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Analysis of Tomato Carotenoids: Comparing Extraction and Chromatographic Methods

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Abstract

Background: Tomatoes (*Solanum lycopersicum*) are an economically and nutritionally important crop colored by carotenoids such as lycopene and β-carotene. Market diversification and interest in the health benefits of carotenoids has created the desire in plant, food, and nutritional scientists for improved extraction and quantification protocols that avoid the analytical bottlenecks caused by current methods.

Objective: Our objective was to compare standard and rapid extraction as well as chromatographic separation methods for tomato carotenoids.

Method: Comparison was based on accuracy and the ability to discriminate between alleles and genetic backgrounds. Estimates of the contribution to variance in the presence of genetic and environmental effects were further used for comparison. Selections of cherry and processing tomatoes with varying carotenoid profiles were assessed using both established extraction and HPLC–diode array detector (HPLC-DAD) methods and rapid extraction and ultra-HPLC-DAD (UHPLC-DAD) protocols.

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Results: Discrimination of alleles in samples extracted rapidly (<5 min/sample) was similar to samples extracted using a standard method (10 min/sample), although carotenoid concentrations were lower due to reduced extraction efficiency. Quantification by HPLC-DAD (21.5 min/sample) and UHPLC-DAD (4.2 min/sample) were comparable, but the UHPLC-DAD method could not separate all carotenoids and isomers of tangerine tomatoes. Random effects modeling indicated that extraction and chromatographic methods explained a small proportion of variance compared with genetic and environmental sources.

Conclusions: The rapid extraction and UHPLC-DAD methods could enhance throughput for some applications compared with standard protocols.

The tomato (*Solanum lycopersicum*), is an economically important and nutritious horticultural crop containing a range of nutrients, vitamins, micronutrients, and phytochemicals with potential health benefits (1, 2). Epidemiological evidence suggests that consumption of tomatoes and tomato products is associated with a reduced risk for development of prostate cancer and other chronic diseases; an outcome which is often ascribed to the presence of lycopene, the predominant pigment in red tomatoes (3–7). Another pigment found in tomatoes, β-carotene, is an important provitamin A carotenoid. Carotenoids are also responsible for the vibrant red and orange colors of tomatoes and tomato products which partly drives consumer acceptability (8).

In order to modulate tomato fruit carotenoid profiles, sources of natural variation needed to be identified and accurately characterized. Examples of natural variation for carotenoid concentration and profiles include but are not limited to the chromoplast-specific allele of lycopene beta cyclase (CYC-B) known as *Beta* (*B*; 9, 10) and alleles of carotenoid isomerase (CRTISO) responsible for *tangerine* (*t*; 11, 12). For both genes, specific alleles lead to orange-colored tomato fruits. However, *Beta* alleles allow for the accumulation of β-carotene in ripe fruits, whereas *tangerine* alleles prevent the biosynthesis of all-*trans* lycopene, and its precursors (phytoene, phytofluene, ζ-carotene, neurosporene, and tetra-*cis*-lycopene) predominate the carotenoid landscape. Plant breeders have created tomato germplasm with these alleles that has been used by seed companies and food scientists to produce various tomato-based products (13–16). In each of these contexts, accurate quantification of carotenoids and fast turnaround time is essential for making decisions about selection, quality control, and dosage delivered.

In order to partition and analyze carotenoids while minimizing enzymatic and oxidative degradation, analytical chemists have developed a wide array of extraction and analysis methods for tomatoes (17–21). Although the solvent systems differ slightly, a common element is that samples are first extracted with a water-miscible solvent and then re-extracted multiple times with nonpolar solvents. Each successive extraction requires centrifugation and liquid-handling steps, which are time consuming. Extracts are then phase separated to remove water and water-miscible solvents prior to sample dry-down. After redissolving in a known volume of solvent, carotenoids and their isomers are typically separated and quantified using liquid chromatography which can take from 15 to >100 min/sample (18, 22–27). Genetic treatments, environmental factors, and sample processing can

profoundly influence carotenoid profiles and these sources of variation speak to the need for rapid and accurate methods to measure carotenoids and their isomers.

