to 6 fold difference in ascorbic acid, total phenolic content, and antioxidant activity was observed both in melon and tomato cultivars. Melons grown organically had significantly higher ascorbic acid when both years were combined while total phenolic content was significant only in the first year. In tomatoes, higher antioxidant activity was observed in organically grown tomatoes while yield and soluble solids concentration was higher under the conventional production system. Lower yield was observed in melon and tomato cultivars with higher antioxidant content and activity suggesting a possible trade- off between yield and nutrients.

Compared to genotype, production system had less influence on antioxidant properties and fruit quality attributes. A significant interaction between cultivar and production system would likewise imply that some cultivars when grown organically or conventionally could have higher antioxidant content and activity. Thus, choice of cultivar was the most important contributor to high antioxidant properties. Based on the antioxidant index that we have developed as a tool to rank the cultivars in terms of antioxidant content and activity, the top melon cultivars, regardless of year or production system, are the following: 'Savor', 'Sweetie#6', 'Early Queen', 'Edonis' and 'Rayan'; while 'Jet Star', 'New Girl', 'Fanstastic', 'First Lady', and 'Celebrity' were determined to be nutritionally superior tomatoes. These nutritionally superior cultivars with high antioxidant levels may provide a competitive marketing and supply niche for small farm producers. Future initiatives could involve screening of tomato and melon cultivars for higher antioxidant content that could be utilized in breeding programs.

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#### ACKNOWLEDGMENT

I would like to extend my deepest appreciation to my advisor, Dr. Cecil Stushnoff, for his support and guidance in every crucial step that I took as a graduate student and researcher. His brilliance and enthusiasm for learning and research had inspired me to critically examine ideas and possibilities in different perspectives.

I would like to acknowledge my committee members, Dr. Stephen Wallner and Dr. Marisa Bunning, for their valuable suggestions during the thesis defense and encouragement throughout the entire period of my graduate work.

I would like to thank Mr. James zumBrunnen who provided sound pieces of advice on statistical procedures used in data analysis; Ms. Jeannette Stushnoff who trained me to do ascorbic acid analysis using HPLC; and Mr. Frank Stonaker who spearheaded the field experiments and for his patience in answering my vegetable production-related inquiries. I would like to acknowledge fellow students that I have worked with in Stushnoff Lab (Ms. Stephanie Brown, Ms. Heather Troxell, and Ms. Amber Yohnar). I am also thankful for the administrative assistance provided by Ms. Gretchen Dewesee, Ms. Bonnie Schilling, and Ms. Kathi Nietfeld.

The Institute of International Education and Philippine-American Educational Foundation that jointly coordinated the Fulbright-Philippine Agriculture Scholarship Program, for providing me the great opportunity to do my graduate studies in CSU and facilitating all the required documents during my stay here. I extend my special thanks to Dr. Esmeralda Cunanan, Ms. Gigi Dizon, Ms. Marjorie Tolentino, and Ms. Nora Yepes.

I would also like to acknowledge my friends with whom I shared the joy, hope, and minor frustrations of living thousands of miles away from home.

I am also indebted to my parents and siblings who gave their full support and prayers when I opted to pursue my master's degree in the U.S.

I dedicate this piece of work to my God who led me to meet the aforementioned supportive people and made this wonderful journey of graduate study possible and enriching.

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#### **CHAPTER I**

#### INTRODUCTION

Consumption of fruit and vegetable crops is expected to rise because of increasing awareness that fruits and vegetables can lower the prevalence of important risk factors for cardiovascular diseases and contribute to the reduction of cancer incidence (Bazzano et al., 2003; van't Veer et al., 2000). Among fruit and vegetable crops, melons and tomatoes are predicted to maintain a stable stronghold in the American diet. There has been an increasing trend in melon and tomato consumption in the last 35 years (Lucier and Jerardo, 2007). With melons (cantaloupe and honeydew), there has been an increase in per capita use from 8.1 lbs in 1970 to 11.7 lbs in 2007. Similarly, there has been an approximately 23% increase in per capita use of fresh and canned tomatoes, from 74.2 lbs in 1970 to 90.9 lbs in 2007. Along with this increase in consumption, the production sector is also growing to meet the demand. In 2004, cantaloupe production was valued at \$300.6 million and honeydew production totaled \$89 million (Boriss et al., 2006). In the same year, nearly 2 million tons and 12 million tons of fresh market and processing tomatoes were produced, respectively.

Although the majority of tomato and melon producers are still interested in increased quantity to meet the growing demand, some small and medium scale producers are taking into consideration the special needs of consumers for organically grown fruits and vegetables. In response to consumer concerns, producers could benefit from the price premiums placed on organic produce that served as a catalyst for the growth of organic crop farmlands and expansion of organic market (Oberholtzer et al., 2005). Most sales of organic foods occur through conventional markets and grocery stores; however, some producers opt to go to farmer's markets for direct selling of their produce to consumers. Dimitri and Greene (2002) of the USDA reported that in 1994, there were only 1,755 farmers' markets, but in 2005, this number doubled to 3,700 markets. This was attributed to the strong and moderate demand for organic products surveyed across the country. The organic food market has been growing steadily since late 1990s. Thilmany (2006) reported that sales of organic food were estimated at \$1 billion in 1990 and reached \$10 billion in 2003 or around 1.8% of total US food market. Moreover, according to the *Nutrition Business Journal*, U.S. sales of organic products were \$15.7 billion in 2005—nearly 2.5 percent of total food sales—and were predicted to reach \$17.8 billion by 2007. Among organically grown food, fruits and vegetables remain as top sellers accounting for 39% of the U.S. organic food sales in 2005. Official USDA data were not available for organic tomato and melon sales.



Figure 1.1. 2005 Organic food sales (Source: (<u>http://www.ers.usda.gov/Briefing/Organic/Demand.htm</u>)

Kortbech-Olesen (2003) also reported that annual growth rates between 15 and 20% for organic retail sales are expected over the next few years, which makes the United States the most vigorous organic growth market.

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Knudson (2007) explained that there are two drivers for increased demand for organic produce. The primary demand driver for the increased consumption of organic food is health concerns, and the second demand driver is the formulation of the USDA Organic Food standards, which appears to have increased consumer awareness of organic foods. With regard to the primary demand driver, common perceptions have been held that the full potential of fruit and vegetable crops to contribute to good health and prevention of chronic diseases would only be realized when crops are produced in a way that would enhance nutritional content and minimize the environmental impact of using synthetic pesticides and fertilizers. This new way of looking at produce quality, health, and safety supports the expanding organic industry.

Does the organic production system enhance nutritional quality of fruit and vegetable crops? This big question demands a special research focus in order to determine the differences and sustain growth of the organic industry. However, before examining comparative studies (organic vs. conventional) related to nutrition considerations, it is crucial to understand antioxidant and quality attributes and how these parameters are affected by several production-related factors (i.e. genotype, environment and management practices).

Antioxidant content is comprised of non-essential phytonutrients/phytochemicals and essential nutrients like vitamins with antioxidant functions. A wide variety of phytochemicals are present in of fruit and vegetable crops and have a significant role in

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protecting plants against biotic and abiotic stresses such as excess light, high temperature, proliferation of reactive oxygen species, and pathogen and insect pest attack (Schreiner, 2005). In humans, phytochemicals are being examined for their ability to provide health benefits by acting as cofactors and/or inhibitors of enzymatic reactions and scavenging reactive or toxic chemicals (Dillard and German, 2000). Harborne (1999) classified the three major types of phytochemicals as terpenoids, phenolic metabolites, and alkaloids. Among these types of phytochemicals, phenolic metabolites are the most abundant and include flavonoids, flavonones and flavonols, isoflavonoids, phenols and phenolic acids (Rice-Evans et al., 1997). Certain groups of terpenoids like carotenoids are also widely available in plants and classified as lycopenes, carotenes, and xanthophylls. These groups of phytochemicals have antioxidant properties capable of inducing specific effects on human physiology. Vitamins, particularly C or ascorbic acid, also contribute to the antioxidant content in fruits and vegetables. It is the first line of defense against reactive oxygen species (i.e. superoxide, hydroxyl radicals, hydrogen peroxide, and singlet oxygen) and works compatibly with antioxidant enzymes such as GSH reductase and ascorbate peroxidase (Kalt, 2001).

Another important parameter that will be examined is antioxidant activity. Studying the mechanism of action is critical for proper assessment of an antioxidant considering that it has many ways to quench free radicals and many factors could influence this process (Parr and Bolwell, 2000). Several mechanisms of antioxidants used in stopping the radical chain reactions could include scavenging free radicals, complexing protein, reducing oxidized antioxidants, and partitioning effects influencing their distribution in different oxidation sites (Frankel, 1999).

Antioxidant content and activity of fruit and vegetable crops are affected by several factors such as genotype (Macheix et al., 1990), ontogeny (plant tissue, fruit size, stage of development, ripening) (Zhao et al., 2006), diseases and pests, and climatic conditions (Howard et al., 2002; Islam et al., 2003). Specifically for tomatoes, differences in the level of antioxidant activity, phenolic content, ascorbic acid, and lycopene have been found among various genotypes (Bhatt et al., 2001; George et al., 2004; Spencer et al 2005). In melons, influences of cultivar, fruit size, soil type and year are also evident as shown by the range of ascorbic acid from approximately 10- 25 mg 100 g<sup>-1</sup> fresh weight in green-fleshed honeydew muskmelons observed by Lester and Crosby (2002). Other literature reported that melons contain approximately 33 mg 100 g<sup>-1</sup> fresh weight of vitamin C and their antioxidant activity is classified as moderate (Lister, 2003). Vouldoukis et al., (2004) also demonstrated that melons have high superoxide dismutase activity contributing to antioxidant properties. Lamikanra and Watson (2001) reported that the total phenolic content in cantaloupe melons was determined as 5.16 mg 100g<sup>-1</sup> dry matter and stated that melon phenolics are all nonflavonoid compounds. Another phytochemical present in melons, but not to be covered in this study, is beta-carotene, a carotenoid that acts as a precursor for vitamin A. Among the commonly consumed fruits, orange-fleshed muskmelon contains a significant amount of beta-carotene (20.4 ug/g fresh weight) (Lester and Eischen, 1992). Likewise, management practices such as fertilization, irrigation, and pesticide application (Bang et al., 2004; Leskovar et al., 2004; Mozafar, 1993; Tovar et al., 2002; Vallejo et al., 2003) and nutrient source (Toor et al., 2006) also influence the level of antioxidants in produce.

Fruit quality is determined by several developmental and biochemical processes that may result in alterations of the color, texture, flavor and aroma (Li et al., 2006). Two quality parameters that are crucial in assessing consumer preference are percent dry matter and soluble solids. Knowledge of the dry matter content is necessary for correct interpretation of the results obtained from antioxidant analysis. Although nutrient content in plants is expressed on a dry weight basis, the antioxidant content should be shown on a fresh weight basis since most fruits and vegetables are consumed fresh and contain a lot of moisture. Moreover, another important parameter is the total soluble solids, which is correlated with flavor and sweetness (Senesi et al., 2005; Pardo et al., 2000). Nevertheless, high soluble solids concentration at harvest does not always correlate with high overall fruit quality (Lester and Shellie, 1992). Greater than 97% of the total soluble solids in melons are soluble sugars wherein 50% of it is sucrose at fruit ripening (Pharr, 1994). McCollum et al., (1988) reported that 24 days after anthesis, glucose and fructose are the predominant sugars present in the ripe fruit. In tomatoes, total soluble solids is made up of sugars (>50%), minerals (8%), organic acids (>10%) and pectin ( $\sim$ 7%) (Davies and Hobson, 1981). Most of the studies available on factors affecting total soluble solids examined genotype (Saftner et al., 2006), plant and fruit size (Stevens, 1986), stage of ripening (Senesi et al., 2002), postharvest conditions (Gutierrez et al., 1994) and management practices (Bhella and Wilcox; 1989; Long et al., 2004).

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With regard to the effect of production method on ascorbic acid, total phenolic content, antioxidant activity, dry matter and soluble solids concentration, some studies have shown that the antioxidant activity of organic spinach was 120% higher compared to conventionally grown spinach (Ren et al., 2001). Increase in total phenolic compounds

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was observed in organically grown leafy vegetables like pac choi (Young et al., 2005). A few studies have been carried out to compare the antioxidant content and fruit quality attributes of tomatoes grown in organic and conventional production systems. Mitchell et al., (2007) reported that the ten-year mean levels of flavonoid (quercetin and kaempferol) were higher in organically grown tomatoes than conventionally grown tomatoes. Lumpkin et al., (2005) showed that significant differences were found between two pairs of conventional and organic farms for ascorbic acid, total phenolics, lycopene content, and soluble solid concentration. Comparisons of the antioxidant activity of tomatoes grown organically and conventionally are lacking. Likewise, comparison studies that investigate differences in nutritional value and fruit quality attributes are lacking in melons.

At present, some comparison studies have been criticized for the way they were designed. In addition, comprehensive reviews of the literature have been carried out, but particular results of known differences on crops grown under two production systems have not been sufficiently consistent. A review by Worthington (2001) concluded that many organically grown crops have significantly more vitamin C, phosphorus, and magnesium than conventionally grown crops. Less, but higher quality protein and lower amounts of heavy metals were also observed in organic crops. Magkos et al., 2003 reported that there is a small amount of evidence that organic crops have higher micronutrients (i.e. minerals and trace elements) than conventional grown leafy vegetables. Brandt and Mølgaard (2001) concluded that there is ample but circumstantial evidence that organic crops had more defense-related secondary metabolites beneficial

for optimal health than conventional crops. However, they suggested that several hypotheses concerning the differences in the health promoting effects of organic and conventional food crops have to be investigated first in order to answer the question whether organic production enhances the nutritional value of foods or not. Assessment of • • the effect of production system on nutritional value is a complex task because of some problems related to study design. Moreover, Bourn and Prescott (2002) commented that a • valid comparison between organic and conventional production system is hard to make because there are only a few well-controlled studies published in peer-reviewed journals. • • Considerable variation has been observed in the experimental design and study • approaches. Among the three study approaches (i.e. retail market, research center, farm) that were used to compare organic and conventional produce, retail market studies have . weaknesses that include the inability to conclude that chemical composition differences were due to production system and uncertainty associated with sampling procedure since • it could not be guaranteed that the produce sold in stores is indeed conventionally or • organically grown or of the same cultivar. Research center and farm studies are • considered better approaches to implement because of easier identification of genetic and • environmental factors affecting nutrient content of crops. The only drawback of the • • former is that the results could not be applied widely and generalized as reflective of the • large and small scale commercial production system. Lester (2006) recommended the • • following guidelines to carry out effective direct comparison studies on the two • : production systems: a) implementation of appropriate study approaches and b) standardized pre-harvest and post-harvest conditions and analyses. ••••• :

Our study focus is to address some of the aforementioned gaps on comparison studies and carry out a well-designed experiment to assess the effect of production system on antioxidant and quality attributes in two warm season crops: tomatoes and melons. The objectives of the study are the following:

- To examine if organic production confers more or less antioxidants using research parameters that minimize experimental variables, thus enabling meaningful comparisons;
- To evaluate the extent of antioxidant diversity for ascorbic acid, total phenolic content and antioxidant activity;
- To identify nutritionally superior melon (*Cucumis melo* L.) and tomato (*Solanum lycopersicum* L.) cultivars that may be grown for specialty markets by small and medium sized farmers; and
- To assess yield and fruit quality attributes (i.e. dry matter, soluble solids content and pH) of organically and conventionally grown tomatoes and melons.

#### **CHAPTER II**

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# COMPARATIVE ANALYSIS OF ANTIOXIDANT PROPERTIES, FRUIT QUALITY ATTRIBUTES, AND YIELD OF ORGANICALLY AND CONVENTIONALLY GROWN MELONS (*Cucumis melo* L.) in Colorado

#### Abstract

This research examined the antioxidant properties, quality attributes, and yield of ten melon (Cucumis melo L.) cultivars grown in 2005 and 2006, under certified organic and conventional plots at the Horticulture Field Research Center, Fort Collins, CO. The melon cultivars were analyzed for their ascorbic acid, total phenolic (TP) content, Trolox equivalent antioxidant capacity (TEAC), dry matter, soluble solids content, and yield. There was a 3-fold difference in ascorbic acid and total phenolic content among melon cultivars. A 6-fold difference was observed in their antioxidant capacity. Melons grown organically had significantly higher ascorbic acid when both years were combined while TP was significant only in the first year. Percent dry matter and soluble solids content varied widely among cultivars but were not significantly affected by production system. Thus, compared to genotype, production system had less influence on the observed diversity of antioxidant levels and quality attributes. Yield was negatively correlated with ascorbic acid. Low yield was observed in cultivars with higher antioxidant content and activity suggesting a possible trade-off between yield and nutrients. Choice of cultivar was the most important contributor to high antioxidant properties. Based on the

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antioxidant index that we developed by taking into account levels of ascorbic acid, TP content and free radical scavenging capacity, the top cultivars, regardless of year or production system, are the following: 'Savor', 'Sweetie#6', 'Early Queen', 'Edonis' and 'Rayan'. This information could be of importance to melon plant breeders who are selecting for increased ascorbic acid, TP content and radical scavenging capacity. These data also suggest that many as yet untested cultivars represent a potentially rich resource for producers wishing to market high antioxidant produce. Nutritionally superior cultivars with high antioxidant levels may provide a competitive marketing and supply niche for small farm producers.

