AN ABSTRACT OF THE THESIS OF

Todd F. Dalotto for the degree of Honors Baccalaureate of Science in Horticulture presented on February 8, 2010.
Title: Variation of Phenolics in Anthocyanin- and nonanthocyanin- fruit tomatoes.

Abstract approved: ______________________________________________________

James R. Myers

Phenolic compounds are known to have biological activity with beneficial effects on human health. Fruit of cultivated tomatoes (Solanum lycopersicum L.) are a major source of phenolics in the U.S. diet because this crop is the second most consumed vegetable per capita, but actual levels are low compared to other fruits and vegetables. Anthocyanins are an important class of phenolic phytonutrients known for their antioxidant properties and color. Tomato fruits do not normally possess anthocyanins, but we have developed lines which express substantial quantities in the fruit. We wished to quantify levels of total phenolics and anthocyanins in anthocyanin expressing tomato fruit in different production systems and compare to that of normal tomatoes. Epidermis and pericarp tissue of three advanced breeding lines of anthocyanin-fruit tomatoes and three lines of nonanthocyanin-fruit tomatoes, both grown in organically and conventionally managed systems, were assayed for total phenolics and total anthocyanin content. The mean phenolic concentration of the highest-ranking anthocyanin-fruit line was 73 mg/100g FW and was significantly greater than that of the highest-ranking nonanthocyanin-fruit line (43 mg/100g FW). The highest anthocyanin concentration was 9.7 mg/100g FW. Total phenolics in tomatoes from the organic production system was not significantly different from that of a conventional production system.

Keywords: Tomato, phenolics, anthocyanins, antioxidant, organic
Corresponding email: dalotto@lifetime.oregonstate.edu
Variation of Phenolics in Anthocyanin- and Nonanthocyanin- Fruit Tomatoes

by

Todd F. Dalotto

A PROJECT

Submitted to

Oregon State University

University Honors College

and Department of Horticulture

in partial fulfillment of the requirements for the degree of

Honors Baccalaureate of Science in Horticulture, Research Option (Honors Associate)

Presented February 8, 2010
Commencement June 12, 2010
Honors Baccalaureate of Science in Horticulture project of Todd F. Dalotto presented on February 8, 2010.

APPROVED:

Mentor, representing Horticulture

Committee member

Committee member

Chair, Department of Horticulture

Dean, University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Todd F. Dalotto, Author
ACKNOWLEDGMENTS

I gratefully acknowledge my daughter, Ophelia, for her love, inspiration, and patience while Dad worked long hours to work on research and get through school. I thank my partner, Sara for her love, support, and patience through the past year. I am also fortunate to have the support and encouragement from my parents, Rosemary and Frank. I thank my mentor, Dr. Jim Myers for a great research experience and for all his help and guidance throughout my research project. Peter Boches provided instruction on HPLC, spectrophotometry, other lab assays, and provided a wealth of technical support. Without Peter, this project wouldn't have been possible for me. Thanks to Miles Barrett, Shawna Zimmerman, and Joel Davis for help in the lab, in the field, and with technical feedback. I appreciate Deborah Kean for her excellent leadership at the Vegetable Farm and for making field work enjoyable. High-fives to the 2008 Vegetable Breeding Program Farm crew, Miles Barrett, Shawna Zimmerman, Charlie Boches, Nick Failing, and Antoine Farmin for their good field work and fun spirit. Thanks to Scott Robbins and Randy Hopson for their excellent management of the research farms. I thank Patti Skinkis and members of her lab, Levi Frederickson and Sunny Lucas for use of their lab for HPLC analysis and -80°C freezer. My academic advisors, Kelly Donegan and Rebekah Lancelin have been tremendously supportive throughout my time at OSU. I thank department head, Anita Azarenko for her encouragement, humor, and for driving the Horticulture Department in a sustainable direction. I am thankful for Stephanie Duckett and ASOSU’s Student Parent Advisory Board for their support of my difficult task of parenting while going to school.
This research was funded by the OSU Vegetable Breeding Program, Baggett-Frazier Vegetable Breeding Endowment, and the E.R. Jackman Internship Support Program. My education on a whole wouldn't be possible without the support of the following scholarship providers: CHS Foundation, Grow and Show, Jim & Dee Davis, Native Plant Society of Oregon, General Dillingham Produce Industry Scholarship, National Garden Clubs, American Society for Horticultural Science, Oregon Community Foundation, Oregon Seed Trade Association, American Seed Trade Association, OSU Foundation, Claire Hanley Scholarship, Grandma Honors, and federal financial aid.
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DEDICATION