To mitigate the bottlenecks created by lengthy extraction and analysis procedures, we developed a rapid extraction and an ultra-HPLC–diode array detector (UHPLC-DAD) method focused on tomatoes. Our goal was to compare and contrast standard extraction and chromatographic methods with our rapid protocols by their ability to accurately discriminate between tomatoes with different genetic backgrounds and carotenoid profiles as well as model the amount of variance these methods contribute in the presence of genetic and environmental effects. To test our methods, we phenotyped selected accessions from populations of tomatoes encompassing the natural range of tomato carotenoids with distinct alleles of *Beta* and *tangerine* in processing and/or cherry tomato backgrounds (28–30). The tomatoes were grown with replication at two locations in order to estimate variance contributed by the effects of genetics, environment, extraction method, and analysis method. Here, we present our results emphasizing the strengths and weaknesses of the standard and rapid extraction and chromatographic analysis approaches. This analysis also provided comprehensive carotenoid profiles for important subpopulations of tomato germplasm.

Methods

Plant Material

Thirty partially inbred tomato lines were assembled to represent two major loci affecting carotenoid content and two genetic backgrounds. These lines were considered genetic treatments, or “genotypes.” The thirty lines were divided into subpopulations based on genetic background and major loci (genes) affecting carotenoid biosynthesis. The first subpopulation consisted of 11 BC_2S_3 lines of cherry tomatoes in a Tainan (PI 647556) genetic background containing one of four alleles of *Beta* (*B*) in the homozygous state (30). *Beta* codes for a fruit-specific *CYC-B* with the high β-carotene alleles conditioned by sequence variation in the 5′ untranscribed region generally associated with the promoter (10). The *Beta* alleles were derived from Purdue 89-28-1 (three independent sibling lines), Jaune Flamme (two independent sibling lines), 97L97 (three independent sibling lines), and Tainan (three independent sibling lines). The second subpopulation consisted of 12 BC_1S_3 selections of processing tomatoes in an OH8245 background containing one of three alleles of *Beta* in the homozygous state (29). These alleles were derived from LA3502 (four independent sibling lines), Jaune Flamme (four independent sibling lines), and OH8245 (*S. lycopersicum*; four independent sibling lines). Based on sequence comparisons, the alleles of *Beta* in Purdue 89-28-1, Jaune Flamme, 97L97, and LA3502 were independent accessions of wild species (29, 31). The third subpopulation consisted of seven F_5 lines in an OH9242 processing tomato genetic background (*S. lycopersicum*) with alleles of *tangerine*. *Tangerine* codes for CRTISO, which converts tetra-*cis*-lycopene to all-*trans* lycopene (12, 32). Lines contained the *tangerine* (*t*) allele from NC99471–4 (three independent sibling lines) or the *tangerine virescent* (t^V) allele from LA0351 (four independent sibling lines; 28).

Experimental Design

Plants were grown in field sites located in Fremont and Wooster, OH, during the summer of 2016. At both field sites, each plot contained 6–10 plants of the same line, and the plots were arranged in a randomized, complete block design with two blocks per location. The samples represented an aggregate of fruits from all plants within a plot with exception of the first and last plant, which were not sampled. Fruits were harvested when ripe and stored whole at −40°C until analysis. Prior to extraction, fruits were thawed at room temperature, blended while partially frozen into homogenate, and partitioned into 50 mL tubes. The carotenoid extractions were then performed as described below and analyzed by HPLC-DAD. Carotenoids extracted from tomato fruits using a standard extraction method were also analyzed using a rapid UHPLC-DAD method, and phenotypic data were statistically compared (Figure 1). Statistical models used for comparisons are described below.

Chemical Reagents

Acetone, ammonium acetate, hexanes, methanol (MeOH), methyl *tert*-butyl ether (MtBE), and water were purchased from Fisher Scientific (Pittsburgh, PA) and of HPLC grade. β-Carotene (≥95%) was purchased from Sigma Aldrich (St. Louis, MO), and lycopene was purified as previously described (33).