#### Introduction

Consumer demand for fruits and vegetables, including *Cucumis melo* L. has increased over the past two decades (Pollack, 2001), much of this is likely due to increasing awareness of contributions to good nutrition and human health. Alongside evidence of nutritional benefits, the organic production industry is also experiencing an increase in demand especially in local markets. Organic produce is perceived to be safe, environmentally friendly, and of high quality (Brandt et al., 2001; Yiridoe et al., 2005). Some reports have shown that organic produce is higher in ascorbic acid, phenolic content, total sugars, and micronutrients (Caris-Veyrat et al., 2004; Ren et al., 2001; Weibel et al., 2000). A recent marketing survey suggests that certain categories of consumers are willing to pay a premium for produce that has been documented by unbiased sources to contain higher nutrient levels and enhanced antioxidant properties (Bond et al., 2007).

While antioxidants are not considered as nutrients *per se*, recent research suggests potentially valuable roles in human nutrition, especially in intervention and prevention of cancer and several chronic diseases (Cheng et al., 2007; Czernichow et al., 2004). They suppress formation of reactive oxygen species and restore integrity of damaged DNA (Lister, 2003). While fruits and vegetables are recognized as the best dietary source of antioxidants, the amount and type of phytochemicals are influenced by a number of factors, including genotype, ontogeny, environment and postharvest handling (Lata, 2007; Schreiner and Huyskens-Keil 2006; Zhao et al., 2007a). Production systems (organic vs. conventional) have been shown to affect antioxidants in some, but not all cases. Choice of cultivars has only been superficially examined as a means of enhancing nutritional attributes.

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Studies that evaluate produce attributes grown under organic and conventional practices have encountered criticism, often for unavoidable but valid reasons because of difficulties in making unambiguous comparisons (Asami et al., 2003; Lombardi-Boccia et al., 2004). Accordingly, after careful examination of the literature (Lester, 2006; Magkos et al. 2003; Zhao et al. 2006), we propose the following factors as guidelines for studies that attempt to make such comparisons. To the extent possible, research targeted at comparing organic production to conventional management should strive to meet the following conditions:

- locate research plots on soils with similar texture, fertility status, drainage, and exposure, as close to each other as practical while meeting organic certification requirements for the organic plots;
- (2) select identical cultivars for each crop to minimize genetic variation as a variable;

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- (3) plan to repeat the experiment at least two or three years;
- (4) apply similar production practices including planting time, planting methods, plot design, spacing, row orientation, irrigation source, application method, and scheduling with care to avoid water stress conditions;
- (5) to the extent possible apply similar quantities of major nutritional elements (organic matter will of necessity differ, as in all likelihood will those minor elements associated with organic sources);
- (6) samples collected for analytical purposes should be subjected to similar postproduction harvest methods including physiological maturity, fruit size, harvest time of day, fruit location on plants, storage conditions and handling;
- (7) multiple samples intended for analytical assays should be freeze-dried and stored desiccated at -20°C or lower;
- (8) utilize similar analytical methodology with sufficient biological replication and laboratory precision to facilitate sound statistical analysis.

Studies that systematically compare antioxidant properties of organic and conventional melons, especially the differences among cultivars, are very limited. This study aimed to fill this gap and therefore examined the level of phytochemical contents and radical scavenging capacity in the same ten cultivars grown in the same general location, on the same soil texture and type, using organic and conventional practices in 2005 and 2006. This study has three objectives:

To evaluate the extent of antioxidant diversity for ascorbic acid, TP, and TEAC; quality attributes and yield among 10 *C. melo* cultivars; To identify nutritionally superior *C. melo* cultivars that may be grown for specialty markets by small and medium sized farmers.

#### **Materials and Methods**

#### Melon production and postproduction

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The study was carried out with field plot trials in 2005 and 2006 at the Horticulture Field Research Center (HFRC), Colorado State University, Fort Collins, Colorado USA. Areas for organic and conventional production were identified early in the project development. For organic production, the soils in the area passed the criteria set by the National Organic Certification Board in 2001. For conventional production, the plots in that part of the HFRC have been applied with inorganic fertilizers, herbicides, and pesticides for many years. The soil texture in HFRC is classified as Nunn clay with a pH of 7.8.

This study is part of a larger project entitled 'Differentiating Small Farm Produce Offerings through Nutritionally Superior Cultivars, Marketing, and Extension Programs' wherein six crops including melons were planted under organic and conventional production systems. The experimental units were laid out in a split plot with the whole plots arranged as a completely randomized design. The whole plot factor is production system while the sub-plot factor is cultivar. Three blocks in each production system served as replications. Ten cultivars were planted in each block of the organic and

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conventional production plots namely: 'Arava', 'Burpee Hybrid', 'Early Queen', 'Edonis', 'Swan Lake', 'Haogen', 'Honey Orange', 'Rayan', 'Savor', and 'Sweetie#6'. All transplants were grown at the CSU Plant Environmental Research Center Greenhouses. The characteristics of each cultivar are described in Table 2.1. Organic planting media (Sunshine Organic Basic) was used. Melons were grown in peat pots (Jiffy peat pots 7.62 cm round). Sowing of melons occurred 21 days prior to being transferred in the field. The transplants were grown on a bottom heated greenhouse floor maintained at 18<sup>o</sup>C. Watering for the transplants was done automatically by overhead misting/sprinkler using a city water source. 'Rootshield®' (*Trichoderma harzianum*, Strain T-22 #9462), approved for use in organic crops, was drenched into the soil immediately after sowing following label rates. Melons were transplanted into black plastic mulched beds at 3.81 centimeters (cm) spacing between plants and 12.70 cm between beds. The field plots measured 45.72 meter (m) long and 10.80 m wide. There was a 0.61 m space between rows.

Table 2.1.	Classification	and description	of melo	on cultivars

Melon Type	Cultivar	Description
Galia	Arava	Fragrant with soft green flesh and yellow netted rind
	Haogen	Green-fleshed (similar to a muskmelon)
Cantaloupe	Burpee Hybrid	Flesh is thick and deep orange in color
	Early Queen	Muskmelon hybrid with orange flesh
Charentais	Edonis	French melon with orange flesh and honey orange in color
	Savor	Classic Charentais type, sweet and aromatic
	Swan Lake	White flesh with some orange swirls
Honeydew	Honey Orange	Pale orange flesh honeydew
Ananas	Rayan	Elongated shape with sweet, greenish white flesh
Butterscotch	Sweetie#6	Sweet and fragrant flavor suggestive of butterscotch

#### Fertilization

Soil sampling was done before and after planting at 7.6 cm off the irrigation drip tape and to a depth of 27.9 cm. Soil samples were submitted to the CSU Soil Testing Lab. Based on these soil tests, 22,407 kilogram (kg) ha<sup>-1</sup> of 'Evergreen' poultry compost (A-1 Organics) was applied to the organic block. The compost was applied with a Mill Creek spreader and disked into the soil immediately following the application. To match the amount of nutrients in the organic block, appropriate amount of nitrogen (N) and phosphorus (P) from urea and superphosphate, respectively, were applied to the conventional block using a broadcast spreader.

#### Pest management

Cucumber beetle (*Acalymma vittatum*) pressure on the transplants was more intense for both organic than conventional plots in 2005. To minimize the infestation, the synthetic insecticide Permethrin was applied in conventional plots while naturally derived pyrethrum ('Entrust') was used in the organic plots.

#### Irrigation management

Domestic water was applied using drip irrigation and was scheduled using 'Watermark' sensors. Drip irrigation was applied at 6.20 liters/min/m per row, and application varied during the season from 0.5 hours/2 days to 2 hours/day depending upon the developmental stage of the crop. During the production period, crops were not permitted to suffer from water stress based on 'Watermark' soil monitors.

#### Harvesting and Postharvest handling

Melons were harvested at physiological maturity. Three fruits were collected from each replication/block per cultivar and cooled after harvest to  $1.1^{\circ}$ C.

#### Sample preparation, extraction, and analysis

Melons were washed twice to remove contamination on their outer surface and cut into halves. Forty milligrams of thin melon slices were obtained from radial slices without the skin. These melon slices were placed in a 'Virtis' freeze dryer and lyophilized for five days to remove water and prolong storage viability. Freeze-dried melon samples were weighed for dry matter content and ground in preparation for extraction. Five ml of 80% acetone was placed in each tube and vortexed until thoroughly suspended. The sample was placed in a refrigerated rotator for one hour and centrifuged at 6,000 rpm for 15 minutes at  $4^{0}$ C. One ml of aliquot clear supernatant was removed and placed in 'Eppendorf' vacufuge<sup>TM</sup> for 2 hours at  $45^{0}$ C. Extracted, dehydrated samples were used for total phenolic content, ABTS and DPPH assays only. Freeze dried samples were used for ascorbic acid analysis. Desiccated samples were stored dry at -20<sup>0</sup> C before analysis.

#### Total phenolic content (TPC)

TPC was standardized against gallic acid (Sigma Chemicals Co., St. Louis, MO) and expressed as milligrams per 100 gram of melon fresh weight (mg GAE  $100g^{-1}$  FW) using a microplate-based Folin-Ciocalteu assay adapted from Spanos and Wrolstad, (1990). Vacufuged stored samples were reconstituted with 1.0 ml 80% acetone (Fisher Chemicals, Fair Lawn, NJ) and 100µl of this extract was diluted with 900µl of nanopure water. In triplicate, 35µl of diluted sample was pipetted in microplate wells. Using a multichannel pipette, 150 µl of 0.2 M Folin-Ciocalteu reagent (Sigma Aldrich) and 115 µl 7.5% (w/v) Na<sub>2</sub>CO<sub>3</sub> (Fisher Chemicals) were added to all wells. The plate was

incubated at 45<sup>°</sup>C, cooled to room temperature for one hour, and read at 765 nm using a (Spectra Max Plus, Molecular Devices Corp., Sunnyvale, CA) spectrophotometer.

## ABTS<sup>+</sup> Trolox equivalent antioxidant capacity (TEAC)

ABTS<sup>++</sup> 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonicacid TEAC was measured using a microplate assay ABTS<sup>++</sup> radical cation assay based on the method developed by Miller and Rice-Evans (1997). The ABTS solution was prepared by mixing 40 mg of ABTS (Calbiochem, EMD Biosciences, La Jolla, CA), 15 ml distilled water and two to three mg of MnO<sub>2</sub> (Sigma-Aldrich). To remove MnO<sub>2</sub>, the samples were first vacuum filtered, then passed through a 0.2  $\mu$ m 'Acrodisk' syringe filter into a flask. The absorbance value of the ABTS solution was read at 734 nm in a spectrophotometer and adjusted to 0.700 absorbance units (AU) by adding 5.0 mM phosphate buffer solution. Twenty-five  $\mu$ l of reconstituted vacufuged samples and 250  $\mu$ l of ABTS solution were mixed well and read at 734nm exactly after 60 seconds at 25<sup>0</sup>C. The absorbance value was expressed as  $\mu$ mol TEAC/ml in assay and compared to a set of Trolox (Calbiochem) standards. This was converted to  $\mu$ mol TEAC/100g sample (FW) taking into account all dilution and concentration factors.

## **DPPH**<sup>.+</sup> Trolox equivalent antioxidant capacity (TEAC)

Antioxidant activity was also measured with a microplate-based 2,2-diphenyl-1picrylhydrazyl or DPPH antioxidant assay based on the method of (Lu and Foo, 2000) with some modifications. A 0.1 mM of DPPH solution was prepared by mixing 7.89 mg of DPPH with 100% methanol adjusting the absorbance value to 0.95 AU. Fifteen  $\mu$ l of the reconstituted vacufuged samples were mixed with 285  $\mu$ l of DPPH<sup>-+</sup> solution and read at 515 nm in the spectrophotometer after exactly three minutes at  $25^{\circ}$ C. Results were expressed as  $\mu$ M trolox equivalent antioxidant capacity, TEAC 100 g<sup>-1</sup> FW.

#### Soluble solids content and percent dry matter

Soluble solids content of melon samples was measured using a temperature compensated 'Reichert' handheld refractometer and results were expressed as <sup>0</sup>Brix. The dry matter percentage was obtained gravimetrically from dried and fresh weights.

#### Ascorbic acid

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Standard solutions were prepared by mixing 100 mg dithiothreitol (Promega Corp., Madison, WI) and 10 mg of ascorbic acid (Sigma-Aldrich Inc., St. Louis, MO) and by diluting to five concentrations to prepare the standard curve. Lyophilized melon tissue was extracted in 5% w/v aqueous solution of metaphosphoric acid containing 1% w/v dithiothreitol. The mixture was vortexed for 15 seconds and rotated for 15 minutes at 4<sup>0</sup>C. To separate the liquid from the solid phase, the mixture was centrifuged for 5 minutes at 4,000 rpm at 4<sup>0</sup>C. This procedure was repeated twice. The supernatant from the first and second extraction was filtered through a 0.45 mm nylon syringe filter, prior to injection onto an Inertsil 4C high performance liquid chromatography (HPLC) column (Agilent Technologies, Santa Clara, CA) and run with a phosphoric acid/methanol gradient. This method was adapted from Esparza et al., (2006).

#### Temperature and solar radiation data collection

Data on temperature and solar radiation for two cropping seasons (2005-2006) were obtained from the Northern Colorado Water Conservancy District (NCWCD) with one of its weather stations being located within 100 m of the research plots. To determine the effect of temperature, daily growing degree-days was computed by subtracting the

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base temperature  $(10^{0}C)$  for warm season crops like melons from the average temperature as daily GDD=[ $(T_{max} + T_{min})/2$ ]- base temperature where  $T_{max}$  and  $T_{min}$  are maximum and minimum daily air temperatures. Each daily GDD is added up over the growing season and 30 days prior to harvest. Solar radiation data was recorded by an 'Epply' pyranometer and expressed as Langleys (1-calorie square centimeter<sup>-1</sup>).

#### Statistical Analysis

Analysis of variance (ANOVA) was carried out using SAS Mixed Procedure (SAS Inc., version 9.1, Cary NC). Correlation analysis to determine the r (Pearson correlation coefficient) value was done using SAS Proc Corr. Differences between means were calculated using Tukey-Kramer ( $P \le 0.05$ ). To determine the differences between the antioxidant indexes of each cultivar, Duncan's multiple range test ( $P \le 0.05$ ) was utilized. Graphs were prepared using the Graph Pad Prism software (Graph Pad Prism Inc., version 4.0, San Diego, CA).

#### Results

#### Temperature and solar radiation

From field planting to harvest, melons accumulated more heat units in 2006 than 2005 (Fig. 2.1). Thirty days prior to harvest, the heat accumulated was slightly higher in some days of 2005 than in 2006 (Fig. 2.2). The number of days with temperature greater than 30<sup>o</sup>C was higher in the early stage of development in 2006 than 2005. Melons exposed to temperatures higher than 30<sup>o</sup>C were considered under heat stressed conditions (Fig. 2.3). In 2005 and 2006, solar radiation (in Langleys) received by crops from planting to harvest and 30 days prior to harvest were almost the same (Fig. 2.4).



Figure 2.1.Heat accumulation (in growing degree-days) from planting to harvest.  $GDD=[(T_{max} + T_{min})/2]-10^{\circ}C$ , where  $T_{max}$  and  $T_{min}$  are maximum and minimum daily air temperatures;  $10^{\circ}C$  is the base temperature or the temperature below which there is no growth or development.



Figure 2.2. Heat accumulation (in growing degree days) 30 days prior to harvest.  $GDD=[(T_{max} + T_{min})/2]-10^{0}C$ , where  $T_{max}$  and  $T_{min}$  are maximum and minimum daily air temperatures;  $10^{0}C$  is the base temperature or the temperature below which there is no growth or development.