This thesis is dedicated to my daughter, Ophelia Rose who made the greatest sacrifice for this research project. When I was young, my father tried to explain to me that his long hours at work are for the good of the family. I didn't understand this until I became a parent.
Variation of Phenolics in Anthocyanin- and nonanthocyanin- fruit tomatoes

Introduction

The center of origin of Tomato (*Solanum lycopersicum*) (2n = 2x = 24) is South America and Mexico. It was then domesticated in Mexico (Simpson, Ogorzaly, 1986), and is now grown in most parts of the world as an annual warm weather and greenhouse crop.

The array of colors of tomato fruit is derived largely from organic compounds which are also known to have biological activity with beneficial effects to human health. The most common tomato fruit colors are red, yellow, and orange, which are derived from the carotenoids lycopene, Δ-carotene, and β-carotene, respectively. Carotenoids are members of the terpenoid class of compounds and are different from phenolics that are the subject of this study.

Phenolics are a large class of organic compounds that are composed of at least one aromatic ring with a hydroxyl group bonded to an unsaturated carbon and are mainly products of the phenylpropanoid pathway. Such compounds with more than one phenol ring are called polyphenolics. Phenolics have many functions in plants including defenses against pests, pathogens, and UV radiation. In the human diet they are strong antioxidants and may serve to reduce the risk of chronic diseases by preventing oxidative damage to DNA, lipids and proteins (Hollman, 2001). There is great interest in increasing levels of polyphenols in our diet because of the wide range of health benefits they offer.
Fruit of cultivated tomatoes are considered a major source of phenolics in the U.S. diet because this crop is the second most widely consumed vegetable on a per capita basis (Economic Research Service, 2008), but actual levels are low compared to other fruits and vegetables. For example, tomato has about 1/10 the total phenolics of garlic by fresh weight (FW), but people eat more tomatoes than garlic and therefore have twice the daily consumption of phenolics from tomatoes compared to garlic in the US (Vinson, et al., 1998).

Anthocyanins are an important class of polyphenolic phytonutrients known for their antioxidant properties and novel colors. They are the common red, purple and blue pigments that occur in all types of plant tissue and are largely responsible for the attractive colors of blueberries, cranberries, cabbage, and many other fruits and vegetables. Although anthocyanins are often produced in tomato vegetative tissues, the fruits of cultivated tomatoes do not normally possess anthocyanins. Some wild tomato relatives express anthocyanins in their fruit skin, and this trait has been transferred into cultivated tomato lines by traditional breeding methods (Rick, 1994). Oregon State University Vegetable Breeding Program has developed lines which express up to 80 mg/100g FW anthocyanins in the fruit skin¹.

The genes that control anthocyanin expression in tomato fruit are the dominant Aft (Anthocyanin fruit), and recessive atv (atroviolaciun) genes, located on chromosomes 10 and 7, respectively (Rick, et al., 1968). The OSU breeding lines used in this study were developed by crossing genetic stocks which had Aft and atv backcrossed separately into

¹ This value is not intended for comparison of values presented in this study. Samples in this study included pericarp tissue in addition to skin, which produced anthocyanin concentrations far lower than those from samples of fruit skin only.
cultivated tomato lines. *Aft* was derived from *S. chilense* and *atv* from *S. cheesemanii* (Jones, et al., 2003).

Anthocyanin expression is photoactivated with production in the cytosol and storage in vacuoles (Tanaka, et al., 2008; Schwartz, et al., 2008). Carotenoids are stored in plastids throughout the fruit, and in anthocyanin-fruit tomatoes, their expression is obscured when anthocyanins are expressed in the epidermis and outer pericarp. Therefore, carotenoid pigments are visible mainly in shaded areas of the fruit, and skin that is covered by the calyx.

In addition to the development of anthocyanin fruit tomatoes through introgression of genes from related wild species, anthocyanin in tomato fruit has also been introduced using transformation. In this case, anthocyanins in a genetically-modified tomato under development in England are expressed throughout the fruit (Butelli, et al., 2008). The genes which control anthocyanin expression were transferred from a snapdragon plant. Genetically-modified tomatoes are not included in this study.