Standard Carotenoid Extraction

Carotenoids were extracted in near darkness (≤1 μmol m^{-2} s^{-1}) as previously described (34). Approximately 1 g tomato puree was weighed into an 11 mL glass vial and extracted with 5 mL MeOH, briefly mixed on a vortex mixer, probe sonicated (Branson Fisher Scientific 150E Sonic Dismembrator) for 8 s, and centrifuged for 5 min at 2000 × *g*. The supernatant was decanted and the pellet was re-extracted with 5 mL hexanes–acetone (1:1), briefly mixed on a vortex mixer, probe sonicated for 8 s, and centrifuged for 5 min at 2000 × *g*. The supernatant was added to the methanolic extract, and the extraction was repeated two more times or until the pellet was colorless. To induce phase separation, 10 mL water was added to the combined supernatants, and 1 mL aliquots organic layer [experimentally determined to be on average 7.94 mL (2.69% CV)] were dried under nitrogen gas and stored at −20°C until analysis. Twenty samples were processed in parallel, and each extraction took an average of 10 min/sample (200 min/batch).

Rapid Carotenoid Extraction

In near darkness, approximately 0.5 g tomato puree was weighed into a 44 mL glass vial and, with 5 mL MeOH, briefly mixed on a vortex mixer and probe sonicated twice at 8 s bursts to disperse the tomato tissue. Fifteen milliliters hexanes–acetone (1:1) were added, and the samples were probe sonicated three times at 8 s bursts. To induce phase separation, 10 mL water was added, and 1 mL aliquots organic layer [experimentally determined to be on average 7.68 mL (1.25% CV)] were dried under nitrogen gas and stored at −20°C until analysis. Twenty samples were processed in parallel, and each extraction took an average of 5 min/sample (100 min/batch).

Standard HPLC-DAD Analysis

Carotenoids were analyzed as previously described and each run lasted 21.5 min (27). Briefly, dried extracts were redissolved in 1 mL of MtBE–MeOH (1:1), filtered with a 0.22 μm nylon filter (CellTreat, Shirley, MA), and 20 μL was injected into a Waters Alliance 2695 HPLC (Waters Corp., Milford, MA) fitted with a 996 DAD. Carotenoids were separated on a 4.6 × 250 mm, 3 μm particle size, C30 column (YMC Inc., Wilmington, NC) maintained at 35°C. A gradient using solvent A: 60% MeOH, 35% MtBE, 3% water, and 2% (w/v) aqueous ammonium acetate and B: 78% MtBE, 20% MeOH, and 2% (w/v) aqueous ammonium acetate at a flow of 1.3 mL/min was used as follows: 100% A to 64.4% A over 9 min, 64.4% A to 0% A over 5.5 min, a hold at 0% A for an additional 3.5 min, and a switch to 100% A for the remaining 3.5 min to recondition the column. Quantification was achieved using a six-point external calibration curve of lycopene and β-carotene. Adjusted slopes were calculated for other carotenoids based on ratios of their molar extinction coefficient to lycopene, as done previously (35).

UHPLC-DAD Analysis

Dried extracts were redissolved in 1 mL MtBE–MeOH (1:1), filtered with a 0.22 μm nylon filter (CellTreat) and 5 μL was injected into an 1290 Infinity II UHPLC-DAD (Agilent; Santa Clara, CA). Carotenoids were separated on a C18 Acquity BEH column (Waters Corp.) 2.1 × 150 mm, 1.7 μm particle size, maintained at 55°C. An isocratic flow using 42% solvent A [80% MeOH, 20% water, and 2% (w/v) aqueous ammonium acetate] and 58% solvent B [78% MtBE, 20% MeOH, and 2% (w/v) aqueous ammonium acetate] at a flow rate of 0.45 mL/min was used, and each run lasted 4.2 min. Quantification was achieved by six-point external calibration curves as described above. Carotenoid identities were confirmed by authentic standards, spectral characteristics, and tandem MS using a 6495 triple quadrupole MS (Agilent) with an atmospheric pressure chemical ionization source operated in positive mode. Source parameters and multiple reaction monitoring experiments were adapted from those previously reported (27) and were as follows: phytoene: 545.5>463.6, 421.6, 395.6, 327.4; phytofluene: 543.5>461.6, 393.6, 325.4; β-carotene: 537.5>455.3, 269.2, 69.0; and lycopene: 537.5>455.3, 269.2, 69.0.

Statistical Analysis

All statistical analysis was conducted in R version 3.31 (36). Analysis of variance (ANOVA) was used to determine significance of model parameters and their contribution to total variance. Prior to analyzing data, visual inspection of histograms, quantile-quantile (Q-Q) plots, and the output of Levene's tests revealed that our data violated the assumptions of ANOVA. Log_{10}, log_2, and natural log transformations were tested. Visual inspection of Q-Q plots and nonsignificant outcomes from Levene's tests determined that natural log transformation of our data satisfied the assumptions of ANOVA. Natural log transformed data were subsequently analyzed, whereas untransformed means and SDs are presented for ease of interpretation.