Figure 2.4. Daily net solar radiation (in Langleys) from planting to harvest

#### Effects of year, cultivar and production system on nutritional quality and yield

Production system had a significant effect on ascorbic acid, TPC, and DPPH but not on ABTS, dry matter and soluble solids (Table 2.2). Year and cultivar effects were significant in all the parameters tested. The interaction between year and cultivar was • : • • .

highly significant indicating that environmental factors had a large effect on some cultivars but not on others. For the other interactions, their effects on the observed parameters varied widely. There was also significant interaction between year and production system for the TPC indicating that it was highly influenced by organic or conventional production in each year. Significance in cultivar and production system interaction implied that some cultivars had different levels of ascorbic acid and ABTS radical scavenging capacity when grown organically or conventionally. Accordingly, some three-way interactions among year, cultivar and production system were significant for ascorbic acid, TPC, soluble solids and antioxidant activity (ABTS). Since year effects are significant for nutritional quality parameters and yield, subsequent analysis on cultivar and production system effects will be examined for each production year.

Table 2.2. Analysis of variance of the effects of year, cultivar and production system and their interactions

Source	AA	TPC	ABTS	DPPH	Dry matter	Soluble solids
Year (Y)	0.0015	0.0016	0.0003	0.0012	0.0096	0.0032
Cultivar (C)	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Y x C	<.0001	0.0008	<.0001	0.0024	0.0001	0.0002
Production system (PS)	0.0215	0.0086	0.4085	0.0261	0.8447	0.6877
Y x PS	0.5196	0.0057	0.1095	0.8694	0.1625	0.6092
C x PS	0.0250	0.0870	<.0001	0.0528	0.8745	0.8911
Y x C x PS	0.0044	0.0007	<.0001	0.3355	0.0921	0.0051

Expressed as p values for statistical significance; significant at  $P \le 0.05$ .

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When the data were analyzed by separate years, cultivar differences had a very prominent effect on all parameters observed (Table 2.3). Production system (PS) had a highly significant effect on the TPC of melons in the first year. It had slight influence on antioxidant activity (DPPH) but definitely no influence on the TPC and antioxidant activity (ABTS) in the second year. In both years, production system did not significantly influence the dry matter content nor percent soluble solids. The interaction between cultivar and production system had a significant effect on ascorbic acid and TPC in the first year while in the second year it influenced almost all parameters except dry matter and ascorbic acid level.

Table 2.3. Analysis of variance on the effects of cultivar and production system on nutritional quality parameters and yield by year

Analysis	2005			2006		
	С	PS	C x PS	С	PS	C x PS
AA	<0.0001 (***)	0.0623 (NS)	0.0001 (***)	<0.0001 (***)	0.0707 (NS)	0.100 (NS)
TPC	<0.0001 (***)	0.0018 (**)	0.0024 (**)	<0.0001 (***)	0.7142 (NS)	0.0188 (*)
ABTS	<0.0001 (***)	0.1136 (NS)	0.8144 (NS)	<0.0001 (***)	0.9154 (NS)	<0.0001 (***)
DPPH	<0.0001 (***)	0.0980 (NS)	0.2695 (NS)	<0.0001 (***)	0.0127 (*)	0.0342 (*)
Dry matter	<0.0001 (***)	0.3474 (NS)	0.5216 (NS)	<0.0001 (***)	0.4019 (NS)	0.3071 (NS)
Soluble solids	<0.0001 (***)	0.6197 (NS)	0.2693 (NS)	<0.0001 (***)	0.9854 (NS)	0.0473 (*)
Yield	<0.0001 (***)	0.3255 (NS)	0.4113 (NS)			

Expressed as p values for statistical significance.

NS, \*, \*\*, \*\*\* Nonsignificant or significant at  $P \le 0.05, 0.01, 0.001$ , respectively.

#### Ascorbic acid

A wide difference in the level of ascorbic acid is very evident among cultivars ranging from 14.12 to 44.21 mg 100  $g^{-1}$  fresh weight. Melon cultivars grown organically

generally had higher ascorbic acid than their conventionally grown counterparts (Fig. 2.5). Cultivars with the highest ascorbic acid content grown in both organic plots and conventional plots are the following: 'Savor', 'Sweetie#6', 'Burpee Hybrid', and 'Edonis' (Table 2.4).



Figure 2.5. Ascorbic acid content determined in organic and conventionally grown cultivars in 2005 and 2006. Data are expressed as mean  $\pm$  SEM.

Production System	Cultivar	Mean as (mg 100 g	corbic acid	
Conventional	Savor	38.45	a	
	Sweetie#6	33.97	а	
	Burpee Hybrid	26.52	b	
	Edonis	23.05	bc	
	Rayan	22.17	bcd	
	Haogen	21.78	bcd	
	Swan Lake	19.91	cde	
	Honey Orange	19.19	cde	
	Early Queen	17.27	de	
	Arava	16.11	e	
Organic	Savor	37.97	a	
	Sweetie#6	37.66	а	
	Burpee Hybrid	33.69	ab	
	Edonis	27.16	bc	
	Early Queen	25.36	cd	
	Rayan	24.46	cd	
	Swan Lake	23.30	cd	
	Honey Orange	22.46	cd	
	Arava	17.98	d	
	Haogen	17.65	d	

Table 2.4. Ascorbic acid in melon cultivars grown in organic and conventional production systems for 2005 and 2006

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Within each production system, least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ ).

In the first year, the top five cultivars in terms of ascorbic acid whether they were planted in organic or conventional plots were 'Savor', 'Sweetie#6', 'Burpee Hybrid', 'Edonis', and 'Rayan' (Table 2.5). Mean ascorbic acid of 'Sweetie#6' and 'Burpee Hybrid' was not significantly different from 'Savor'. In the second year, 'Savor' had the highest ascorbic acid followed by 'Sweetie#6'.

Mean ascorbic acid of the other cultivars (i.e. 'Honey Orange', 'Burpee Hybrid', 'Swan Lake', 'Rayan', 'Early Queen', 'Haogen', 'Edonis' and 'Arava') were significantly
different from 'Savor' and 'Sweetie#6'. For both years, 'Arava' had the lowest amount of ascorbic acid while 'Savor' and 'Sweetie#6' had the highest amounts among the cultivars planted.

Year	Cultivar	Mean as	corbic acid	
		(mg 100	$g^{-1}$ FW)	
2005	Savor	40.77	а	
	Sweetie#6	37.78	ab	
	Burpee Hybrid	37.78	ab	
	Edonis	32.62	b	
	Rayan	25.60	С	
	Swan Lake	22.04	cd	
	Early Queen	21.85	cd	
	Haogen	21.38	cd	
	Honey Orange	19.19	d	
	Arava	18.50	d	
2006	Savor	35.65	а	
	Sweetie# 6	33.85	а	
	Honey Orange	22.45	b	
	Burpee Hybrid	22.45	b	
	Swan Lake	21.16	b	
	Rayan	21.03	b	
	Early Queen	20.78	b	
	Haogen	18.01	b	
	Edonis	17.60	b	
	Arava	15.59	b	

Table 2.5. Year to year variability in ascorbic acid content of melon cultivars

Within each year, least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ ).

# **Total phenolic content**

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The level of total phenolics in melon cultivars was highly influenced by production system in the first year but not in the second year. Organically grown melons had higher TPC than those grown in conventional plots in the first year (Fig. 2.6). Cultivar effect was highly significant in both years and a wide range of TPC was observed from 30.82 to 88.85 mg gallic acid equivalent (GAE) 100 g<sup>-1</sup> fresh weight. Mean TPC of 'Edonis' was significantly different from the mean TPC of 'Early Queen', 'Burpee Hybrid', 'Honey Orange', and 'Rayan' (Table 2.6).



Figure 2.6. Total phenolic content of organically and conventionally grown cultivars. Data are expressed as mean  $\pm$  SEM.

Cultivar-production system	Mean TP concentration <sup>z</sup>
	$(mg GAE 100 g^{-1} FW)$
Edonis-Organic <sup>y</sup>	88.85 a
Early Queen-Organic	84.75 ab
BurpeeHybrid-Organic	81.57 abc
Honey Orange-Organic	77.18 abcd
Rayan- Organic	76.03 abcd
Haogen -Organic	73.78 abcd
Sweetie#6- Conventional	73.63 abcd
Savor- Conventional	66.65 abcde
Sweetie#6- Organic	64.87 abcde
Swan Lake- Organic	62.55 bcdef
Rayan – Conventional	62.46 bcdef
Burpee Hybrid- Conventional	62.31 bcdef
Savor- Organic	62.16 bcdef
Edonis- Conventional	60.38 bcdef
Early Queen- Conventional	59.09 cdef
Honey Orange- Conventional	55.85 def
Arava - Organic	53.65 def
Haogen – Conventional	46.78 ef
Swan Lake- Conventional	45.56 ef
Arava – Conventional	38.00 f

Table 2.6. Concentrations of total phenolics identified in cultivars planted in organic and conventional plots in 2005

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<sup>z</sup> Least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ ) <sup>y</sup> Cultivars in bold letters were statistically similar to the top cultivar and are in the top tier for TPC.

When Edonis, Early Queen, and Haogen were grown in conventional plots, they had less total phenolics. Organically grown cultivars ('Edonis', 'Early Queen', 'Burpee Hybrid', 'Honey Orange', and 'Rayan') had higher TPC compared to the other cultivars planted in conventional plots in 2005 and 2006 (Table 2.7). In both years, highest TPC was observed in 'Edonis', 'Early Queen', and 'Sweetie#6' regardless of the production system where they were grown. The lowest levels of total phenolics were detected in 'Haogen' and 'Arava' (Galia type). In 2005, mean TPC of 'Edonis', 'Burpee Hybrid', and 'Early Queen' were significantly different from 'Swan Lake' and 'Arava'. In 2006,

'Savor' had the highest TPC but it was not significantly different from 'Edonis', 'Sweetie#6', and 'Early Queen' (Table 2.8).

Production	Cultivar	Mean TP	concentration		
System		$(mg GAE 100 g^{-1} FW)$			
Conventional	Savor	71.69	a		
	Edonis	64.74	ab		
	Sweetie#6	63.30	ab		
	Early Queen	60.94	abc		
	Burpee Hybrid	59.28	abc		
	Rayan	53.92	bcd		
	Honey Orange	51.73	bcd		
	Swan Lake	45.90	cd		
	Arava	41.63	d		
	Haogen	40.24	d		
Organic	Edonis	74.87	а		
	Early Queen	69.71	а		
	Honey Orange	69.46	а		
	Sweetie#6	68.25	а		
	Rayan	66.75	ab		
	Savor	65.75	abc		
	Burpee Hybrid	63.50	abc		
	Haogen	52.30	bcd		
	SwanLake	51.17	cd		
	Arava	44.62	d		

Table 2.7. Total phenolic content of melon cultivars by production system

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Within each production system, least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ ).

Year	Cultivar	Mean TP concentration		
		(mg GAE	$100 \text{ g}^{-1} \text{ FW}$ )	
2005	Edonis	74.62	а	
	Burpee Hybrid	71.94	a	
	Early Queen	71.92	a	
	Sweetie#6	69.25	ab	
	Rayan	69.24	ab	
	Honey Orange	66.51	ab	
	Savor	64.40	ab	
	Haogen	60.28	abc	
	Swan Lake	54.06	bc	
	Arava	45.83	с	
2006	Savor	73.04	a	
	Edonis	64.99	ab	
	Sweetie# 6	62.31	ab	
	Early Queen	58.73	abc	
	Honey Orange	54.67	bcd	
	Rayan	51.42	bcd	
	Burpee Hybrid	50.84	bcd	
	Swan Lake	43.07	cde	
	Arava	40.43	de	
	Haogen	32.26	e	

Table 2.8. Total phenolic content of organic and conventionally grown melon cultivars by year

Within each year, least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ ).

#### Antioxidant activity

Trolox-equivalent antioxidant activity of cultivars was determined using two radical scavenging cation assays: ABTS and DPPH.

# ABTS<sup>+</sup> Trolox equivalent antioxidant capacity (TEAC)

Antioxidant activity of melon cultivars was significantly different in each year. Higher antioxidant activity was observed in 2006 than 2005. Genotype greatly affected antioxidant activity. A range of 24.4 to 386.9  $\mu$ M TEAC 100 g<sup>-1</sup> fresh weight was observed among cultivars (Fig. 2.7). Neither organic nor conventional production highly influenced antioxidant activity (Table 2.2).



Figure 2.7.ABTS<sup>+</sup> Trolox equivalent antioxidant capacity (TEAC) of melon cultivars grown in conventional and organic plots. Data are expressed as mean  $\pm$  SEM.

In 2005, the top five cultivars with high antioxidant activity were 'Savor', 'Edonis', 'Early Queen', 'Honey Orange', and 'Rayan'. Mean antioxidant activity of 'Savor', 'Edonis', and 'Early Queen' was significantly different from the other cultivars (Table 2.9). In 2006, cultivars with higher antioxidant activity included the following: 'Sweetie#6', 'Savor', 'Rayan', 'Early Queen', and 'Honey Orange'. Mean antioxidant activity of these cultivars was significantly different from 'Swan Lake', 'Haogen' and 'Arava'.

Year	Cultivar	Mean antioxidant activity		
		(µM TEAC/100 g fresh weight)		
2005	Savor	140.00	a	
	Edonis	122.62	ab	
	Early Queen	119.82	abc	
	Honey Orange	69.58	bcd	
	Rayan	65.92	cd	
	Burpee Hybrid	59.38	d	
	Sweetie#6	59.32	d	
	Swan Lake	58.98	d	
	Haogen	47.50	d	
	Arava	35.02	d	
2006	Sweetie# 6	257.91	а	
	Savor	199.71	ab	
	Rayan	198.20	ab	
	Early Queen	186.91	ab	
	Honey Orange	153.38	bc	
	Edonis	138.88	bc	
	Burpee Hybrid	137.41	bc	
	Haogen	86.39	с	
	Swan Lake	82.43	c	
	Arava	69.17	с	

Table 2.9. ABTS<sup>• +</sup> Trolox equivalent antioxidant capacity (TEAC) of melon cultivars grown in conventional and organic plots

Within each year, least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ ).

# DPPH<sup>+</sup> Trolox equivalent antioxidant capacity (TEAC)

Results of the DPPH radical cation assay showed that the antioxidant activity of melon cultivars was highly affected by year, genotype, and production system. Genotypic differences among cultivars is a key factor influencing the rate of antioxidant activity as indicated by a wide range from 57.6 to 348.9  $\mu$ M TEAC 100 g<sup>-1</sup> fresh weight. A higher rate of antioxidant activity was also observed in 2005 than in 2006. Cultivars grown in conventional plots had a higher rate of antioxidant activity than those that were planted in organic plots (Fig. 2.8). Although genotype, year, and production system had individual



significant effects, the interactions among these factors did not influence the rate of antioxidant activity.

Figure 2.8. DPPH<sup>+</sup> Trolox equivalent antioxidant capacity (TEAC) of melon cultivars grown in conventional and organic plots. Data are expressed as mean <u>+</u> SEM.

In 2005, top cultivars in terms of rate of antioxidant activity measured by DPPH were 'Savor', 'Sweetie#6', 'Rayan', 'Edonis,' and 'Early Queen' (Table 2.10). Mean antioxidant activity of 'Savor' was significantly different from the other four cultivars. In 2006, these cultivars were also identified with high antioxidant activity.

Year	Cultivar	Mean antioxidant activity ( $\mu$ M TEAC 100 g <sup>-1</sup> fresh weight)		
2005	Savor	322.69	a	
	Sweetie#6	251.65	b	
	Rayan	214.38	bc	
	Edonis	198.29	bc	
	Early Queen	192.10	bc	
	Burpee Hybrid	188.85	с	
	Swan Lake	171.74	cd	
	Haogen	162.64	cd	
	Honey Orange	161.99	cd	
	Arava	122.12	d	
2006	Savor	210.94	а	
	Sweetie#6	186.53	ab	
	Early Queen	171.48	abc	
	Edonis	151.96	bcd	
	Rayan	135.93	cd	
	BurpeeHy	126.10	cde	
	HoneyOra	125.61	cde	
	Arava	112.40	de	
	SwanLake	109.20	de	
	Haogen	79.80	e	

Table 2.10. DPPH<sup>· +</sup> Trolox equivalent antioxidant capacity (TEAC) of melon cultivars grown in conventional and organic plots

Within each year, least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ ).