The heirloom cultivars with ‘black’ or ‘purple’ in their cultivar name are not pigmented by anthocyanins, nor can their colors be accurately described as 'black' or 'purple.' Rather, their color is derived from red and pink carotenoids mixed with brownish pheophytin. The *green flesh (gf)* gene prevents normal chlorophyll breakdown during ripening and thus produces pheophytin (Mes, et al., 2008). The heirloom cultivar ‘Black Prince’ with *gf* was selected as a nonanthocyanin control for comparison of total phenolics in this study. In addition, two red/nonanthocyanin tomato cultivars were selected as controls in this study to compare with OSU anthocyanin-fruit breeding lines for total phenolics.
Organically produced foods are becoming increasingly popular because they are perceived to be more nutritious, environmentally friendly, socially just, and better tasting compared with conventionally produced foods. However, research is not conclusive on the influence such cultural practices have on nutritional differences. Many studies supporting both sides are available in literature. In addition to increasing phenolics in crops through genetic means, there is great interest in doing so by optimizing cultural practices. The fundamental differences between organic and conventional agriculture and the popularity of each provide a good basis for studying their cultural and biochemical differences.

**Research objectives.** The main objectives of this research were to evaluate three known nonanthocyanin-fruit cultivars and three anthocyanin-fruit breeding lines of tomato for total phenolics, anthocyanin concentration, and characterization of important phenolic compounds. The aim is to select one anthocyanin-fruit line for release based upon concentration of phenolics and anthocyanins and checking against the known nonanthocyanin-fruit cultivars. Other horticultural characteristics such as yield, flavor, and disease resistance are also important considerations for the released cultivar and have been evaluated outside of this study. To test the variation of phenolics due to cultural practices along with that due to genetic variation, a comparison of total phenolics and anthocyanins produced in organic systems vs. conventional systems was another objective of this study.
Expected results and significance. For the tomato trial, the null hypothesis was that each tomato line would show a similar profile of chlorogenic acid, rutin, and naringenin. If experimental observations contradict the null hypothesis, the alternative hypothesis may be that anthocyanin-fruit type tomato lines are higher in phenolic acids than non anthocyanin-fruit type cultivars (in addition to uniquely possessing anthocyanins). Higher levels of other phenolics are predicted because *Aft* and *atv* are putative regulatory genes that potentially up regulate the whole biosynthetic pathway. For the production systems comparison, the null hypothesis is that no differences in phenolic acid and anthocyanin profiles between organic and conventionally-grown fruits will be observed. If significant differences are discovered, an alternative hypothesis may be that such differences are due to environmental interaction with genetic control of phenolic acid biosynthesis.
Materials and Methods

Plant materials. Six lines of tomatoes were selected for evaluation. Three are nonanthocyanin-fruit type cultivars: 'Early Girl' (red fruit), 'Siletz' (red fruit), and 'Black Prince' ('black' (gf) fruit). The other three, P20-3-1, P20-3-2, and P20-4, are breeding lines of anthocyanin-fruit tomatoes from the Oregon State University Vegetable Breeding Program and are candidates for release. Their genotype is inferred to be $AftAft/atvatv$ (Boches, 2009). All materials were highly inbred pure lines with the exception of 'Early Girl,' which is an F1 hybrid (Tracy, 1998).

Plant growth. Tomato plants evaluated in this study were grown outdoors during the summer of 2008 at Oregon State University's Lewis-Brown Farm on two test fields, one managed organically, and the other conventionally. For each field, three replications of five plants per plot were arranged in a randomized complete block design. Seeds were sown in the greenhouse on April 14, 2008 in Sunshine SB40 professional growing mix (Sun Gro Horticulture, Bellevue, WA) in 5-cm-diameter plastic transplant cells. Seedlings were transplanted outdoors on June 4, 2008 into rows 0.91 m apart with 60 cm within-row spacing. Five hundred and five kg·ha$^{-1}$ of 12N-29P-10K-4S fertilizer was banded before transplanting in the conventional plot, and 814 kg·ha$^{-1}$ of BioGro 8N-5P-3K (BioOregon, Warrenton, OR) fertilizer was banded in the organic plot two weeks after transplanting. Plants were irrigated at weekly intervals until mid-August when water was withheld. Both plots were maintained using standard cultural practices for fresh market production and copper hydroxide fungicide (Kocide; Griffen L.L.C.)
Valdosta, GA) was applied at label rates late in the growing season for prevention of late blight (*Phytophora infestans*).