Linear models were used to determine if the population should be subdivided based on presence/absence of *tangerine* alleles, genetic background, and analysis method. Variance components were estimated considering each model parameter a random effect using the R

package “lme4” (37). The analysis was first conducted in a hierarchical process on data generated by HPLC-DAD and UHPLC-DAD. A graphical representation of the data analysis workflow is presented in Figure 1. The initial linear model used for the entire population was

$$Y = \mu + G + G{:}L + L + AM + AM{:}G + BLK(L) + \varepsilon$$

where Y = the concentration of a given carotenoid; G = genotype, or specific partially inbred line as a measure of genetic variation; L = location as a measure of environmental variation; AM = analysis method; and $BLK(L)$ = block nested within location as a measure of environmental variation as a result of within-field variation.

To investigate major effects because of the presence of allelic variation at *Beta* or *tangerine* or because of genetic background, the datasets were split to test the significance of these effects. First, within the *Beta* material, we tested for differences between cherry and processing tomatoes using the linear model

$$Y = \mu + BG + BG{:}L + L + AM + AM{:}BK + BLK(L) + \varepsilon$$

where BG = genetic background (cherry or processing) and all other terms held the same meaning as the previous model. After the main effects suggested significance as a result of genetic background, cherry and processing tomatoes were analyzed separately. Further processing populations were split based on *tangerine* and *Beta* subpopulations. The following linear model was used to test for allele differences, analysis method differences and potential interactions for each of the three subpopulations

$$Y = \mu + A + A{:}L + L + AM + A{:}AM + BLK(L) + \varepsilon$$

where A = allele of either *Beta* or *tangerine* depending on the subpopulation being analyzed, and all other terms held the same meaning as the previous models. Finally, data from each subpopulation was separated by analysis method and compared using the linear model

$$Y = \mu + A + A{:}L + L + BLK(L) + \varepsilon$$

The means separation tests were carried out using Tukey’s honest significance test (HSD; $\alpha = 0.05$) using the R package “Agricolae” (38). The means and significance patterns generated from Tukey’s HSD tests were then compared by the extraction method. Moreover, data for each subpopulation generated by standard extraction and HPLC-DAD analysis were compared with the same samples analyzed using a rapid UHPLC-DAD method and statistically compared using the previous model followed by a Tukey’s HSD ($\alpha = 0.05$) post-hoc tests. Finally, phenotypic data and population structure were visualized by principal components analysis (PCA) with the R packages “FactoMineR” and “factoextra” using covariance matrixes (39).

A similar statistical analysis workflow was performed for phenotypic data generated by the two extraction methods compared in this study. However, it was determined from the

previous dataset that BLK(L) was not significant for any carotenoid measured in subpopulations with the allelic variation for *Beta*. Therefore, samples from only one block per location were used for extraction method comparison in the two subpopulations containing alleles of *Beta*.

Results and Discussion

Assessing extraction and chromatographic methods was done based on ability to separate treatments, accuracy, variance partitioning, and throughput. Although uncommon, variance partitioning assesses the contribution to total variance of the individual factors or sources of variation.

Extraction Methods

Unsupervised learning using PCA was performed to visualize population structure based on carotenoid profiles as a function of genetic background, allele of *Beta* or *tangerine*, and extraction method (Figure 2A). Similar clustering patterns were observed regardless of extraction method. In principal component 1 (PC1), processing tomatoes with alleles of *tangerine* clearly separate from other types of tomatoes. This is because of the presence of carotenoids unique to tangerine tomatoes (e.g., ζ-carotene, neurosporene, tetra-*cis*-lycopene) and altered concentrations of those normally found in red tomatoes (e.g., phytoene and phytofluene). In PC2, tomatoes with various alleles of *Beta* clustered separately. Red tomatoes (OH8245 and Tainan) separated from orange tomatoes (JF, PU, and 97L97) with alleles of *Beta*. Orange tomatoes with the LA716 allele of *Beta* bridged the clusters of red tomatoes and orange tomatoes high in β-carotene in PC2 (Figure 2A).