## Dry matter

Percent dry matter differed among cultivars ranging from 9.39 to 16.80 percent and was significantly different between 2005 and 2006 (Fig. 2.9). The dry matter content of cultivars was 9.52% higher in 2005 than that of 2006. Production system did not significantly affect dry matter percentage (Table 2.2).



Figure 2.9. Percent dry matter of melon cultivars grown in conventional and organic plots. Data are expressed as mean  $\pm$  SEM.

'Sweetie#6' had the highest percent dry matter in 2005 followed by 'Savor', 'Edonis', 'Early Queen', and 'Honey Orange' (Table 2.11). Lowest percent dry matter was observed with 'Haogen', 'Swan Lake', and 'Arava'. In the second year, cultivars with high percent dry matter were 'Honey Orange', 'Savor', 'Sweetie#6', 'Swan Lake', and 'Rayan'. Mean dry matter percentage of 'Honey Orange' was statistically the same with 'Savor' and 'Sweetie#6'. 'Haogen' and 'Arava' remained on the bottom of the list with the lowest amount of dry matter.

Year	Cultivar	Mean dry (%)	matter content	
2005	Sweetie#6	15.82	a	
	Savor	14.98	ab	
	Edonis	13.95	abc	
	Early Queen	13.46	abed	
	Honey Orange	13.07	abcd	
	Burpee Hybrid	12.90	abcd	
	Rayan	12.58	bcde	
	Haogen	11.31	cde	
	Swan Lake	10.75	de	
	Arava	9.79	e	
2006	Honey Orange	15.43	a	
	Savor	14.46	ab	
	Sweetie#6	13.02	abc	
	Swan Lake	11.96	bcd	
	Rayan	11.38	bcd	
	Early Queen	10.97	cd	
	Edonis	10.40	cd	
	Haogen	9.76	d	
	Burpee Hybrid	9.60	d	
	Arava	9.41	d	

Table 2.11. Dry matter percentage of melon cultivars grown in conventional and organic plots

Within year, least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ ).

#### Soluble Solids Content

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Production system did not significantly influence the soluble solids content (SSC) of cultivars. Likewise, interactions involving production system have yielded non-significant differences on SSC. A wide difference in the amount of soluble solids was observed among cultivars ranging from 8.61 to 17.23 <sup>0</sup>Brix (Fig. 2.10). <sup>0</sup>Brix was higher in 2005 than in 2006.



Figure 2.10. Soluble solids content of melon cultivars grown in conventional and organic plots. Data are expressed as mean  $\pm$  SEM.

In 2005, Sweetie#6, 'Savor', 'Edonis', and 'Honey Orange' had the highest amounts of soluble solids (Table 2.12). Mean differences among these cultivars were not statistically significant, but they did differ from the bottom two cultivars (i.e. 'Arava' and 'Swan Lake'). In 2006, the top five cultivars that had high SSC were 'Honey Orange', 'Savor', 'Sweetie#6', 'Swan Lake', and 'Rayan'. Mean SSC of 'Honey Orange', 'Savor', and 'Sweetie#6' were significantly different from other cultivars.

Year	Cultivar	Mean so ( <sup>0</sup> Brix)	luble solids content
2005	Sweetie#6	16.18	а
	Savor	15.12	ab
	Edonis	14.75	ab
	Honey Orange	14.17	abc
	Burpee Hybrid	12.73	bc
	Rayan	12.48	bc
	Haogen	12.32	bc
	Early Queen	11.82	bc
	Swan Lake	11.08	с
	Arava	10.89	c
2006	Honey Orange	15.66	а
	Savor	14.14	ab
	Sweetie#6	12.94	abc
	Swan Lake	11.89	bcd
	Rayan	10.92	cd
	Early Queen	10.56	d
	Edonis	10.17	d
	Haogen	10.00	d
	Arava	9.36	d
	Burpee Hybrid	9.33	d

Table 2.12. Soluble solids content of melon cultivars grown in conventional and organic plots

Within year, least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ ).

# Yield

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Yield of melon cultivars was significantly affected by cultivar. There were no significant yield differences among cultivars grown under conventional and organic production systems. In 2005, 'Honey Orange' had the highest yield and its mean was not significantly different from 'Rayan', 'Early Queen', and 'Arava'. The lowest yield was observed in 'Sweetie #6'. A complete set of yield data was unavailable for the 2006 crop (Table 2.13).

Cultivar	Yield (kg h	ia <sup>-1</sup> )	
Honey Orange	2732.10	а	
Rayan	2675.84	ab	
Early Queen	1944.96	abc	
Arava	1929.31	abc	
Swan Lake	1778.36	bcd	
Haogen	1555.67	cd	
Savor	1553.66	cd	
Burpee Hybrid	1549.81	cd	
Edonis	1105.41	cd	
Sweetie#6	953.08	d	

Table 2.13. Yield of organic and conventional melons grown in 2005

Least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ ).

#### **Correlation analysis**

Although many parameters measured were significantly correlated, relationships between factors were rather weak, except for dry matter with ascorbic acid and soluble solids. Two methods of measuring the antioxidant activity (ABTS and DPPH) were not strongly related at  $P \le 0.05$ . Ascorbic acid and total phenolic content are highly correlated with the level of antioxidant activity, soluble solid content, and percent dry matter of melon cultivars (Table 2.14). Moreover, antioxidant activity is highly correlated with dry matter. Among all the variables examined, soluble solid content and dry matter content were highly correlated (Fig. 2.11). Yield was negatively correlated with all nutritional parameters and fruit quality attributes. Strong negative correlation as observed between yield and ascorbic acid levels.

	ABTS	DPPH	AA	TP	Soluble solids	Dry matter
ABTS						
DPPH	0.18 NS					
AA	0.24**	0.55***				
ТР	0.24**	0.53***	0.39***			
Soluble solids	0.18 NS	0.52***	0.54***	0.44***		
Dry matter	0.28**	0.59***	0.67***	0.51***	0.88***	
Yield (2005 only)	-0.06 NS	-0.18 NS	-0.47***	-0.16 NS	-0.17 NS	-0.28 NS

Table 2.14. Correlation matrix of r (Pearson correlation coefficient) values

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NS, \*, \*\*, \*\*\* Nonsignificant or significant at  $P \le 0.05, 0.01, 0.001$ , respectively.



Figure 2.11. Correlation plot of soluble solids content and dry matter percentage. Data include all cultivars for both production systems over two years.

#### Discussion

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This study provides comparative data on antioxidant properties and fruit quality attributes for ten melon cultivars, grown two years using conventional and organic practices. Our study showed that genotype, production system, and year significantly affected the antioxidant composition of cultivars. Higher levels of ascorbic acid, total phenolic content and antioxidant activity were observed among cultivars in 2005 than 2006. Year effect could be attributed to biotic stress in the form of insect pest attack. In 2005, melon transplants were heavily challenged by western cucumber beetle (Acalymma *vittatum*). The effect of biotic stress could have triggered increased synthesis of ascorbic acid and total phenolics. When the plants are subjected to stresses, these antioxidants are produced by the plants to minimize and suppress the production of reactive oxygen species (ROS) (Smirnoff, 1998). In a study conducted by Young et al., (2005), increase in the level of total phenolic contents in organic pac choi samples was observed and attributed to the flea beetle infestations in organic plots. These results suggested that effect of year differences on antioxidant composition and activity may be attributed to pest infestation, but this would be more convincing with additional studies.

Another environmental effect that influenced the antioxidant content of melon cultivars is the production system. No previous comparison studies have been done regarding the influence of production system on the ascorbic acid content, TPC, and antioxidant activity of melon cultivars. To the best of our knowledge, this is the first reported study that compared antioxidant content and activity of organic and conventionally grown melon cultivars. The results suggest that melon cultivars grown in organic plots have higher ascorbic acid than those which were grown in conventional

plots when both years were combined. Organic melons had higher TPC than conventional • • Among the factors that were examined, genotype played a major role in • • • 

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melons but only in the first year. Production system had no significant effect on TPC during the second year. The free radical scavenging capacity was likewise not significantly influenced by the method of production, but this depends on the laboratory analysis used. ABTS<sup>++</sup> and DPPH<sup>++</sup> TEAC assays were not highly correlated. Results obtained from the ABTS<sup>++</sup> TEAC assay showed that production system did not influence the antioxidant activity, however the DPPH + TEAC indicated otherwise.

determining the amount of antioxidants present in melons. The ten melon cultivars varied significantly in terms of their ascorbic acid, total phenolic content and free radical scavenging capacity for both years suggesting high antioxidant diversity among melon cultivars. Our results complemented the conclusion of the study conducted by Lester and Crosby (2002) on green fleshed honeydew muskmelons wherein they reported that cultivar (genetics) was highly significant effect (P=0.001) on ascorbic acid while year (environment) was not. Other studies also reported considerable genetic variability in the antioxidant content and activity of cultivars of tomato (Spencer et al, 2005), black currant (Tabart et al., 2006), apples (Stushnoff et al., 2003), and pears (Galvis Sanchez et al., 2003).

Although the effect of production system on phytochemical content is not consistent, some cultivars exhibited superior nutritional quality over both cropping seasons. An antioxidant index is being proposed to integrate the overall antioxidant potential of melon cultivars. This index integrates the overall antioxidant potential of tomato cultivars by combining the ascorbic acid and total phenolics representing the

antioxidant content and taking the average of TEAC values obtained from DPPH and ABTS assays to represent the antioxidant activity. In 2005, cultivars with the highest antioxidant index (AI) include Savor, Edonis, Sweetie#6, Early Queen, and Rayan (Table 2.15). AI value of Savor was significantly different from the other cultivars. In 2006, the top cultivars were Sweetie#6, Savor, Early Queen, Rayan and Honey Orange. For both years, Savor, Sweetie #6, Early Queen, Edonis, and Rayan rank as the five highest cultivars of the ten we tested (Fig. 2.12). This information may be very beneficial to specialty crop growers interested in marketing high antioxidant melons. In addition, breeders may wish to consider these germplasm sources in developing melons with high nutritional quality.

Year	Cultivar	Antioxida	nt index	
2005	Savor	112.18	a	
	Edonis	89.23	b	
	Sweetie#6	87.50	b	
	Early Queen	83.24	b	
	Rayan	78.33	bc	
	Burpee Hybrid	77.89	bc	
	Honey Orange	67.16	cd	
	Swan Lake	63.82	d	
	Haogen	62.24	d	
2006	Sweetie#6	106.12	a	
	Savor	104.67	a	
	Early Queen	86.24	ab	
	Rayan	79.84	b	
	Honey Orange	72.20	be	
	Edonis	72.17	bc	
	Burpee Hybrid	68.34	bed	
	Swan Lake	53.33	cde	
	Arava	48.91	de	
	Haogen	43.39	e	

Table 2.15. Antioxidant index of ten melon cultivars

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Within each year, least square means with the same letter are not significantly different by Duncan's multiple range test  $(P \le 0.05)$ .



Figure 2.12. Antioxidant index [( $\Sigma$ Vit C+TPC +Antioxidant capacity)/3] of ten melon cultivars (Bars = Mean & S.E.M.).

Swan Lake, Arava and Haogen ranked the lowest AI for two consecutive years. These cultivars have greenish white flesh. Melon cultivars that obtained the highest AI have orange colored flesh. The orange hue in melons is due to high concentration of  $\beta$ -carotene (Lester and Eischen, 1996; Robinson and Decker-Walters, 1999). Saftner et al., (2006) suggested that orange-netted melons have higher  $\beta$ -carotene concentration than in the green honeydews. Although we only measured ascorbic acid and total phenolics,  $\beta$ -carotene may have played a role in higher antioxidant content and radical scavenging capacity of orange-colored melon cultivars.

The individual and interactive effects of cultivar, year, and production system were also examined in our study. This would be the first comparative study on the effect of production method on dry matter and soluble solids content of melons. As pointed out by Magkos et al., (2003), there were only a few comparative studies on selected fruit crops (i.e. apples, strawberries, oranges, lemons and pineapples) that have been done to evaluate the qualitative differences of crops grown in conventional and organic production systems. The results showed that melons grown in organic and conventional plots did not display significant differences in dry matter percentage. This is in agreement with Bordeleau et al., (2002) as cited by Magkos et al., (2003) explaining that significant differences in dry matter could not be expected between organic and conventional produce because fruits have low ability to absorb and assimilate nitrogen. Soluble solids content was not significantly influenced by production system differences. On one hand, cultivar, year, and the interactive effects (Y x C) were observed to have a significant effect on dry matter and soluble solids. Our study also looked at the correlation between fruit quality attributes and antioxidant content and activity. Dry matter and soluble solids correlated significantly with ascorbic acid, total phenolic content and antioxidant activity (DPPH). These fruit quality attributes also moderately correlated with ABTS, another measure of antioxidant activity.

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This study also examined the yield of ten melon cultivars, which was observed to be significantly affected by genotype. Correlation analysis revealed that yield is highly negatively correlated with ascorbic acid (r=-0.47\*\*\*). Savor and Sweetie#6 have the highest antioxidant index; however, these were also the cultivars with the lowest yield. Davis et al., (2004) reported that trade-off between yield and nutrient concentrations could occur because of genetic dilution effect. Nutrient decline may result from decades of selecting for one trait such as high yield, thereby leaving less resource available for other functions like the crop's capacity to synthesize vitamins, minerals and other nutrients (Davis, 2005). Trade-offs between yield and dry weight and between yield and ascorbic acid has been reported for other vegetable crops like tomatoes (Stevens, 1986). As far as we know, there are no previous studies of genetic trade-off between yield and nutrient concentrations in melons that have been reported.

Drawing appropriate conclusion on the effect of production system on antioxidant content and activity; fruit quality attributes and yield lies on sound experimental design and sampling. There is a need to take into account all production and postproduction factors to ensure that the results obtained from this kind of study are conclusive. The results of our study have indicated that in general, production system has less effect than genotype (cultivar) and year differences. A follow-up study is suggested to be carried out which will have a proposed duration of greater than or equal to 3 years (Lester, 2006) to account for extraneous production variables such as year to year weather variation.

#### CHAPTER III

# ANTIOXIDANT PROPERTIES, FRUIT QUALITY ATTRIBUTES AND YIELD OF TEN COMMERCIAL CULTIVARS OF TOMATO (*Solanum lycopersicum* L.) GROWN UNDER ORGANIC AND CONVENTIONAL PRODUCTION SYSTEMS

#### Abstract

In 2005 and 2006, ten commercial cultivars of tomatoes were grown under organic and conventional production systems at the Horticulture Field Research Center, Colorado State University, Colorado USA. These cultivars were examined for their ascorbic acid levels, total phenolic content, and antioxidant activity using two radical cation assays: ABTS and DPPH. Likewise, fruit quality attributes such as percent dry matter, pH, and soluble solids content; and yield was determined for each cultivar. Genotype had significant influence on all parameters. A 3-fold difference in antioxidant properties and dry matter was observed in all cultivars. Higher antioxidant activity was observed in organically grown tomatoes while yield and soluble solids concentration was higher in tomatoes cultivated under a conventional production system. Ascorbic acid was strongly correlated with fruit quality attributes. Antioxidant activity was strongly correlated with total phenolic content and dry matter. Yield was negatively correlated with fruit quality attributes and ascorbic acid suggesting a possible 'dilution effect'. Compared to genotype, production system had less effect on antioxidant content and activity. A significant interaction between cultivar and production system would also imply that

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some cultivars when grown organically or conventionally could have higher antioxidant content and activity. Thus, choice of cultivars is a fundamental approach in producing tomatoes with high phytochemical content. Future initiatives could involve screening of tomato cultivars for higher antioxidant content that could be utilized in breeding programs and selection of nutritionally superior cultivars that could provide a competitive niche for small and medium sized producers.

#### Introduction

Tomato, one of the most economically important vegetable crops in many parts of the world, has been the subject of a long-standing controversy in terms of origin, domestication, and taxonomy. It originated from the Andean region but the place and time of domestication are not yet identified with certainty (Bai and Lindhout, 2007). Taxonomically, the genus has been assigned to Lycopersicum or Solanum. Hypotheses of interspecific relationships based on molecular data support treatment of tomato in Solanum (Peralta and Spooner, 2007). Despite these debates on taxonomy and origin, tomato has gained its niche as a common produce vegetable in the American diet thereby boosting the field and greenhouse production industry all over the country. At present, it is the fourth most popular fresh-market vegetable behind potatoes, lettuce, and onions.