*Tissue extraction.* Two fruits representative of average size, color, and ripeness were selected for analysis from each of the plots at approximately four weeks after first ripe date. A longitudinal slice of epidermis and pericarp tissue, excluding seeds, placenta, and core, approximately 5-7 mm wide was made for each sample. Slices from anthocyanin-fruits included both shaded areas (lower anthocyanin expression) when present and those that received full sun (higher anthocyanin expression). Samples were preserved at -80°C. Samples were frozen in liquid nitrogen and ground to a fine powder using a mortar and pestle. 1000 mg of powder was immediately extracted in 3 mL of acidified methanol (1% HCl) at 4°C in 15 mL glass tubes. 2.0 mL of deionized H$_2$O and 5.0 mL chloroform were added to the extraction, and centrifuged at 2,900 rpm for twelve minutes. The aqueous upper layer was transferred to 2.0 mL Eppendorf tubes and centrifuged at 13,000 RPM for 6 minutes to achieve a more complete separation. The aqueous supernatant was transferred to 2.0 mL Eppendorf tubes and stored at -20°C for the following analyses (Boches, personal communication describing lab techniques for analyzing phenolics and anthocyanins, 2008).

*Folin-Ciocalteau analysis.* For measuring total phenolics, Folin-Ciocalteau assays were performed according to the method of Singleton and Rossi (1965) and Waterhouse (2005) with modifications. Reactions were performed on a microscale: each containing 198 μL nanopure H$_2$O, 2.5 μL sample or gallic acid standard, 12.5 μL Folin-
Ciocalteu reagent (Sigma Chemical Corp., St. Louis, Mo.), and 37.5 NaCO₃ solution. Reactions were incubated for one hour at room temperature, and absorbance at 765 nm was read using a SPECTRAmax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA). Folin-Ciocalteu values were expressed as gallic acid equivalents (GAE) based upon a gallic acid standard curve in the range of 0-1000 mg·L⁻¹ and reported as mg/100g fresh weight (FW).

**PH differential analysis.** To measure monomeric anthocyanin content, pH differential assays were performed according to Giusti and Wrolstad (2005) using a SPECTRAmax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA). Two dilutions were made of each sample, one with potassium chloride buffer, pH 1.0, and the other with sodium acetate buffer, pH 4.5, diluting each by a 1:4 dilution factor. Dilutions were equilibrated for 1 hour at room temperature. Absorbance of each dilution was observed at 540 nm and at 700 nm. The monomeric anthocyanin pigment concentration was calculated with molecular weight (MW)=934 and a molar absorptivity (ε) of 17000, corresponding to petunidin-3-(p-coumaryl)-rutinoside-5glucoside in acidified methanol (Price and Wrolstad, 1995), which has been reported to be the predominant anthocyanin in tomato fruit (Mes et al., 2008; Boches, 2009). Values were expressed as mg/100g FW.

**HPLC analysis.** To characterize phenolic compounds, high performance liquid chromatography (HPLC) assays were performed. Buffers were filtered with 0.5 µM MPS membrane filters and nanopure water with 0.45 µM Tuftryn membrane filter.
Samples were filtered with 0.45 µM Acrodisc LC13 membrane syringe filters (Pall Corp., Port Washington, NY). A HP 1040 photodiode array detector (HPLC-PDA) coupled to a Hewlett-Packard (Palo Alto, Ca) 1050 series autosampler and pump were used. An ES Industries (West Berlin, NJ) reversed phase LiChrospher RP18 endcapped column (25 cm x 4.6 mm, 5 µM particle size) was used with a guard column of the same material. The separation gradient and buffers followed that of the tomato metabolome database (Moco, et al., 2006). Solvent A was formic acid:water (1:1000). Solvent B was formic acid:acetonitrile (1:1000). The flow rate was 1.0 mL·min⁻¹, and the injection volume was 40.0 µL. The elution conditions were as follows: 0-45 minutes, linear gradient from 5% B to 35% B; 45-50 minutes, isocratic at 85% B to wash the column; 50-60 minutes, isocratic at 5% B to equilibrate the column. Peak spectra were monitored at 280 nm. UV spectra were recorded from 190 to 600 nm in 0.5 nm intervals. HPLC data were analyzed using Agilent ChemStation Rev.A.09.01 (Santa Clara, CA). Standards were used in the range of 50-500 mg·L⁻¹ for each compound. Twelve samples were analyzed, one from each line grown in the organic system, and one from each line grown in the conventional system. Samples were selected from reps with phenolic and anthocyanin concentrations closest to the mean of its respective line.