Estimates of carotenoid concentrations for both subpopulations are presented in Table 1. In a cherry tomato background, concentrations of phytoene and phytofluene were similar regardless of extraction method (Table 1). However, concentrations of all-*trans* lycopene and total lycopene measured in this subpopulation were negatively affected by the extraction method. Patterns of significance across the four alleles of *Beta* present in the cherry tomato subpopulation were identical between extraction methods for phytoene, all-*trans*-β-carotene, all-*trans* lycopene, and total β-carotene, indicating that either extraction method would yield the same outcome in terms of differentiating alleles of *Beta*. Significance trends for other carotenoids measured, such as phytofluene and total lycopene, tended to be similar between extraction methods. Most trends of allelic variation were similar regardless of extraction method. Similar to cherry tomatoes, concentrations of all-*trans* lycopene and total lycopene were lower in processing tomato samples extracted rapidly (Table 1). Carotenoid concentration data indicate that the amount of lycopene and β-carotene in fruits with the LA716 allele of *Beta* are intermediate between red tomatoes and high β-carotene accumulating tomatoes such as those with the JF allele (Table 1), providing a basis for the separation seen in Figure 2A. Concentrations of phytoene, phytofluene, *cis*-β-carotene, all-*trans*-β-carotene, and total β-carotene (all-*trans*-β-carotene + *cis* isomer species) were similar regardless of extraction method used (Table 1).

All-*trans* lycopene and β-carotene accumulate as crystalline structures in chromoplasts in tomato fruits (40, 41). However, all-*trans* lycopene and β-carotene crystals have different

structures. We hypothesize that the lower extraction efficiency of all-*trans* lycopene using the rapid method is because of a decreased ability to disrupt and solubilize tightly packed H-aggregates of all-*trans*-lycopene (42). β-Carotene extraction was not affected to the same extent. We hypothesize that its nonplanar structure, which does not lend well to H-aggregate formation (42), allowed for similar solubility of β-carotene between extraction methods.

For processing tomatoes with allelic variation at the *tangerine* locus, concentrations of phytoene, phytofluene, neurosporene, and all-*trans*-lycopene were similar between the two extraction methods (Table 2). Lower extraction efficiency in the rapid extraction method influenced the estimated concentrations of tetra-*cis*-lycopene and ζ-carotene. Separation between alleles of *tangerine* were identical for phytoene, phytofluene, tetra-*cis*-lycopene, *cis*-lycopene, and all-*trans*-lycopene. Distinguishing differences between *t* and t^{V} for ζ-carotene, neurosporene, and total lycopene were trending towards significance ($0.05 < P < 0.07$), but these carotenoids only significantly differentiated when the samples were extracted using the rapid method (Table 2).

Beyond contrasting the accuracy of the extraction methods, we also took a novel approach of modeling the contribution of genetic, environmental, and extraction effects on total variance. For most carotenoids, genotypic differences (“G”) tended to account for the majority of variance (up to 96.7%). The contribution of extraction method (“EX”) to total variance depended on the carotenoid (Table 3). The proportion of variance as a result of extraction was between 15.6 and 28.3% for all-*trans* lycopene, other *cis* lycopene, and total lycopene, compared with between 0.0 and 1.3% for all other carotenoids (Table 3). Genotype by extraction method (“G:EX”) contributed a relatively high proportion of variance for these carotenoids and may reflect differences in extraction efficiency between the two methods as discussed above.

The concentrations of carotenoids extracted using the standard method correlated with those extracted using the rapid method (Table 4). Correlations for phytoene and phytofluene were statistically significant ($P < 0.001$) with high correlations (r = 0.937 for both). Most other carotenoids measured followed similar trends. Tetra-*cis*-lycopene and other *cis*-lycopene isomers were found to have a moderately strong (r = 0.604 and 0.672, respectively) relationship and were also statistically significant (Table 4). *cis*-β-Carotene estimates generated by both extractions were statistically significant ($P < 0.05$) but correlated weakly (r = 0.336). Linear models could be used to partially compensate for loss of extraction efficiency. The rapid extraction method could be used in contexts where many samples need to be extracted and profiled for carotenoids. If specific samples require additional accuracy or confirmation, they could be extracted using the standard method.