The United States ranks second as top producer of tomatoes in the world. In 2007, more than 4,000 million pounds of fresh market tomatoes were produced and 335 million pounds of the total produce were exported to Canada, South America, Europe, and Asia. The largest export destinations for fresh market tomatoes are Mexico and Canada. Florida ..... 

and California are the top two fresh market tomato producing states comprising over two thirds of total U.S. fresh-market tomato acreage (Boriss et al., 2005).

Annual per capita use of fresh tomatoes increased 34 percent from 15.5 pounds in 1990 to 20.8 pounds in 2007, while use in processed products declined 6 percent to about 71 pounds (fresh-weight basis) (Lucier and Jerardo, 2007). Based on ERS estimates, the expansion of the domestic greenhouse/hydroponic tomato industry since the mid-1990s has added more than 1 pound per person to fresh-market tomato use. The increase in per capita consumption is also due to improved cultivars, marketing of tomato as nutritional food, and promoting it as a good source of vitamin C, vitamin A and antioxidants (Boriss, 2005). In a study carried out by Chun et al., (2005) on the daily consumption of total phenolics and antioxidant capacity from 34 fruits and vegetables in the American diet, their team reported that tomato contributed a moderate amount of total phenolics measured at 24.4 mg gallic acid equivalent (GAE) person<sup>-1</sup> day<sup>-1</sup> and antioxidant intake from daily consumption measured at 30.3 mg vitamin C equivalents (VCE) person<sup>-1</sup> day<sup>-1</sup>.

Tomato has been widely recognized as a crucial supplier of essential nutrients and antioxidants such as ascorbic acid, carotenoids, phenolic acids, vitamin E, and flavonoids. Although other carotenoids such as  $\alpha$  and  $\beta$  carotene and lutein are present in tomato, major interest is focused on lycopene, an acyclic carotenoid consisting of 11 conjugated double bonds and classified as a lipid soluble antioxidant. Tomato has a lycopene content ranging from 8.0 to 42.0 µg g<sup>-1</sup> wet weight (Rao et al., 2006). Lycopene is considered the most efficient biological carotenoid singlet oxygen quencher since it exhibits the highest physical quenching rate constant with singlet oxygen among the carotenoids studied by Di Mascio et al., 1989. Other important biological activities of lycopene include

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scavenging of peroxyl radicals and induction of cell-to-cell communication and growth control (Stahl and Sies, 1996). Based on epidemiological data, animal studies, and human clinical trials (Bowen et al., 2002; Franceshi et al., 1994; Giovanucci et al., 1995; Heath et al., 2006, Jain et al., 1999), the high antioxidant activity of lycopene in tomatoes revealed its potential in the prevention of cancer in certain organ sites (i.e. prostate, breast, cervical, ovarian, and liver). Proliferation of cancer cells is inhibited in the presence of lycopene in the growth media as shown by tissue culture studies using human cancer cell lines (Karas et al., 2000; Prakash et al., 2001). In addition, studies have shown that lycopene also has potential in the prevention of cardio-vascular diseases by reducing the levels of oxidized LDL (LDL<sub>ox</sub>) and serum total cholesterol levels (Agarwal and Rao, 1998; Arab and Steck, 2000; Fuhramn et al., 1997).

Although lycopene is one of the most studied antioxidant compounds, other antioxidants present in tomatoes are now being examined to complete the picture of tomato being a repository of important phytochemicals. Ascorbic acid in tomato is estimated at 22 mg 100 g<sup>-1</sup> fresh weight (Lister, 2003). Other studies have shown that vitamin C ranged from 15.7 to 28.6 mg 100 g<sup>-1</sup> fresh weight (Singh et al, 2004). Moreover, studies have shown that tomato also contains phenolic antioxidants such as chalcones, flavanones, flavanols and hydroxycinnamic acids functioning as free radical terminators and metal chelators (Iijima et al., 2007; Shahidi and Wanasundara, 1992). Martinez-Valverde et al., (2002) characterized the phenolic compounds in tomato as flavonoids (quercetin, kaempferol and naringenin) and hydroxycinnamic acids (caffeic, chlorogenic, ferulic and p-coumaric acids). Based on this study, the most abundant flavonoid in tomato is quercetin, ranging between 7.19 and 43.59 mg kg<sup>-1</sup> fresh weight while the most abundant hydroxycinnaic acid is chlorogenic acid, with concentration ranging from 14 to 32 mg kg<sup>-1</sup> fresh weight. Other studies have measured total phenolics in tomato as  $68.0 \pm 1.6$  mg catechol 100 g<sup>-1</sup> fresh weight (Kaur and Kapoor, 2002) or as  $23.69\pm 0.21$  mg GAE 100 g<sup>-1</sup> fresh weight. These phenolic compounds have been shown to be more effective antioxidants *in vitro* than vitamins C and E on a molar basis; and might contribute significantly to the antioxidant (radical-scavenging) activity (Rice-Evans et al., 1995; Rice-Evans et al., 1997).

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Antioxidant activity has also been studied extensively in recent years using several analytical methods such as 2.2'-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical cation assay; 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay and oxygen radical absorbance capacity (ORAC) assay. ABTS + radical cation assay is a decolorization assay measuring both lipophilic and hydrophilic antioxidants, including carotenoids, flavonoids and hydroxycinnamic acids (Re et al., 1999). In this assay, the pre-formed ABTS radical cation is generated by chemical reduction using manganese dioxide prior to the addition of antioxidant test systems (Miller and Rice-Evans, 1997). Another decolorization assay uses DPPH<sup>++</sup> radical cation as a stable free radical that is deep violet in color due to the 'delocalization' of a spare electron over the molecule as a whole. When DPPH solution and a plant extract containing antioxidants are mixed together, the hydrogen atom from the antioxidants reduces DPPH and results in loss of deep violet color (Molyneux, 2003). The results from ABTS assay could be expressed as Trolox Equivalent Antioxidant Capacity (TEAC) or as Vitamin C Equivalent Antioxidant Capacity (VCEAC) while DPPH assay results are often expressed as EC<sub>50</sub> value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by

50% (Lu and Foo, 2000). Another way to measure antioxidant activity is the ORAC assay which uses antioxidant.

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 $\beta$ -phycoerythrin ( $\beta$ -PE) as an indicator protein, 2,2'-azobis(2amidinopropane) dihydrochloride (AAPH) as a peroxyl radical generator, and 6-hydroxy-2.5.7.8-tetramethylchroman-2-carboxylic acid (Trolox) as a control standard. This assay estimates the total antioxidant capacity of a sample by taking the oxidation reaction to completion or combining into a single quantity the inhibition time and inhibition degree of the free radical action (Cao et al., 1993). Results are expressed as one ORAC unit, which is equivalent to 1µM of Trolox. Since the antioxidant activity of fruit extracts is a function of the array of antioxidants present, accurate comparisons could be done by using more than one assay to describe the total antioxidant activity of fruit samples (Ozgen et al., 2006). In addition, Parr and Bolwell (2000) stated that an effective antioxidant in one assay system is not necessarily an effective antioxidant in another assay system; therefore, there is no single assay that can capture the total efficacy of an Antioxidant activity of tomatoes has been determined in several studies. Toor et

al., (2005) reported that the antioxidant activity of the four tomato cultivars that they have tested ranged from 2329 to 3268 umole TEAC 100g<sup>-1</sup> dry matter (DM) in the hydrophilic extracts and from 178 to 303 µmole TEAC 100g<sup>-1</sup> DM in the lipophilic extracts (mainly carotenoids) using the ABTS<sup>+</sup> radical cation assay. Ascorbic acid contributed 28-38% while flavonoids contributed 29-34% to the antioxidant activity of the hydrophilic extract of tomatoes. Using automated ORAC assay to measure the total antioxidant capacity of tomato fruit, Wang et al., (1996) reported the total ORAC as  $1.89 \pm 0.12$  µmole TEAC g<sup>-1</sup> (wet weight) and 37.8 µmole TEAC g<sup>-1</sup> DM.

Several factors can affect the antioxidant properties of vegetable crops. Research and review articles have reported the influence of genotype (Premier, 2002; Tsao et al., 2006); climatic factors (i.e. temperature, light intensity) (Lee and Kader, 2000; Weston and Barth, 1997); seasonal variations (Howard et al., 2002); crop management practices (Robbins et al, 2005; Schreiner, 2005); type and source of fertilizers (Mozafar, 1993; Toor et al., 2006a) and postharvest handling practices (Javanmardi and Kubota, 2006; Schreiner and Huskeyns-Keil, 2006). In tomatoes, variation for lycopene, ascorbic acid, total phenolic content and free radical scavenging capacity have been observed in different tomato cultivars (Abushita et al., 2002; Hanson et al., 2004; Spencer et al., 2005) suggesting an important genotypic effect. Temperature effect based on the • calculation of growing degree days (GDD) have been carried out by Helyes et al., (2006) who reported that lycopene concentrations increased on the second harvest, which was attributed to cooler weather conditions preceeding that harvest. In relation to this, Rosales et al., (2006) showed that high temperature and overall solar radiation could lower the carotenoid content of exocarp fraction of tomato cultivars grown in a greenhouse despite an increase in ascorbic acid oxidation by APX (ascorbic acid peroxidase enzyme). Seasonal variations could also affect the carpometric characteristics, antioxidant (2006) and Toor et al., (2006b).

composition, and radical scavenging capacity of tomatoes as reported by Raffo et al., Agricultural techniques such as fertilizer and growth regulator application, and irrigation regimes could also influence the level of antioxidants in tomatoes (Dumas et al., 2003). Based on their review of published reports, their team concluded that high application rate of supplementary nitrogen (N) generally tends to decrease the vitamin C

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in tomato fruits through an indirect effect of increased foliage due to high N and consequently less exposure to sunlight. Dumas et al., (1993) also reported that low level of N availability resulted in an increase in leaf polyphenol content of young tomato plants. Graham and Ballesteros (1980) showed that gibberellic acid, cycocel and phosphon (2,4-dichlorobenzyl tributyl phosphonium chloride) increased the ascorbic acid content of field grown tomato fruits. The effect of water availability and occurrence of salt stress on tomato antioxidants have also been studied. Dastane et al (1963) reported that vitamin C content of field-grown tomato fruit in sandy loam soils increased when soil moisture was depleted (40 and 50%) and Naphade (1993) also showed that 40-70% soil moisture depletion can increase ascorbic acid, total soluble solids, sugars and sugar/acid ratio. Rudich et al., (1977) showed that there was a decrease in vitamin C and soluble solids concentration although an increase in yield was observed when low water tensions were maintained in the soil by daily drip irrigation during fruit development. Moreover, moderate salt stress when applied in hydroponically grown tomatoes can increase lycopene as reported by Kubota et al., (2006), and soluble solids concentration (Saito et al., 2008). De Pascale et al., (2001) likewise showed that irrigation with saline water increased the antioxidant activity and carotenoid content of tomato.

Although it is crucial to examine the effect of individual factors on the level of antioxidants, there is also an urgent need to investigate the effect of whole production system (organic vs conventional) to evaluate the potential of organic farming in enhancing the nutritional quality of fresh produce. On the consumer's end, consumer panelists in the study carried out by Zhao et al., (2007b), considered organic produce to be healthier compared to conventionally grown produce. Moreover, Yiridoe et al., (2005)

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concluded from review of several literature sources that consumers are willing to pay 10-20% higher price premium for organic products that have shorter shelf life (i.e. fruits and vegetables). Organic producers who are selling their produce through direct markets are faced with the challenge to enhance their produce' appeal to consumers by focusing on nutritional properties, freshness, and uniqueness. Interest in these qualities may explain increasing awareness in new and nutritionally superior cultivars and organically produced fresh vegetables (Thilmany et al., 2007). In this study, a systematic comparative analysis of the antioxidant content and properties, quality attributes and yield was carried out to contribute to the growing body of knowledge on nutritional quality of tomatoes and organic agriculture.

This study has three objectives:

- To examine if organic production confers more or less antioxidants using research parameters that minimize experimental variables enabling meaningful comparisons;
- To evaluate the extent of antioxidant diversity for ascorbic acid, TP, and TEAC; quality attributes and yield among ten S. lycopersicum L cultivars;
- To identify nutritionally superior S. lycopersicum L. cultivars that may be grown for specialty markets by small and medium sized farmers.

#### **Materials and Methods**

## Tomato production and postproduction

The study was carried out at the Horticulture Field Research Center (HFRC), Colorado State University, Fort Collins, Colorado USA from 2005 to 2006. Areas for organic and conventional production were identified early in the project development phase. For organic production, the soils in the area passed the criteria set by the National Organic Certification Board in 2001. For conventional production, the soils in that part of the HFRC have been applied with inorganic fertilizers, herbicides and pesticides for many years. The soil texture in HFRC is classified as Nunn clay with a pH of 7.8. This study is part of a larger project entitled 'Differentiating Small Farm Produce Offerings through Nutritionally Superior Cultivars, Marketing, and Extension Programs'

Offerings through Nutritionally Superior Cultivars, Marketing, and Extension Programs' wherein six crops including tomatoes were planted under organic and conventional production systems. The experimental units were laid out in a split plot with the whole plots arranged as completely randomized design. The whole plot factor is production system while the sub-plot factor is cultivar. Three blocks in each production system served as replications. Ten cultivars were planted in each block of the organic and conventional production plots namely: 'Big Beef', 'Early Girl', 'Celebrity', 'Fantastic', 'First Lady', 'Husky Red', 'Jet Star', 'Red Sun', 'New Girl', and 'Roma'. All cultivars are Beefsteak type except Roma (a plum type).

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All transplants were grown at the CSU Plant Environmental Research Center Greenhouses. Organic planting media (Sunshine Organic Basic) was used. Sowing of tomato seeds occurred 42 days prior to field planting. The transplants were grown on a bottom heated greenhouse floor maintained at 18<sup>o</sup>C. Watering for the transplants was done automatically by overhead misting/sprinkler using a city water source. 'Rootshield®' (*Trichoderma harzianum*, Strain T-22 #9462), approved for organic cropping systems, was drenched into the soil immediately after sowing following label rates. Organic liquid fertilizer Omega 6-6-6 (Peaceful Valley Farm Supply) was applied

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to tomatoes at four weeks after sowing. Tomatoes were transplanted into black plastic mulched beds at 5.1 cm in row spacing and 12.7 cm between beds. The field plots measured 45.0 meter (m) long and 10.8 m wide.

## Fertilization

Soil sampling was done before and after planting at 7.6 cm off the irrigation drip tape and to a depth of 27.9 cm. Soil samples were submitted to the CSU Soil Testing Lab. Based on these soil tests, 22,407 kilogram (kg) ha<sup>-1</sup> of 'Evergreen' poultry compost (A-1 Organics) was applied to the organic block. The compost was applied with a Mill Creek spreader and disked into the soil immediately following the application. To match the amount of nutrients in the organic block, appropriate amount of nitrogen (N) and phosphorus (P) from urea and superphosphate, respectively, were applied to the conventional block.

#### Pest management

In 2005, there was a combination of pressure from potato psyllid (*Paratrioza cockerelli*) and beet leafhopper (*Circulifer tenellus*). Psyllid yellows expression was variable and curly top virus killed some plants. Potato psyllids were controlled in the organic plot using an approved botanically derived pyrethrum ('Pyganic') insecticide. In the conventional plots, 'Provado' (Imidicloprid) was applied to control psyllids and leafhoppers. There was no psyllid infestation in the 2006 cropping season.

## Irrigation management

Domestic water was applied using drip irrigation and was scheduled using 'Watermark' sensors. Drip irrigation was applied at 6.20 liters/min/m of row, and application was varied during the season from 0.5 hours/2 days to 2 hours/day depending

upon the developmental stage of the crop. During the production period, crops were not permitted to suffer from water stress based on 'Watermark' soil monitors.

## Harvesting and Postharvest handling

Tomatoes were harvested after reaching physiological maturity. Three fruits were collected from each replication/block per cultivar and cooled after harvest to 8.9 <sup>o</sup>C.

#### Sample preparation, extraction and analysis

Tomatoes were washed well to remove contamination on their outer surface and cut into half following a standard procedure. Forty milligrams of thin tomato slices were obtained from radial slices. These tomato slices were placed in a 'Virtis' freeze dryer and lyophilized for five days to remove water and prolong storage viability. Freeze-dried tomato samples were weighed to determine dry matter content and ground in preparation for extraction. Five ml of 80% acetone was placed in each tube and vortexed until thoroughly suspended. The samples were placed in a refrigerated rotator in the dark for one hour and centrifuged at 6,000 rpm for 15 minutes at  $4^{\circ}$ C. One ml aliquots of clear supernatant was removed and placed in a Eppendorf vacufuge<sup>TM</sup> for a minimum of 2 hours at  $45^{\circ}$ C. Extracted, dehydrated samples were used for total phenolic content, ABTS and DPPH assays only. Freeze dried powdered samples were used directly for ascorbic acid analysis. Desiccated samples were stored dry at -20<sup>o</sup> C before analysis.