**Statistical analysis.** SAS software (Cary, NC) was used for analysis of variance and means separation using Fisher's Least Significant Difference (LSD) using a 95% significance level, except where specified.
For total phenolics and anthocyanin concentration, values were calculated as means of all replicates for each line across production systems as determined by Folin-Ciocalteau and pH differential assays, respectively (n=35).

Non anthocyanin phenolics were obtained by the difference between the total anthocyanins as determined by the pH differential method and total phenolics as determined by Folin-Ciocalteau assays. Data are means of all replicates of all lines for each fruit type. Statistical analysis and 2-sample t-test with a 95% confidence interval performed using Minitab 15 (State College, PA) (n=35).

For characterization of selected phenolics and sum of 'other' phenolics by HPLC absorbance, values were calculated as means of two replicates of each line (one replicate from each production system; n=12).

For production system comparison of total phenolics, values were calculated as means of all replicates of all lines within each production system (n=35). For production system comparison of selected and 'other' phenolics, values were calculated as means of one replicate of all lines within each production system (n=12).
Results

*Total phenolics.* Total phenolics were measured by Folin-Ciocalteau assay on 3 replicates of each line averaged over organic and conventional production systems. Phenolic concentrations of two of the anthocyanin-fruit breeding lines, P20-3-1 (\(\bar{x}=68.4\) mg/100g FW) and P20-3-2 (\(\bar{x}=73.2\) mg/100g FW) were significantly higher than those of all other lines (Figure 1, \(P<0.05\)). Phenolic concentration of the third anthocyanin-fruit breeding line, P20-4 (\(\bar{x}=47.2\) mg/100g FW) was comparable to all three nonanthocyanin-fruit cultivars (\(P>0.05\)). Phenolic levels in P20-3-1 reps ranged from 61.1 to 74.8 mg/100g FW, while those of P20-3-2 ranged much more widely (53.9 to 117.0 mg/100g).

*Anthocyanin concentration.* Anthocyanin concentration measured by the pH differential method revealed that P20-3-1 (\(\bar{x}=9.7\) mg/100g FW) and P20-3-2 (\(\bar{x}=7.3\) mg/100g FW) were significantly higher than that of P20-4 (\(\bar{x}=2.9\) mg/100g) (Figure 2, \(P<0.0001\)). As expected, nonanthocyanin types showed no anthocyanins.

*Non anthocyanin phenolics.* Non anthocyanin phenolics were obtained by calculating the difference between total anthocyanins as determined by the pH differential method and total phenolics as determined by Folin-Ciocalteau assays. Non anthocyanin concentrations of anthocyanin lines (\(\bar{x}=57.0\) mg/100g) were significantly higher than that of non anthocyanin lines (\(\bar{x}=36.6\) mg/100g) (Figure 3, \(P=0.001\)).
Characterization of phenolics. Phenolic compounds were characterized by HPLC. Due to an error in the assay for naringenin we were not able to calculate an accurate standard curve for this compound. For this reason, the HPLC data were reported in peak area of milli-absorbance units by elution time (mAU*s) rather than in actual concentration (mg/100g FW) as was reported for the pH Differential and Folin-Ciocalteau assays. The data presented in peak area (mAU*s) is useful for determining what compounds are present or absent from the profile and provide an idea of the relative concentrations of compounds. The HPLC chromatograms (Figure 4) demonstrate the variability of selected phenolics (chlorogenic acid, rutin, and naringenin) and all other phenolic compounds between different lines and production systems by the variability in peak elution times and respective peak sizes. It can be seen in the chromatograms that the phenolic profiles of the same line can differ greatly between production systems. Standards were used to identify the three selected phenolic compounds and the peaks were labeled in each chromatogram.

Although differences in values for chlorogenic acid were not determined to be significant (P=0.06), it is worth noting the lines found to be highest in chlorogenic acid were P20-3-2 (365.1 mAU*s), P20-3-1 (274.5 mAU*s), and 'Black Prince' (165.1 mAU*s) (Table 1). Those lowest in chlorogenic acid were 'Siletz' (20.2 mAU*s), 'Early Girl' (54.3 mAU*s), and P20-4 (70.1 mAU*s). Although none of the lines were found to be significantly different in rutin concentration (P=0.22; Table 1), the values ranged widely from 69.8 mAU*s ('Siletz') to 367.2 mAU*s (P20-3-1). The concentration of naringenin was low (0 to 40.5 mAU*s) and did not differ significantly for any of the six lines (P=0.31; Table 1).
The sum of all phenolic compounds other than chlorogenic acid, rutin, and naringenin ranged between 1832 mAU*s ('Early Girl') and 4374 mAU*s (P20-3-2) and were not significantly different for any of the six lines (Table 1; P=0.7).