Although rapid extractions exist for tomatoes and tomato products (43, 44), our extraction method was able to be completed faster, at less than 5 min/sample (2 times faster than the standard method we used). Typically, tomato carotenoid extractions maximize mass transfer by subjecting samples to multiple rounds of extraction (17–19, 21, 34). Our rapid method aimed to capitalize on the time savings of using a bulk extraction and probe sonication while eliminating time-consuming steps involved with centrifuging and liquid transfer. Although omitting multiple steps of solvent addition reduced the duration of the rapid extraction, the

capacity to partition analytes into solvent was diminished. This effect explains the difference in carotenoid concentration estimates between the standard and rapid extraction methods (Tables 1 and 2), particularly for less soluble carotenoids like all-*trans* lycopene.

HPLC-DAD and UHPLC-DAD Analysis Methods

PCA was used to visualize overall similarities and differences in carotenoid phenotypic data generated by HPLC-DAD or UHPLC-DAD (Figure 2B). The phenotypic data generated by both analysis methods display similar patterns of clustering. Different alleles of *Beta* clustered into three distinct groups containing red tomatoes (OH8245 and Tainan), orange tomatoes (IF, PU, and 97L97), and LA716 as an intermediate between highly pigmented orange and red tomatoes. Tomatoes with alleles of *tangerine* were excluded from PCA analysis in this context because of the inability to quantify all carotenoids in tangerine tomatoes using the rapid UHPLC-DAD method.

Concentrations were similar for all carotenoids measured and significance trends were similar between the two analysis methods (Table 5). The discrimination of alleles of *Beta* in a cherry tomato background was almost completely unaffected by the chromatographic analysis method used. The only deviation in significance trends in this subpopulation can be seen in *cis*-β-carotene and *cis*-lycopene isomers. This observation is likely a result of the lack of chromatographic resolution for these minor carotenoid species in contrast to the longer HPLC method (Figure 3). Similar trends were observed in the processing tomato subpopulation with allelic variation for *Beta* (Table 5). Concentrations of all carotenoids measured were similar between analysis methods, and the significance trends among different alleles of *Beta* were identical between analysis methods. The exception was *cis*-lycopene isomers. An inability to separate *cis* isomers was also observed in tomatoes with *tangerine* alleles using the rapid UHPLC-DAD analysis method.

The subpopulation of processing tomatoes with allelic variation at the *tangerine* locus was also analyzed both by a standard HPLC-DAD and the rapid UHPLC-DAD analysis method. We were unable to adequately resolve ζ-carotene, neurosporene, and tetra-*cis*-lycopene found in *tangerine* tomatoes using the rapid UHPLC-DAD method. Tetra-*cis*-lycopene, ζ-carotene, neurosporene, and their geometrical isomers differ by minor structural alterations that result from desaturation events during their biosynthesis (12, 32). C30 columns were invented to separate geometrical isomers of carotenoids (45). However, no UHPLC columns are currently available with this stationary phase. To separate and quantify these carotenoids using HPLC, long separations (often between 15 and 100 min) are generally employed using C18 or C30 stationary phases packed into 250 mm columns (10, 18, 22–27, 35). This rapid, isocratic UHPLC-DAD method was not able to separate carotenoids from tangerine tomatoes during its 4.2 min run time (Figure 3). Thus, the rapid UHPLC-DAD method is best suited for tomatoes that primarily contain lycopene and β-carotene.

Similar to the comparisons of extraction methods, we used random effects modeling to estimate contributions to variance. Random effects modeling indicated that analysis method (“AN”) contributed between 0 and 14.9% of the variation for all carotenoids measured by UHPLC-DAD. Genotypic (“G”) and environmental conditions {genotype by location [“G:L”], location [“L”], and block within location [“BLK(L)”]} were stronger and

influenced carotenoid profiles substantially more than the analysis method. Genotypic contributions to total variance were as high as 82.1% for all carotenoids measured by both HPLC-DAD and UHPLC-DAD, demonstrating that variance due to biological conditions often overwhelms variance from analytical sources. The analysis method explained almost 15% of the total variance for other *cis*-β-carotene isomers (Table 3), which is reflected in higher values when measured by HPLC-DAD as compared with UHPLC-DAD (Table 5). We hypothesized that this difference is a result of an inability to fully resolve *cis*-β-carotene isomers from all-*trans* using the rapid UHPLC-DAD method as discussed above. Ultimately, these isomers constitute a small proportion of the total carotenoid content in most tomato fruits and may not be of importance in many contexts. Overall, genetic and environmental factors overwhelmed the effects of chromatographic method.