#### Total phenolic content (TPC)

TPC was standardized against gallic acid (Sigma Chemicals Co., St. Louis, MO) and expressed as milligrams per 100 gram of tomato fresh weight (mg GAE 100g<sup>-1</sup> FW) using a microplate-based Folin-Ciocalteu assay adapted from Spanos and Wrolstad, (1990). Vacufuged stored samples were reconstituted with 1.0 ml 80% acetone (Fisher

Chemicals, Fair Lawn, NJ) and 100µl of this extract was diluted with 900µl of nanopure water. In triplicate, 35µl of diluted sample was pipetted in microplate wells. Using a multichannel pipette, 150 µl of 0.2 M Folin-Ciocalteu reagent (Sigma Aldrich) and 115 µl 7.5% (w/v) Na<sub>2</sub>CO<sub>3</sub> (Fisher Chemicals) were added to all wells. The plate was incubated at  $45^{0}$ C, cooled to room temperature for one hour, and read at 765 nm using a (Spectra Max Plus, Molecular Devices Corp., Sunnyvale, CA) spectrophotometer.

# ABTS<sup>+</sup> Trolox equivalent antioxidant capacity (TEAC)

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ABTS<sup>+2</sup>,2'-azinobis(3-ethylbenzthiazoline-6-sulfonicacid) TEAC was measured using a microplate ABTS<sup>+</sup> radical cation assay based on the method developed by Miller and Rice-Evans (1997). The ABTS solution was prepared by mixing 40 mg of ABTS (Calbiochem, EMD Biosciences, La Jolla, CA), 15 ml distilled water and two to three mg of MnO<sub>2</sub> (Sigma-Aldrich). To remove MnO<sub>2</sub>, the samples were first vacuum filtered, then passed through a 0.2  $\mu$ m 'Acrodisk' syringe filter into a flask. The absorbance value of the ABTS solution was read at 734 nm in a spectrophotometer and adjusted to 0.700 absorbance units (AU) by adding 5.0 mM phosphate buffer solution. Twenty-five  $\mu$ l of reconstituted vacufuged samples and 250  $\mu$ l of ABTS solution were mixed well and read at 734 nm exactly after 60 seconds at 25<sup>o</sup>C. The absorbance value was expressed as  $\mu$ mol Trolox equivalent antioxidant capacity (TEAC)/ml in assay and compared to a set of Trolox (Calbiochem) standards. This was converted to  $\mu$ mol TEAC/100g sample (FW) taking into account all dilution and concentration factors.

# **DPPH**<sup>+</sup> Trolox equivalent antioxidant capacity (TEAC)

Antioxidant activity was also measured with a microplate-based (2,2-diphenyl-1picrylhydrazyl) or DPPH antioxidant assay based on the method of Lu and Foo, 2000 with some modifications. A 0.1 mM DPPH solution was prepared by mixing 7.89 mg of DPPH with 100% methanol adjusting the absorbance value to 0.95 AU. Fifteen  $\mu$ l of the reconstituted vacufuged samples were mixed with 285  $\mu$ l of DPPH<sup>++</sup> solution and read at 515 nm in the spectrophotometer exactly after three minutes at 25<sup>o</sup>C. Results were expressed as  $\mu$ M trolox equivalent antioxidant capacity, TEAC 100 g<sup>-1</sup> FW.

## Soluble solids content, pH, and dry matter

Percent soluble solids of tomato samples was measured using a temperature compensated 'Reichert' handheld refractometer and results were expressed as <sup>0</sup>Brix. The dry matter (%) was obtained gravimetrically from dried and fresh weights. The pH of tomato samples was measured using a 'Beckman' pH meter.

#### Ascorbic acid

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Standard solutions were prepared by mixing 100 mg dithiothreitol (Promega Corp., Madison, WI) and 10 mg of ascorbic acid (Sigma-Aldrich Inc., St. Louis, MO) and by diluting to five concentrations to prepare the standard curve. Lyophilized tomato tissue was extracted in 5% w/v aqueous solution of metaphosphoric acid containing 1% w/v DTT. The mixture was vortexed for 15 seconds and rotated for 15 minutes at 4<sup>o</sup>C. To separate the liquid from the solid phase, the mixture was centrifuged for 5 minutes at 4,000 rpm at 4<sup>o</sup>C. This procedure was repeated twice. The supernatant from the first and second extraction was filtered through a 0.45 mm nylon syringe filter, prior to injection onto an Inertsil 4C high performance liquid chromatography (HPLC) column (Agilent Technologies, Santa Clara, CA) and run with a phosphoric acid/methanol gradient. This method was adapted from Esparza et al., (2006).

#### Temperature and solar radiation data collection

Data on temperature and solar radiation for two cropping seasons (2005-2006) were obtained from the Northern Colorado Water Conservancy District (NCWCD) where one of its weather stations is located within 100 m of the research plots. To determine the effect of temperature, daily growing degree-days was computed by subtracting the base temperature ( $10^{0}$ C) for warm season crops like tomatoes from the average temperature as daily GDD=[( $T_{max} + T_{min}$ )/2]- base temperature where  $T_{max}$  and  $T_{min}$  are maximum and minimum daily air temperatures. Each daily GDD is added up over the growing season. Solar radiation data was recorded by an 'Epply' pyranometer and expressed as Langleys (1-calorie square centimeter<sup>-1</sup>).

# Statistical Analysis

Analysis of variance (ANOVA) was carried out using SAS Mixed Procedure (SAS Inc., version 9.1, Cary NC). Correlation analysis was done using Pearson-Gaussian distribution (SAS Proc Corr). Differences between means were calculated using Tukey-Kramer ( $P \le 0.05$ ). To determine the differences between the antioxidant indexes of each cultivar, Duncan's Multiple Range Test ( $P \le 0.05$ ) was carried out. Graphs were prepared using the Graph Pad Prism version 4.0 software.

#### Results

#### Temperature and solar radiation

Higher temperatures were observed prior to 17 days before harvest in 2005 than in 2006; however, the heat accumulated in the last 13 days before harvest in both years was similar (Fig.3.1). From field planting to harvest, GDD was higher in 2006 than 2005
suggesting that tomatoes grown in 2006 were exposed to higher temperatures (Fig. 3.2). Solar radiation received by tomato plants from planting to harvest was almost the same in 2005 and 2006 (Fig.3.3).



Figure 3.1. Heat accumulation (in growing degree-days) 30 days prior to harvest.  $GDD=[(T_{max} + T_{min})/2]-10^{\circ}C$ , where  $T_{max}$  and  $T_{min}$  are maximum and minimum daily air temperatures;  $10^{\circ}C$  is the base temperature or the temperature below which there is no growth or development.



Figure 3.2. Heat accumulation (in growing degree-days) from planting to harvest  $GDD=[(T_{max} + T_{min})/2]-10^{0}C$ , where  $T_{max}$  and  $T_{min}$  are maximum and minimum daily air temperatures;  $10^{0}C$  is the base temperature or the temperature below which there is no growth or development.



Figure 3.3. Daily net solar radiation (in Langleys) from planting to harvest

# Effects of year, cultivar and production system and their interactions on nutritional quality and yield

Results from split plot analysis of variance showed that production system (PS) had a significant effect on antioxidant capacity measured using the ABTS + radical cation assay: soluble solids concentration, and yield (Table 3.1). No significant effect of PS was observed on dry matter percentage and pH. Environmental effects from 2005 to 2006 (Y) greatly influenced the level of ascorbic acid (AA); Trolox equivalent antioxidant capacity (TEAC) measured using ABTS and DPPH<sup>+</sup> radical cation assays; fruit quality attributes (i.e. soluble solids, pH and dry matter); however, Y had less effect on the level of total phenolic content (TPC). Among the factors that were examined, cultivars (C) had significantly influenced all parameters (P <0.0001) suggesting high genotypic variability in ten commercial cultivars in terms of antioxidant properties, quality attributes, and vield. The interaction effects among cultivar, year and production system on the parameters varied widely. Y x C interaction had a significant effect on antioxidant content and activity but not on fruit quality attributes. Greater effect of Y x PS interaction was observed in TPC and dry matter than other parameters tested suggesting that their levels in organic or conventional tomatoes may vary every year. C x PS interaction significantly affected the level of AA, TPC, and antioxidant activity (ABTS) implying that some cultivars had different levels of AA, TPC and antioxidant activity (ABTS) when grown organically or conventionally. A three-way interaction effect (Y x C x PS) was significant for AA, TPC, antioxidant activity (ABTS) and soluble solids but not on DPPH, dry matter, pH, and yield. Simply put, some cultivars were less stable than others from year to year, depending on if they were grown organically or conventionally.

Source	AA	TPC	ABTS	DPPH	Dry matter	Soluble solids	рН	Yield
Year (Y)	<.0001	0.0531	0.0173	<.0001	<.0001	0.0003	<.0001	0.0023
Cultivar (C)	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Y x C	0.0037	0.0005	0.0004	0.0029	0.3670	0.0512	0.0225	<.0001
Production system (PS)	0.0617	0.0856	0.0020	0.1605	0.7944	0.0210	0.3169	0.0053
Y x PS	0.7135	0.0015	0.3921	0.2701	0.0094	0.2774	0.0433	0.0743
C x PS	0.0273	<.0001	<.0001	0.7970	0.5243	0.0671	0.2179	0.2892
Y x C x PS	0.0010	<.0001	0.0002	0.4854	0.2643	0.0204	0.1665	0.2852

Table 3.1. Analysis of variance of the effects of year, cultivar and production system and their interactions

Expressed as p values for statistical significance; significant at  $P \le 0.05$ .

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Since Y significantly influenced all the observed parameters except TPC, subsequent analysis on C, PS, and C x PS interaction was carried out for each year. When the data were analyzed by year, cultivar remained the most significant factor in determining the level of antioxidants, attributes of fruit quality and yield (Table 3.2). PS had a significant effect on antioxidant capacity (ABTS) and yield in both years. In 2005, PS did not alter DM and soluble solids, while in 2006; PS significantly influenced all parameters except soluble solids. The interaction between C x PS had a significant effect on antioxidant activity (ABTS) and AA in both years which suggests that cultivars grown in conventional or organic production system may have different levels of AA and antioxidant capacity. Yield response, soluble solids, and pH varied in both years depending on cultivar and production system. Antioxidant activity (DPPH) and dry matter were not affected by C x PS interaction effect.

Analysis		2005			2006	
	С	PS	C x PS	C	PS	C x PS
AA	<0.0001 (***)	0.1599(NS)	0.0100 (**)	<0.0001 (***)	0.0568 (NS)	0.0060 (**)
ABTS	<0.0001	0.0097 (**)	0.0001 (***)	<0.0001 (***)	0.0053(**)	<0.0001(***)
DPPH	<0.0001 (***)	0.7729 (NS)	0.6576 (NS)	<0.0001 (***)	0.0474 (*)	0.6173 (NS)
Dry matter	<0.0001 (***)	0.0670 (NS)	0.6689 (NS)	<0.0001 (***)	0.0258 (*)	0.1057 (NS)
Soluble	<0.0001 (***)	0.0289 (*)	0.0033 (**)	<0.0001 (***)	0.1398 (NS)	0.6123 (NS)
рН	<0.0001 (***)	0.4601 (NS)	0.8010 (NS)	<0.0001 (***)	0.0232 (*)	0.0027 (**)
Yield	<0.0001	0.0083(**)	0.0942 (NS)	0.0011	0.0491 (*)	0.9718 (NS)

Table 3.2. Analysis of variance on the effects of cultivar and production system on nutritional quality parameters and yield (by year)

Expressed as p values for statistical significance; NS, \*, \*\*, \*\*\* Nonsignificant or significant at  $P \le 0.05$ , 0.01, 0.001 respectively.

#### Ascorbic acid

When grown in the organic production system, almost all tomato cultivars except for 'Celebrity', 'Husky Red', and 'Early Girl' had higher ascorbic acid than those grown in the conventional production system (Table 3.3). In both production systems, 'New Girl' had the highest ascorbic acid level and it was significantly different from 'Red Sun', 'Big Beef', 'Early Girl', and 'Roma'. In terms of cultivar effect, there was a 3-fold difference in the level of ascorbic acid content ranging from 9.65 to 29.72 mg 100 g<sup>-1</sup> fresh weight (Fig.3. 4).

Ascorbic acid levels were significantly different in 2005 and 2006. All cultivars grown in 2006 had higher ascorbic acid than those that were grown in 2005 (Table 3.4). In 2006, the top cultivars were 'New Girl', 'First Lady', 'Celebrity', 'Fantastic', and 'Jet Star'. 'New Girl' was significantly different from 'Jet Star'. In 2005, 'New Girl' had the

highest ascorbic acid level and it was significantly different from 'Celebrity' and the bottom five cultivars namely 'Red Sun'. 'Roma', 'Husky Red', 'Big Beef', and 'Early Girl'. In both years, the top four cultivars that were significantly different from the other cultivars were 'New Girl', 'First Lady', 'Fantastic', and 'Jet Star'.

Production System	Cultivar acid	Mean ascorbic (mg 100 g <sup>-1</sup> FW)	
Conventional	New Girl	21.29	а
	Celebrity	20.48	ab
	First Lady	19.90	ab
	Fantastic	18.56	abc
	Jet Star	18.24	abc
	Husky Red	18.06	abc
	Red Sun	17.81	bc
	Big Beef	17.62	bc
	Early Girl	17.03	bc
	Roma	15.43	с
Organic	New Girl	23.44	а
	Jet Star	22.11	ab
	First Lady	21.69	ab
	Fantastic	21.67	ab
	Celebrity	20.16	bc
	Red Sun	19.10	bcd
	Roma	18.55	cd
	Husky Red	17.25	cd
	Big Beef	17.20	cd
	Early Girl	16.77	d

Table 3.3. Ascorbic acid in tomato cultivars grown in organic and conventional production systems for 2005 and 2006

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Within each production system, least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ ).



Figure 3.4. Ascorbic acid content determined in organic and conventionally grown cultivars in 2005 and 2006. Data are expressed as mean  $\pm$  SEM.

Year	Cultivar	Mean ascorbic acid $(m = 100 \text{ s}^{-1} \text{ FW})$		
2006	New Cirl	(mg 100 g FW)		
2006	New Girl	26.28	а	
	First Lady	24.31	ab	
	Celebrity	24.03	ab	
	Fantastic	23.21	abc	
	Jet Star	22.57	bc	
	Red Sun	22.57	bc	
	Early Girl	22.20	bc	
	Husky Red	21.49	bc	
	Big Beef	2006	bc	
	Roma	20.12	c	
2005	New Girl	18.44	а	
	Jet Star	17.78	а	
	First Lady	17.28	ab	
	Fantastic	17.02	abc	
	Celebrity	16.62	abcd	
	Red Sun	14.34	bcde	
	Roma	13.86	cde	
	Husky Red	13.82	cde	
	Big Beef	13.53	de	
	Early Girl	11.60	e	

Table 3.4. Year effect on ascorbic acid content of tomato cultivars

Within each year, least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ ).

# Total phenolic content

Year had a moderate effect on the total phenolic content (TPC) of tomato cultivars (P=0.0531) suggesting that variation on TPC in both years was not very wide. Genotype significantly affected the TPC since it ranged from 41.84 to 117.00 mg gallic acid equivalent (GAE) 100 g<sup>-1</sup> fresh weight indicating an almost 3-fold difference among cultivars (Fig. 3.5).



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Figure 3.5. Total phenolic content of organically and conventionally grown tomato cultivars. Data are expressed as means<u>+</u>SEM.

In 2005 and 2006, the highest TPC was observed in 'Fantastic', 'Jet Star', and 'New Girl' (Table 3.5). These cultivars were significantly different from 'Red Sun', 'Roma', 'Husky Red', and 'Early Girl'. Production system had a moderate effect on the level of TPC. Most cultivars had higher TPC when they were grown using organic production compared to conventional production except for 'Fantastic' and 'Roma' (Table 3.6).