*Organic vs. conventional production systems.* Total phenolics in organic (\(\bar{x}=57.1\) mg/100g FW) and conventional (\(\bar{x}=47.8\) mg/100g FW) systems were not significantly different (Figure 5, P=0.62). Each of the means for chlorogenic acid, rutin, naringenin (P=0.21, 0.92, and 0.69, respectively), and 'other' phenolic compounds (P=0.67) did not differ significantly between production systems.

*Evaluate candidates for release.* P20-4 was excluded from candidacy for release due to having significantly lower total phenolics and anthocyanins compared to the other two anthocyanin-fruit lines (Figures 1 and 2). There was no significant difference between the levels of total phenolics and anthocyanins of P20-3-1 and P20-3-2 (Figures 1 and 2, P>0.05). Therefore, both P20-3 lines were equal candidates for release in terms of total phenolics and anthocyanins.
Discussion and Conclusions

In addition to genetic factors, the levels of phenolics in tomatoes vary widely depending upon the stage of maturation, growing conditions (Robbins, 2003), and sampling technique. The bias resulting from each of these factors was reduced by uniform cultural practices, replication, randomized complete block design, and uniform sampling technique as described in materials and methods.

Phenolics are not homogenously distributed throughout the fruit. Rather they are largely concentrated in the epidermal tissue of tomato fruit. It is reported that ninety eight percent of flavonols (the most abundant group of phenolics in vegetables [Kushad, et al., 2003]) detected in tomatoes were found to occur in the skin (Stewart, et al., 2000). Additionally, light quantity, particularly that of UV-B radiation, influences the concentration of phenolics since flavonol and phenolic concentration is increased in response to elevated light levels (Bieza and Lois, 2001).

Anthocyanin expression has been found to be photoactivated by visible light (Schwartz, et al., 2008; Dixon and Paiva, 1995) so its accumulation in tomato fruits is limited mainly to tissue exposed to adequate light, namely the epidermis and one or two outer cell layers of the pericarp (Figures 6 and 7). With all else being equal, smaller fruits have higher concentrations of anthocyanins and total phenolics due to their higher surface/volume ratio and decreased self-shading ability compared with larger fruits. An inverse-logarithmic relationship between total anthocyanin content and tomato fruit weight has been found (Mes, et al., 2008). For this reason sampling of fruits were based upon average size and light exposure for the plot it was harvested from.
Variations of light quality and quantity from year to year and between different geographic locations were likely to be responsible for some degree of variation in anthocyanin concentration of tomatoes grown in different years or locations. Therefore, the data presented in this study do not represent the minimum nor maximum potential phenolics produced by these tomato lines.

Open canopy habit and leaf curl, which are usually thought to be undesirable characteristics for tomatoes, were stable traits in the anthocyanin-fruit breeding lines in this study and work favorably for anthocyanin expression and flavonoid production without having observed the negative effects often associated with these traits, such as sunburn and ripening disorders.

The low anthocyanin concentration of the P20-4 line, as determined by pH differential assay, correlated with the observation of lighter purple coloration of both the fruit skin and extract compared with that of the two P20-3 lines with higher anthocyanin concentrations.

It was here that the two P20-3 anthocyanin-fruit breeding lines were higher in total phenolics than all the other lines in this study and produced up to 9.7 mg/100g FW anthocyanins. Because the anthocyanin fruit lines had higher average concentration of non anthocyanin phenolics than did nonanthocyanin-fruit cultivars (Figure 3), it was inferred that the increase in total phenolics of anthocyanin-fruit types was attributable to non anthocyanin phenolics in addition to anthocyanins.

Further characterization by HPLC analysis showed that the anthocyanin breeding lines tested were higher in some phenolics and lower in others compared with the nonanthocyanin cultivars (Table 1). Although there were only three peaks positively
identified and labeled on the HPLC chromatograms (Figure 4), there appeared to be much variation of peak heights and elution times for each of the samples, even between samples of the same fruit type. Because the sample size was small (n=12), significant differences in the concentrations of selected and 'other' phenolics were not determined when comparing between lines and between production systems. These data (Table 1) showed the P20-3 lines were higher in chlorogenic acid and rutin, and lower in naringenin compared with all the other lines, although data from a larger sample size is required to determine whether these differences were statistically significant.