To explore the two datasets further, we used linear regression again to determine their relationship (Table 4). We found that all carotenoid concentrations in our *Beta* processing and cherry tomato subpopulations measured by UHPLC-DAD were strongly related to those measured by HPLC-DAD. The regression equations presented in Table 4 could be used as a starting point to convert values generated by the UHPLC-DAD method to those generated by the HPLC-DAD method we used.

Because of the structural and chemical similarity of tomato carotenoids as well as the presence of geometrical isomers, chromatographic separation methods tend to be time consuming (10, 18, 22–27, 35). The UHPLC-DAD method presented here was able to resolve major tomato carotenoids and to an extent, some *cis* isomers of lycopene and β-carotene in only 4.2 min (Figure 3). Other HPLC-DAD and UHPLC-DAD methods have been recently developed to separate carotenoids but are considerably longer and do not resolve lycopene precursors such as phytoene and phytofluene (46–48). Furthermore, the rapid UHPLC-DAD method requires 93% less solvent compared with the standard method because the reduction in sample run time and lower flow rate. Given the data we presented here, this UHPLC-DAD analysis method could greatly enhance the analytical throughput in many applications including but not limited to breeding, quality control, and food product development. The UHPLC-DAD method was unable to resolve the complex mixture of carotenoids and geometrical carotenoid isomers found in tangerine tomatoes, although it can still be used to rapidly determine if a sample is from a tangerine tomato and those samples could be profiled using the standard method we presented in this work.

Conclusions

Here, we developed new carotenoid extraction and analysis methods and applied them to assess diverse selections of tomatoes grown in multiple environments. The rapid extraction protocol was able to distinguish between alleles of *Beta* and *tangerine* similarly to standard methods, although extraction efficiency was lower for some carotenoids. This extraction method may be best suited for qualitative, high-throughput phenotyping in which rapid turnaround time is required. The novel UHPLC-DAD method presented here separates carotenoids 5 times faster compared with the standard method. The UHPLC-DAD method was able to separate genetic background, allele effects, and environmental effects as well as the standard method. Although the UHPLC-DAD method is the fastest tomato carotenoid

separation protocol to date, carotenoids and geometrical isomers unique to tangerine tomatoes could not be separated and quantified. If a high degree of accuracy is required for carotenoid phenotyping, a subset of samples could be extracted using a standard method and analyzed using the rapid UHPLC-DAD method to capitalize on its time and resource savings. In many cases, genetic and environmental effects tended to contribute more to the variation in our samples than that of the extraction or chromatography methods. The rapid carotenoid extraction and analysis platform we outlined here could be adopted by plant breeders and food product developers interested in making fast, data-driven decisions.

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References

(1). Frusciante L, Carli P, Ercolano MR, Pernice R, Di Matteo A, Fogliano V, & Pellegrini N (2007) Mol. Nutr. Food Res 51, 609–17. doi:10.1002/mnfr.200600158 [PubMed: 17427261]

(2). Raiola A, Rigano MM, Calafiore R, Frusciante L, & Barone A (2014) Mediators Inflamm 139873. doi:10.1155/2014/139873 [PubMed: 24744504]

(3). Clinton SK (2009) Nutr. Rev 56, 35–51. doi:10.1111/j.1753-4887.1998.tb01691.x

(4). Friedman M (2013) J. Agric. Food Chem 61, 9534–9550. doi:10.1021/jf402654e [PubMed: 24079774]

(5). Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, & Willett WC (1995) JNCI, J. Natl. Cancer Inst 87, 1767–1776. doi:10.1093/jnci/87.23.1767 [PubMed: 7473833]

(6). Wei MY, & Giovannucci EL (2012) J. Oncol e271063. doi:10.1155/2012/271063

(7). Wu K, Erdman JW Jr, Schwartz SJ, Platz EA, Leitzmann M, Clinton SK, DeGroff V, Willett WC, & Giovannucci E (2004) Cancer Epidemiol. Biomarkers Prev 13, 260–269. doi:10.1158/1055-9965.EPI-03-0012 [PubMed: 14973107]