Cultivars	Mean TP concentration			
	(mg GAE 100 g <sup>-1</sup> F	W)		
Fantastic	90.77	a		
Jet Star	90.26	а		
New Girl	82.27	ab		
Celebrity	73.74	bc		
First Lady	73.26	bcd		
Big Beef	72.54	bcd		
Red Sun	68.96	cd		
Roma	68.64	cd		
Husky Red	63.69	cd		
Early Girl	62.53	С		

Table 3.5. Total phenolic content of tomato cultivars in both years

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Least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ )

Cultivars	Production	Mean TP concentration	
	System	(mg GAE 100 g <sup>-1</sup> FW)	
Fantastic	Conventional	93.24	a
Jet Star	Organic	91.65	ab
Jet Star	Conventional	88.88	ab
Fantastic	Organic	88.30	ab
New Girl	Organic	83.08	abc
New Girl	Conventional	81.46	abcd
First Lady	Organic	80.15	abcde
Big Beef	Organic	79.35	abcde
Celebrity	Organic	78.37	abcde
Roma	Conventional	73.51	bcde
Red Sun	Organic	73.41	bcde
Husky Red	Conventional	72.49	bcdef
Early Girl	Organic	69.82	cdefg
Celebrity	Conventional	69.10	cdefg
First Lady	Conventional	66.36	defg
Big Beef	Conventional	65.74	defg
Red Sun	Conventional	64.50	defg
Roma	Organic	63.78	efg
Early Girl	Conventional	55.25	fg
Husky Red	Organic	54.89	g

Table 3.6. Total phenolic content of tomato cultivars by production system

Least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ ).

#### Antioxidant activity

Trolox-equivalent antioxidant activity of cultivars was determined using two radical scavenging cation assays: ABTS and DPPH.

# ABTS<sup>+</sup> Trolox equivalent antioxidant capacity (TEAC)

Analytical evaluation revealed over a 3-fold difference in antioxidant activity among the ten cultivars grown in two production systems. Their antioxidant activity ranged from 66.29 to 247.77  $\mu$ M TEAC 100g <sup>-1</sup> fresh weight (Fig. 3.6). Higher antioxidant activity was also observed in 2006 than in 2005. Production system significantly affected the level of antioxidant activity among cultivars. Organically produced tomatoes had higher antioxidant activity than conventionally produced ones (Table 3.7). Under the organic production system, 'Fantastic' and 'First Lady' had the highest antioxidant activity and they were significantly different from the other eight cultivars. In contrast, antioxidant activity of most tomato cultivars, except for 'Fantastic' and 'Celebrity', was not significantly different when they were grown under conventional production.

Percentage difference between organic and conventional production for each cultivar was computed using the formula: organic-conventional/conventional \*100, as described by Worthington (2001). The highest percentage difference (>30%) was observed in 'Celebrity', 'First Lady', 'New Girl', and 'Fantastic'. Four cultivars ('Roma', 'Husky Red', 'Big Beef', and 'Early Girl') were not consistent in terms of percentage difference in each year (Table 3.8).



Figure 3.6. ABTS<sup>.+</sup> Trolox equivalent antioxidant capacity (TEAC) of tomato cultivars grown in conventional and organic plots Data are expressed as means<u>+</u>SEM.

Production System	Cultivar	Mean anti (µM TEA	oxidant activity C/100 g fresh weight)
Organic	First Lady	207.17	a
	Fantastic	194.85	ab
	New Girl	174.47	abc
	Celebrity	165.70	bcd
	Jet Star	148.96	cde
	Red Sun	145.53	cde
	Big Beef	136.34	de
	Roma	127.87	e
	Husky Red	119.72	e
	Early Girl	117.30	e
Conventional	Fantastic	149.61	а
	First Lady	143.84	ab
	Red Sun	140.86	ab
	Jet Star	136.85	ab
	Roma	135.91	ab
	New Girl	128.79	ab
	Big Beef	122.63	ab
	Husky Red	119.57	ab
	Early Girl	115.18	ab
	Celebrity	108.40	b

Table 3.7. ABTS<sup>+</sup> Trolox equivalent antioxidant capacity (TEAC) of tomato cultivars grown in conventional and organic plots

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Within each production system, least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ )

Cultivar	Year	Organic	Conventional	Percentage Difference
First Lady	2005	227.62	123.50	84.31
,	2006	186.72	164.18	13.73
	Average	207.17	143.84	44.03
Fantastic	2005	165.91	152.83	8.56
	2006	223.79	146.38	52.88
	Average	194.85	149.605	30.24
New Girl	2005	183.40	152.75	20.07
	2006	165.54	104.84	57.90
	Average	174.47	128.795	35.46
Red Sun	2005	145.24	121.57	19.47
	2006	145.81	160.15	-8.95
	Average	145.525	140.86	3.31
Jet Star	2005	165.65	155.93	6.23
	2006	132.27	117.77	12.31
	Average	148.96	136.85	8.85
Celebrity	2005	133.21	126.25	5.51
	2006	198.18	90.56	118.84
	Average	165.70	108.41	52.85
Roma	2005	85.95	119.73	-28.21
	2006	169.80	152.09	11.64
	Average	127.875	135.91	-5.91
Big Beef	2005	114.94	120.64	-4.72
	2006	157.75	124.62	26.58
	Average	127.875	135.91	11.18
Husky Red	2005	115.38	112.50	2.56
	2006	124.06	126.63	-2.03
	Average	119.72	119.565	0.13
Early Girl	2005	120.49	67.34	78.93
	2006	114.11	163.02	-30.00
	Average	117.3	115.18	1.84

Table 3.8. Percentage difference in antioxidant activity of tomato cultivars in 2005-2006

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# DPPH<sup>.+</sup> Trolox equivalent antioxidant capacity (TEAC)

Antioxidant activity of tomato cultivars was significantly different in each year. Higher antioxidant activity was observed in 2006 than 2005 (Table 3.9). In 2005, the top three cultivars with higher antioxidant activity were 'New Girl', 'Jet Star', and 'First Lady'. Mean antioxidant activity of 'New Girl' was not significantly different from 'Jet Star' and 'First Lady' while it was significantly different from the other seven cultivars. In 2006, cultivars with higher antioxidant activity were 'First Lady', 'Fantastic', 'New Girl', 'Husky Red', 'Jet Star', and 'Red Sun'. Mean antioxidant activity of 'First Lady' and 'Fantastic' was significantly different from 'Big Beef', 'Celebrity', 'Early Girl', and 'Roma'. This result could be attributed to effect of storage conditions on antioxidant content of tomatoes since 2005 tomato freeze-dried samples were analyzed a year after harvest. Possibly, carotenoids or phenolic compounds contributing to antioxidant activity may have been reduced while in storage though they were freeze-dried and kept at -20<sup>o</sup>C in sealed tubes in the dark.

For the effect of other factors, genotype greatly affected antioxidant activity. More than 3-fold difference ranging from 98.33 to 319.72  $\mu$ M TEAC 100 g<sup>-1</sup> fresh weight was observed among cultivars (Fig.3.7). Neither organic nor conventional production system highly influenced antioxidant activity. Apart from C x Y interaction, other two-way and three-way interactions among cultivar, year and production system were not significant.

Year	Cultivar	Mean antioxidant	activity	
		( $\mu$ M TEAC 100 g <sup>-1</sup> fresh		
		weight)		
2005	New Girl	260.03	а	
	Jet Star	221.10	ab	
	First Lady	202.60	abc	
	Fantastic	179.96	bcd	
	Husky Red	152.00	cd	
	Big Beef	147.61	cd	
	Red Sun	146.29	cd	
	Celebrity	144.01	cd	
	Roma	143.35	cd	
	Early Girl	123.55	d	
2006	First Lady	297.61	а	
	Fantastic	288.87	а	
	New Girl	282.12	ab	
	Husky Red	277.46	ab	
	Jet Star	269.59	abc	
	Red Sun	258.64	abcd	
	Big Beef	241.78	bcd	
	Celebrity	232.52	cd	
	Early Girl	222.26	d	
	Roma	217.42	d	

Table 3.9. DPPH<sup>• +</sup> Trolox equivalent antioxidant capacity (TEAC) of tomato cultivars grown in conventional and organic plots

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Within each year, least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ ).



Figure 3.7. DPPH<sup>· +</sup> Trolox equivalent antioxidant capacity (TEAC) of tomato cultivars grown in conventional and organic plots Data are expressed as means<u>+</u>SEM.

# Dry matter content

Production system did not significantly affect the dry matter percentage. Nevertheless, dry matter content of cultivars was significantly affected by genotype and year. Wider variation, ranging from 2.99 to 8.56 percent or almost a 3-fold difference, was observed among cultivars (Fig. 3.8). Tomatoes harvested in 2006 had higher dry matter content than those harvested in 2005 (Table 3.11).



Figure 3.8. Dry matter content of tomato cultivars grown in conventional and organic plots. Data are expressed as mean  $\pm$  SEM.

In 2005, cultivars with high percent dry matter were 'First Lady', 'New Girl', 'Jet Star', 'Big Beef', and 'Fantastic' wherein 'First Lady' was significantly different from the other cultivars. In 2006, 'First Lady' had the highest dry matter content followed by 'New Girl', 'Fantastic', 'Jet Star', and 'Big Beef'. It was significantly different from 'Red Sun', 'Celebrity', 'Roma', and 'Early Girl'. Although there was no significant interaction between cultivar and production system, interaction effect between year and production system was significant as shown in Table 3.10. Tomatoes grown in 2006 using organic

production had higher dry matter content that those planted in 2005 with the organic production system. The same held true in conventional production system.

Year	Production system	Dry mat	ter content (%)	
2006	Organic	7.56	a	
2006	Conventional	7.21	a	
2005	Organic	5.49	b	
2005	Conventional	5.09	b	

Table 3.10. Year influence on dry matter content of tomatoes in organic and conventional production systems

Least square means with the same letter are not significantly different by Tukey-Kramer (P $\leq$  0.05).

Table 3 11	Year to year	variability in dr	v matter con	tent of tomato	cultivars
1 abic 5.11.	i cai to year	variability in ui	y matter con	tent of tomato	cultivars

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Year	Cultivar	Percent dry matter		
2006	First Lady	8.08	а	
	New Girl	7.89	ab	
	Fantastic	7.86	ab	
	Jet Star	7.80	ab	
	Big Beef	7.56	abc	
	Husky Red	7.52	abc	
	Red Sun	7.21	bc	
	Celebrity	6.86	cd	
	Roma	6.81	cd	
	Early Girl	6.28	d	
2005	First Lady	6.24	а	
	New Girl	6.12	ab	
	Jet Star	5.86	abc	
	Big Beef	5.42	abc	
	Fantastic	5.34	abc	
	Husky Red	5.09	bc	
	Red Sun	5.04	bcd	
	Roma	4.97	cd	
	Celebrity	4.88	cd	
	Early Girl	3.97	d	

Within each year, least square means with the same letter are not significantly different by Tukey-Kramer (P $\le$  0.05).

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Soluble solids content was significantly influenced by year, genotype, and production system. More than a 2-fold difference for soluble solids was observed among cultivars ranging from 2.50-6.00 <sup>0</sup>Brix (Fig. 3.9). Production year had less effect on soluble solids content than cultivar. Production system also influenced soluble solids content in different tomato cultivars. Conventionally produced 'New Girl', 'First Lady', and 'Fantastic' had the highest soluble solids. (Table 3.12).



Figure 3.9. Soluble solids content of tomato cultivars grown in conventional and organic plots. Data are expressed as mean  $\pm$  SEM.

Production System	Cultivar	Soluble solids content ( <sup>0</sup> Brix)		
Organic	First Lady	4.83	а	
	Big Beef	4.67	ab	
	Jet Star	4.62	ab	
	Fantastic	4.61	ab	
	Husky Red	4.42	abc	
	New Girl	4.41	abc	
	Red Sun	4.25	abc	
	Celebrity	4.07	bcd	
	Roma	3.80	cd	
	Early Girl	3.50	d	
Conventional	New Girl	5.33	а	
	First Lady	5.25	ab	
	Fantastic	5.15	ab	
	Big Beef	4.96	abc	
	Jet Star	4.61	abcd	
	Red Sun	4.52	bcd	
	Husky Red	4.34	cd	
	Roma	4.06	de	
	Celebrity	4.05	de	
	Early Girl	3 49	e	

Table 3.12. Soluble solid content of tomato cultivars grown in conventional and organic plots

Within each production system, least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ )

However, two- way interactions that involved year and production system or cultivars were not significant suggesting that soluble solids content would not significantly change in each production year. Moreover, this would also imply that there would be a narrow variation among cultivars when they are grown organically or conventionally (Table 1).

pH

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Production system including two-way interactions with year and cultivar (C x PS, Y x PS) did not significantly influence the level of pH suggesting that cultivars may have a

similar pH level whether grown organically or conventionally in each year. Genotype and year did significantly alter pH level of ten cultivars where pH ranged from 3.60 to 4.43 (Fig. 3.10). The most significant trend was that all cultivars had a lower pH in 2005 than 2006, indicating an important environmental effect. 'Roma' had the highest pH followed by 'Jet Star' and 'Husky Red'. These cultivars were significantly different with 'Big Beef', 'New Girl', 'Celebrity', 'Early Girl', and 'First Lady' (Table 3.13).

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Figure 3.10. pH of ten commercial tomato cultivars. Data are expressed as mean  $\pm$  SEM.

Year	Cultivar	Mean pH		
2006	Roma	4.32	a	
	Jet Star	4.27	ab	
	Husky Red	4.20	ab	
	Red Sun	4.17	abc	
	Fantastic	4.12	bc	
	Big Beef	4.11	cd	
	New Girl	4.11	cd	
	Celebrity	4.08	cd	
	Early Girl	4.05	d	
	First Lady	4.04	d	
2005	Jet Star	4.05	а	
	Roma	3.90	ab	
	Big Beef	3.88	abc	
	Fantastic	3.87	abc	
	Red Sun	3.87	abc	
	Husky Red	3.85	bc	
	New Girl	3.80	bc	
	First Lady	3.78	bc	
	Celebrity	3.70	с	
	Early Girl	3.70	с	

Table 3.13. pH of ten tomato cultivars grown in 2005 and 2006

Within each year, least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ )

# Yield

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Genotype, year, and production system significantly affected the yield of tomato cultivars. An almost 10-fold difference ranging from 1,071 to 10,355 kg hectare <sup>-1</sup> was observed among cultivars that were grown with the two production systems in 2005-2006 (Fig. 3.11). Production year had likewise a major effect on yield. Tomatoes harvested in 2005 had higher yield than those that were harvested in 2006. Conventionally produced tomatoes had yielded 25% higher than organically grown tomatoes (Table 3.14). In both

production systems, Early Girl had the highest yield and was significantly different from cultivars with the lowest yield namely Big Beef and Roma.

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Figure 3.11. Yield of tomato cultivars under organic and conventional production systems in 2005-2006. Data are expressed as mean  $\pm$  SEM.

Production System	Cultivar	Yield (kg/ha)	
Conventional	Early Girl	1151.93	а
	Fantastic	1050.11	ab
	Red Sun	990.14	ab
	First Lady	971.46	ab
	Husky Red	940.39	ab
	Jet Star	892.27	ab
	Celebrity	870.78	ab
	New Girl	857.38	ab
	Big Beef	723.39	bc
	Roma	485.72	с
Organic	Early Girl	983.40	а
	First Lady	902.13	ab
	New Girl	817.54	abc
	Husky Red	782.01	abc
	Red Sun	738.19	abc
	Celebrity	702.95	bc
	Jet Star	659.49	bc
	Fantastic	637.36	bc
	Big Beef	558.06	cd
	Roma	318.68	d

Table 3.14. Yield of tomato cultivars grown in conventional and organic plots

Within each production system, least square means with the same letter are not significantly different by Tukey-Kramer ( $P \le 0.05$ ).

#### **Correlation analysis**

Strong correlation was observed between two methods of antioxidant activity measurement (ABTS and DPPH radical cation assays). Antioxidant activity (ABTS, DPPH) was significantly correlated with ascorbic acid, total phenolic content, and dry matter (Table 3.15). Ascorbic acid and total phenolic content were not significantly correlated. Total phenolic content was not strongly correlated with quality attributes (i.e. dry matter, soluble solids content, and pH). In contrast, ascorbic acid was strongly correlated with quality attributes and antioxidant activity. Yield was negatively correlated

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with ascorbic acid, dry matter, and pH and weakly correlated with soluble solids content.

Yield and antioxidant activity were not at all correlated.