Standards are necessary for quantifying compounds by HPLC. Standards were available for chlorogenic acid, rutin, and naringenin which aided in the identification of their respective peaks. Methanol extracts containing naringenin are known to be stable for several months at -20°C (Moco, et al., 2006). The extracts used in this study were stored at -20°C for less than a month, so they were assumed to be good sample material. Naringenin chalcone was observed by Moco, et al. (2006) to decay slowly into naringenin while in the autosampler at 20°C at the rate of about 1.4 μg g⁻¹ FW h⁻¹. Extracts were in the autosampler for no more than 11 hours before analysis, so any decay would not have been significant.

Environmental stresses, such as herbivory, pathogens, wounding, and UV radiation have been found to activate the biosynthesis of phenolics which function as plant defense mechanisms (Dixon and Paiva, 1995). Because pest management practices in organic systems favor the use of biocontrols and ecological management practices while those in conventional systems favor the use of chemical controls, organic crops tend to experience higher degrees of herbivory. This a common explanatory factor when
organically grown crops are found to be higher in phenolics. No insect pest problems were observed during the study, so no chemical or biological controls were applied to the test fields in either production system. Therefore, the level of herbivory and other environmental stresses experienced by tomato plants in both production systems was assumed to be equal.

Tomatoes can be grown on a wide variety of soils and prefer slightly acidic (pH 6.2-6.8) well-drained loamy-soil high in organic matter. Cultural practices for both organic and conventional systems vary widely depending upon region, climate, soil type, pests, and pathogens. Fertility management in conventional systems emphasizes use of soluble inorganic nutrients which are directly available to plants in the root zone. In contrast, organic systems emphasize a diverse soil ecosystem by building organic matter and soil fertility over time by cover cropping, adding compost, enhancing microbial symbioses, and using slow-release fertilizers derived from naturally-occurring plant, animal, and mineral sources.

Mitchell, et al. (2007) conducted a ten-year comparison on the influence of organic and conventional management practices on the flavonoid content in tomatoes where organic matter was added to the organic system annually. They found that the increase in flavonoids over time in the organic system correlated with increasing amounts of soil organic matter and with reduced available nitrogen.

The main shortcoming of this production systems comparison was that the organic test field in this study was not characteristic of organic farms as previously described. The organic test field was until three years prior to this study under conventional management practices, had been cover cropped similarly to the
conventional test field, and had no history of organic matter additions. The management practices of both the conventional and organic test fields were identical for this study with the sole exception of the type of fertilizer applied (total N kg⁻¹ ha⁻¹ was nearly equal for both systems). For this reason it is no surprise that no significant difference in phenolic content was found between tomatoes grown in the organic system and those grown in the conventional system. However, it has been shown here that with total nitrogen being equal in both systems, the level of phenolics in tomato fruits produced in a system amended with an organic fertilizer is not significantly different than that amended with a soluble inorganic fertilizer.

Furthermore, the test fields at Oregon State University's Lewis Brown Farm, being low in soil organic matter content, provide a good opportunity for a long term study that tests phenolics in crops over several years in a production system that is transitioning from conventional to organic management practices while gradually increasing soil organic matter.

In terms of anthocyanin and total phenolic content, P20-3-1 and P20-3-2 have been found to be equal top candidates to be selected for release (plant variety protection) and P20-4 has been excluded from candidacy. Naturally, many other horticultural characteristics that have been evaluated outside of this study have been considered. Both breeding lines have been found to be equal for traits such as mature plant size, morphology of leaf, flower and inflorescence. However, P20-3-2 has been found to have much variation in fruit size, number of locules in fruit (2 to many), and determinacy (indeterminate to semi-determinate). Because P20-3-1 has been found to be stable in all the above-mentioned horticultural traits (Dalotto, unpublished data, 2009), it has been
selected to be released. The Plant Variety Protection application will soon be submitted to the USDA Plant Variety Protection Office.
**Figure 1.** Comparison of anthocyanin and nonanthocyanin fruit tomatoes grown in organic and conventional production systems at the Lewis Brown Research Farm, Corvallis, OR in 2008 for total phenolics as determined by Folin-Ciocalteau assay. Each value is the mean of 3 replicates averaged over production systems. P20-3 lines show significantly higher levels of total phenolics compared to P20-4 and nonanthocyanin types. Bars with same letter are not significantly different as determined by Fisher's Least Significant Difference (P=0.05).