	AA	TPC	ABTS	DPPH	DM	SSC	pН
AA							
TPC	0.11 (NS)						
ABTS	0.45 (***)	0.47 (***)					
DPPH	0.83 (***)	0.30 (*)	0.52 (***)				
DM	0.81 (***)	0.12 (NS)	0.44 (***)	0.86 (***)			
SSC	0.57 (***)	0.21 (*)	0.30 (**)	0.69 (***)	0.79 (***)		
pН	0.66 (***)	-0.05 (NS)	0.08 (NS)	0.61 (***)	0.66 (***)	0.48 (***)	
Yield	-0.36(***)	-0.004 (NS)	-0.16 (NS)	-0.18 (NS)	-0.36 (***)	-0.19 (*)	-0.46(***)

Table 3.15. Correlation matrix of r (Pearson correlation coefficient) values

NS, \*, \*\*, \*\*\* Nonsignificant or significant at  $P \le 0.05, 0.01, 0.001$ , respectively.

# Discussion

Although nutrient concentrations in crops are expressed on a dry weight basis and considered as more relevant to use when comparing the effect of production system on plant composition (Caris-Veyrat et al., 2004), we expressed the level of ascorbic acid, total phenolic content, and antioxidant activity on a fresh weight basis. Our results showed that the dry matter content of tomatoes whether grown conventionally or organically is not statistically different; thereby it is reasonable to compare accurately the nutritional value of the ten commercial cultivars on a fresh weight (FW) basis.

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In this study, the influences of genotype, production system and year, as well as their complex interactions on antioxidant content and free radical scavenging capacity, were investigated in ten commercial cultivars grown using organic and conventional production systems for two years. Year to year variability is evident since ascorbic acid and antioxidant activity was higher in 2006 than 2005. Fruit quality attributes such as dry matter, pH, and soluble solids concentration were likewise significantly higher in 2006 than 2005. Weston and Barth (2002) stated that atmospheric environmental conditions including temperature and light intensity are unmanageable in field production but they have important implications in crop quality and nutritional value. In addition, Lee and Kader (2000) stated that when looking at temperature effect, total available heat and the extent of minimum and maximum temperatures are the most important factors in determining chemical composition of horticultural crops.

In our study, environmental factors such as temperature and solar radiation data were examined in both years. From planting to harvest, fluctuations in solar radiation are almost similar in 2005 and 2006. Upon examination of temperature and heat accumulation effects expressed as growing degree days (GDD) for a 30-day period before harvest, slightly higher GDD was observed in the first 17 days in 2005 than in 2006; but in the last 13 days before harvest in both years, GDD were the same. However, when we looked at the GDD from planting to harvest, more heat was accumulated in 2006 than 2005. Increase in ascorbic acid and antioxidant activity of tomato samples in 2006 could be partly attributed to higher temperature and heat accumulation. Tomatoes may have been exposed to stress leading to production of reactive oxygen species (ROS) and increased action of antioxidants to scavenge ROS. However, we cannot entirely relate the

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increase in ascorbic acid and antioxidant capacity to temperature and solar radiation because we did not measure the exact level of stress experienced by tomato plants in the field nor their interactions with other environmental and production variables and also not discounting the effect of genotype. Some greenhouse studies have been done that examined the effect of temperature and solar radiation on antioxidant properties. Liptay et al., (1986) have shown that increased day temperature from 24 to 31°C can increase vitamin C content. Rosales et al., (2006) reported that when higher temperature and solar radiation were beyond the optimum level in the greenhouse and the plants were subjected to stressful conditions, there was an increased oxidation of reduced ascorbic acid by ascorbic acid peroxidase and increased capacity of active oxygen species (AOS) detoxification but they clarified that this could not be related to nutritional quality since this was accompanied by the reduction in lycopene content. Gautier et al., (2005) obtained a different result. Their team showed that there was a reduction in accumulation of vitamin C, lycopene, and β-carotene under increased temperature but these effects were confounded and became more complex when fruit load or fruit position was taken into account.

In terms of temperature and solar radiation effects on quality attributes, Riga et al., (2008) explained that their influence on tomato fruit quality are not yet well established and still being debated because of interactions with physiological factors like source-sink balance. In addition, Anza et al., (2006) reported that the effect of environmental variables on the quality of hydroponically grown tomatoes is cultivar-dependent.

Genotype played the most important role in influencing all parameters that we evaluated. A 3-fold difference in the levels of ascorbic, total phenolic content, and free radical scavenging capacity was observed among cultivars. Our results agreed with other studies that evaluated the inherent variation in antioxidant properties of tomato cultivars. Toor et al., (2005) reported that the antioxidant activity in four New Zealand cultivars ranged from 2329 to 3268 umole TEAC 100 g-1 dry matter in the hydrophilic extracts and from 178 to 303 µmole TEAC 100 g-1 dry matter in the lipophilic extracts. Lenucci et al., (2006) also reported that different cherry and high pigment tomato cultivars are significantly different in terms of ascorbic acid, total phenolics, flavonoids and hydrophilic and lipophilic antioxidant activities. Moreover, a study by George et al., (2004) found significant differences in lycopene, ascorbic acid, phenolic contents, and antioxidant activity among the 12 genotypes. Ascorbic acid and lycopene showed 1-2 fold and 1-4 fold variation on both dry and fresh weight basis. Likewise, Spencer et al., (2005) reported genotypic variation in 37 tomato cultivars for their level of rutin, a flavonol that contributes greatly to the antioxidant capacity of tomatoes. Fruit quality attributes such as pH, soluble solids concentration, and dry matter content of cultivars varied by 1, 2, and 3 fold, respectively. Bhatt et al., (2001) reported that significant differences in terms of soluble solids were observed among the genotypes evaluated. Likewise, Garcia and Barrett (2006) also reported large variations in quality attributes including pH and soluble solids when they examined six selected tomato genotypes.

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Compared to genotype and year, production system had less effect on antioxidant content and activity. In our study, ascorbic acid and total phenolic content was observed to be slightly higher but not statistically significant in organic production than in

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conventional production (P<0.10). The antioxidant activity measured using  $ABTS^{++}$ radical cation assay is statistically higher in organically grown than conventionally grown tomatoes (P=0.002). This increase in antioxidant capacity may be partly attributed to high ascorbic acid and total phenolics of tomatoes grown in an organic production system. Kaur et al., (2004) stated that antioxidant potential of tomato is due to the presence of antioxidant biomolecules like ascorbic acid, vitamin E, phenolics, flavonoids and lycopene. Ascorbic acid and total phenolics significantly correlated with the antioxidant capacity with values of r=0.45\*\*\* and r=0.47\*\*\*, respectively (Table 3.15). Expressing these correlation coefficients as  $r^2$ , ascorbic acid, and total phenolic content contributed 20 and 22%, respectively to the antioxidant capacity. Other antioxidant compounds that had possibly contributed to more than half of the antioxidant capacity of tomatoes may be lycopene and B-carotene contributing 80-90% and 7-10% of the total carotenoid content, respectively (Gould, 1974; Nguyen et al., 1999). Carotenoids are known contributors to antioxidant capacity of tomatoes particularly lycopene which has the highest physical quenching rate constant with singlet oxygen among other carotenoids that have been studied (Di Mascio et al., 1989).

Several production system comparison studies have been carried out to determine if organic production can increase the level of phytochemicals with antioxidant properties in tomatoes. Chassy et al., (2006) reported that cropping system significantly influenced the level of ascorbic acid on a fresh weight basis but not the total phenolics (both on fresh and dry weight bases). Moreover, they determined that the level of ascorbic acid and total phenolics are cultivar-dependent and variable from year to year. In another study conducted by UC Davis researchers for 10 years (1994-2004), higher levels of flavonoid aglycones (quercetin and kaempferol) were observed in organic than conventional dried tomatoes. Some studies have obtained a different result. The World Vegetable Center had carried out an on-farm study /trial on 10 matched pairs of organic and conventional farms in Taiwan to evaluate influences of production system on tomato fruit quality. When farms were aggregated by type, there were no significant differences between organic and conventional production systems for nutritional parameters but when matched pairs of farms were evaluated as individual case studies, there were significant differences between two pairs of organic and conventional farms for antioxidant content and quality attributes. Inconsistencies in these results imply that production system effects on antioxidant content of tomatoes were not uniform and therefore it is difficult to arrive at a valid conclusion.

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The effect on quality attributes varied by production system. In our study, dry matter and pH were not significantly influenced by production system. However, for soluble solids content, higher SS was observed in conventionally grown than organically grown tomatoes. Results of McCollum et al., (2005) indicated otherwise; little difference was observed when soluble solids concentration of organic and conventionally grown tomatoes in Florida was compared. On one hand, Barrett et al., (2007) found that juice from organic tomatoes has higher soluble solids than conventionally grown ones; however, their results were not very conclusive since this study was done on four farms with different soil types and nutrients, cultivars, environmental conditions and production related factors.

Since genotype significantly influenced the antioxidant properties of cultivars and some tomato cultivars have performed better in either production system or year, an antioxidant index is being proposed that will assist consumers and producers in choosing cultivars with higher antioxidant quality. This index integrates the overall antioxidant potential of tomato cultivars by combining the ascorbic acid and total phenolics representing the antioxidant content and taking the average of TEAC values obtained from DPPH and ABTS assays to represent the antioxidant activity. The antioxidant index is computed as follows:  $A_{index}$ = ascorbic acid + total phenolics +antioxidant capacity/3. This is a unitless index intended to assist in ranking cultivars based on several important antioxidant criteria carried out using published reliable laboratory assays.

'Jet Star' had the highest antioxidant index (AI) followed by 'New Girl', 'Fantastic', and 'First Lady' (Fig. 3.12). AI of 'Jet Star' is significantly different from the other six cultivars namely 'Celebrity', 'Big Beef', 'Red Sun', 'Husky Red', 'Roma', and 'Early Girl' (Table 3.16). These results suggest that careful selection of cultivars for enhanced antioxidant properties should be an integral part of the decision making process that has to be made by producers in each cropping season. Producers could place higher price premium for tomatoes marketed or promoted as cultivars with higher nutritional value. Moreover, in the future, seed producing companies may consider the possibility of incorporating antioxidant quality parameters in their cultivar trials and indicate in their information booklets the cultivars, which are found to be nutritionally superior.

For breeders who are interested in selecting and characterizing tomato germplasm resources into high, intermediate or low antioxidant quality, Frusciante et al., (2007) proposed an index of nutritional quality (I<sub>QUAN</sub>) based on their analysis of several advanced breeding lines and open pollinated varieties of tomatoes and a literature survey on tomato composition. This index is computed as:  $I_{QUAN} = \sum_{x} C_x K_x/C_{opt}$  where  $C_x$  is the

concentration of the component in the sample;  $C_{opt}$  is the optimal concentration of the component and  $K_x$  is the coefficient of relative weight of the component.



Figure 3.12. Antioxidant index [( $\Sigma$ Vit C+TPC +Antioxidant capacity)/3] of ten tomato cultivars (Bars = Mean & S.E.M.).

Table 3.16. Antioxidant	index of ten	tomato cultivars	
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Cultivar	Antioxidant in	dex	
Jet Star	98.87	a	
New Girl	96.06	ab	
Fantastic	91.68	abc	
First Lady	90.79	bcd	
Celebrity	80.50	bcd	
Big Beef	79.73	bcd	
Red Sun	77.82	cd	
Husky Red	74.95	cd	
Roma	71.85	d	
Early Girl	67.08	d	

Means are significantly different at P< 0.05 based on Duncan's Multiple Range Test.

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Relationships between yield and antioxidant properties and quality attributes were variable. Yield and ascorbic acid were strongly negatively correlated. When pH and dry matter were correlated with yield, the same result was obtained. Weak correlation with yield and soluble solids concentration was observed. In a comprehensive review carried by Stevens et al. (1986), on inheritance of tomato fruit quality components, he pointed out the negative correlation between yield and soluble solids concentrations acond ascorbic acid. Davis et al (2004) reported that tradeoff between yield and nutrient concentrations could occur because of genetic dilution effect. Nutrient decline may result from decades of selecting for one trait such as high yield, thereby leaving less resource available for other functions like the crop's capacity to synthesize vitamins, minerals and other nutrients (Davis, 2005).

The complex interaction among production system, cultivar and year should be taken into account to evaluate effectively the different factors that may influence antioxidant properties and fruit quality attributes. One of the critical considerations is the experimental design that will take into account accurate replication and randomization to minimize the effects of confounding variables when we are trying to examine the effect of a specific factor. Considerable attention should also be placed on the observed genotypic variation among cultivars in terms of their antioxidant properties. This information is of particular importance to breeders, producers and consumers.

#### **CHAPTER IV**

#### GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

#### **General Conclusions:**

1.) Genotype highly influenced the antioxidant properties of melons and tomatoes. A 3 to 6 fold difference in the ascorbic acid, total phenolic content, and antioxidant activity suggest a wide diversity in the available antioxidant compounds among cultivars. The large variation for antioxidant content and activity for melons and tomatoes represents untapped resources for nutritional quality improvement. Breeding programs for decades or centuries have focused on yield, biotic and abiotic stress resistance, shelf life, taste, and improved fruit morphology resulting in genetic bottleneck or narrowing of diversity for these traits. Characterization, evaluation, and development of cultivars for nutritional quality have been left behind and just slowly catching up at the turn of this century backed up by increasing awareness of possible health benefits from fruits and vegetables.

2) Although antioxidant properties were also influenced by other factors aside from genotype, some melon and tomato cultivars showed more stability in different years and both production systems suggesting that they were more nutritionally superior to other cultivars. Nutritionally superior melon cultivars include 'Savor', 'Sweetie#6', 'Early Queen', 'Edonis', and 'Rayan' while 'Jet Star', 'New Girl', 'Fanstastic', 'First Lady' and 'Celebrity' were nutritionally superior tomatoes. This finding would imply that getting the utmost benefit from growing or consuming a tomato or melon lies in the choice of

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cultivars. A paradigm shift could occur if producers and consumers will be made aware that not all tomatoes or melons are the same. The antioxidant index that we have developed could serve as a tool in measuring and ranking the nutritional superiority of commercial cultivars. Vegetable breeding and seed companies may opt to include antioxidant content as an evaluation criterion in their cultivar trials. In the future, it would be a good idea to place a cultivar under different categories such as high, intermediate, or low antioxidant-containing cultivar if it is not possible to put a range of values for antioxidant compounds present in the cultivar.

**3)** Compared to genotype and year, production system had less influence on antioxidant properties and fruit quality attributes. However, there is a potential for organic production to be used as a strategy for enhancing antioxidant properties since cultivar and production system interaction was evident. Some melon and tomato cultivars have higher ascorbic acid, total phenolic content, and free radical scavenging capacity when they were grown in organic than conventional production system.

**4)** Comparison of the effect of whole agricultural production system (organic vs conventional) on antioxidant properties, fruit quality attributes and yield was associated with complex interactions between genotype and year (environment). Genotypic and year-to-year variability was minimized by using proper replication and randomization and standardized production and postharvest practices. Moreover, as a recommendation, a long-term study similar to the Long Term Research Agricultural System Project similar to UC Davis could be initiated to fully and completely assess the effect of whole production system over time and different environmental conditions.
**5)** Yield is negatively correlated with ascorbic acid and antioxidant activity (using the DPPH radical cation assay) both in melons and tomatoes. Moreover, some nutritionally superior melon and cultivars have low yield suggesting a possible trade off between yield and nutrient content.

6) Correlation between the two assays (ABTS and DPPH radical cation assays) may be strong or weak depending on the crops being tested for free radical scavenging capacity. These two assays were strongly correlated when used in tomatoes but not in melons. This finding emphasized the importance of using at least two or more assays when measuring antioxidant activity of crops.

## **Recommendations for future studies:**

1. Since tomatoes and melons are rich in lycopene and  $\beta$ -carotene, respectively, an analysis of the carotenoid content (using reversed phase HPLC) will provide significant information on the antioxidant/free radical scavenging capacity of different cultivars of these crops.

**2.** A follow up study could be carried out to determine the individual phenolic compounds present in tomatoes and melons using gas chromatography-mass spectrometry (GC-MS) and evaluate the contribution of each phenolic compound to the total antioxidant activity of a tomato or melon sample.

**3.** At present, research on the development of organic fertilization regimes for greenhouse tomatoes is on-going. This is an interesting prospect because of a possibility of comparing the antioxidant properties and fruit quality attributes of organically and conventionally produced greenhouse tomatoes in the future. It would be easier to

minimize the year effect since environmental conditions such as temperature and solar radiation could be manipulated.

4. Organic production system has been considered as a strategy to improve soil ecology on a long term basis. A study on the development of a method for accurate and fast measurement of soil microbiological activity in organic and conventional fields is recommended. Moreover, it is also possible that a study on the effect of different types of cover crops on microbiological activity and antioxidant properties of tomatoes and melons could be carried out in the future.

## CHAPTER V

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