**Figure 2.** Comparison of anthocyanin and nonanthocyanin fruit tomatoes grown in organic and conventional production systems at the Lewis Brown Research Farm, Corvallis, OR in 2008 for anthocyanin concentration as determined by the pH differential method. The two P-20-3 lines show significantly higher anthocyanin concentration than the P-20-4 line as determined by Fisher's Least Significant Difference (P<0.0001). Nonanthocyanin types show no anthocyanin concentration.

**Figure 3.** Comparison of anthocyanin and non anthocyanin fruit tomatoes grown in organic and conventional production systems at the Lewis Brown Research Farm, Corvallis, OR in 2008 for anthocyanin concentration and total phenolics. Each value is the mean of 3 replicates of 3 lines averaged over production systems. Non anthocyanin phenolics obtained by the difference between total anthocyanins as determined by the pH differential method and total phenolics as determined by Folin-Ciocalteau assays. Anthocyanin lines show significantly higher average non anthocyanin phenolics than non anthocyanin cultivars, suggesting increased phenolics in anthocyanin lines are both anthocyanins and non-anthocyanin phenolics (as determined by 2-sample t-test with a 95% confidence interval, P=0.001).
Figure 4. HPLC chromatograms (280 nm) of anthocyanin-fruit (P20-3-1, P20-3-2, and P20-4) and nonanthocyanin-fruit ('Early Girl', 'Siletz', and 'Black Prince') tomatoes grown in organic and conventional production systems at the Lewis Brown Research Farm, Corvallis, OR in 2008. Units on y-axis: mAU at 280nm.
Figure 4. Continued...
Figure 4. Continued.
Figure 5. Comparison of anthocyanin and non anthocyanin fruit tomatoes grown in organic and conventional production systems at the Lewis Brown Research Farm, Corvallis, OR in 2008 for total phenolics as determined by Folin-Ciocalteau assay. Values are the mean of 3 reps per production system. Total phenolics produced in organic production system not significantly different compared to conventional system (P=0.62).
Figure 6. Morphology and distribution of anthocyanin cells in tomato fruit. Cross section of tomato fruit indicates anthocyanin expression is photoactivated and thus is only expressed in epidermis (darker purple pigmentation on left of photo) and outer pericarp (lighter purple in center of photo) of tomato fruit. Photo: Peter Boches. Scale bar is approximately 50μm.

Figure 7. P20-3-1 produced at the Lewis Brown Research Farm, Corvallis, OR in 2008 for analysis of anthocyanins and phenolics. Anthocyanins are expressed only in epidermis and outer pericarp of tomato fruit. Remainder of fruit and epidermis of shaded side is normally pigmented by carotenoids. Scale bar is approximately 1cm.
### Table 1. Comparison of anthocyanin and non-anthocyanin fruit tomatoes grown in organic and conventional production systems at the Lewis Brown Research Farm, Corvallis, OR in 2008 for characterization of phenolics as determined by HPLC. Characterization of phenolics by tomato line and production system in milli-absorbance units (mAU*s).

<table>
<thead>
<tr>
<th>Line</th>
<th>Chlorogenic Acid</th>
<th>Rutin</th>
<th>Naringenin</th>
<th>Other Phenolics</th>
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<tbody>
<tr>
<td>'Black Prince'</td>
<td>165.13</td>
<td>176.3</td>
<td>9.73</td>
<td>2635</td>
</tr>
<tr>
<td>'Early Girl'</td>
<td>54.27</td>
<td>117.8</td>
<td>3.64</td>
<td>1832</td>
</tr>
<tr>
<td>'Siletz'</td>
<td>20.19</td>
<td>69.8</td>
<td>40.54</td>
<td>2920</td>
</tr>
<tr>
<td>P20-4</td>
<td>70.1</td>
<td>197.8</td>
<td>13.67</td>
<td>2733</td>
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<tr>
<td>P20-3-1</td>
<td>274.48</td>
<td>367.2</td>
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<td>4144</td>
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<tr>
<td>P20-3-2</td>
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<td>312.5</td>
<td>0</td>
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<td>System</td>
<td></td>
<td></td>
<td></td>
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<td>Organic</td>
<td>195.98</td>
<td>203.67</td>
<td>13.35</td>
<td>2881</td>
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<td>Conventional</td>
<td>120.45</td>
<td>210.17</td>
<td>9.172</td>
<td>3332</td>
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References