(8). Stommel J, Abbott JA, Saftner RA, & Camp MJ (2005) J. Am. Soc. Hortic. Sci 130, 244–251

(9). Lincoln RE, & Porter JW (1950) Genetics 35, 206–211 [PubMed: 17247343]

(10). Ronen G, Carmel-Goren L, Zamir D, & Hirschberg J (2000) Proc. Natl. Acad. Sci. U. S. A 97, 11102–11107. doi:10.1073/pnas.190177497 [PubMed: 10995464]

(11). MacArthur JW (1934) J. Genet 29, 123–133. doi:10.1007/BF02981789

(12). Isaacson T, Ronen G, Zamir D, & Hirschberg J (2002) Plant Cell 14, 333–342. doi:10.1105/tpc.010303 [PubMed: 11884678]

(13). Grainger E, Schwartz S, Wang S, Unlu N, Boileau T, Ferketich A, Monk JP, Gong M, Bahnson R, DeGroff V, & Clinton SK (2008) Nutr. Cancer 60, 145–154. doi:10.1080/01635580701621338 [PubMed: 18444145]

(14). Bohn T, Blackwood M, Francis DM, Tian Q, Schwartz SJ, Clinton SK, Blackwell M, Francis DM, Tian Q, Schwartz SJ, & Clinton SK (2013) Nutr. Cancer 65, 919–929. doi:10.1080/01635581.2011.630156 [PubMed: 22098224]

(15). Misra R, Mangi S, Joshi S, Mittal S, Gupta SK, & Pandey RM (2006) J. Obstet. Gynaecol. Res 32, 299–304. doi:10.1111/j.1447-0756.2006.00410.x [PubMed: 16764620]

(16). Aust O, Stahl W, Sies H, Tronnier H, & Heinrich U (2005) Int. J. Vitam. Nutr. Res 75, 54–60. doi:10.1024/0300-9831.75.1.54 [PubMed: 15830922]

(17). Ferruzzi MG, Nguyen ML, Sander LC, Rock CL, & Schwartz SJ (2001) J. Chromatogr. B 760, 289–299. doi:10.1016/S0378-4347(01)00288-2

(18). Ferruzzi MG, Sander LC, Rock CL, & Schwartz SJ (1998) Anal. Biochem 256, 74–81. doi:10.1006/abio.1997.2484 [PubMed: 9466800]

(19). Rodríguez-Amaya DB, & Kimura M (2004) HarvestPlus Handbook for Carotenoid Analysis, International Food Policy Research Institute, Washington, DC

(20). Britton G (1996) in Natural Food Colorants, Houghton JD & Hendry GAF (Eds), Springer US, Boston, MA, pp 197–243

(21). Kopec RE, Cooperstone JL, Cichon MJ, & Schwartz SJ (2012) in Analysis of Antioxidant-Rich Phytochemicals, Xu Z & Howard LR (Eds), Wiley-Blackwell, Oxford, United Kingdom, pp 105–148

(22). Bijttebier S, D'Hondt E, Noten B, Hermans N, Apers S, & Voorspoels S (2014) J. Chromatogr. A 1332, 46–56. doi:10.1016/J.CHROMA.2014.01.042 [PubMed: 24534422]

(23). Daood HG, Bencze G, Palotás G, Pék Z, Sidikov A, & Helyes L (2014) J. Chromatogr. Sci 52, 985–991. doi:10.1093/chromsci/bmt139 [PubMed: 24046161]

(24). Lesellier E, Tchapla A, Marty C, & Lebert A (1993) J. Chromatogr. A 633, 9–23. doi:10.1016/0021-9673(93)83133-D

(25). Bramley PM (1992) Phytochem. Anal 3, 97–104. doi:10.1002/pca.2800030302

(26). Kean EG, Hamaker BR, & Ferruzzi MG (2008) J. Agric. Food Chem 56, 9918–9926. doi:10.1021/jf8018613 [PubMed: 18937488]

(27). Cooperstone JL, Ralston RA, Riedl KM, Haufe TC, Schweiggert RM, King SA, Timmers CD, Francis DM, Lesinski GB, Clinton SK, Clinton SK, & Schwartz SJ (2015) Mol. Nutr. Food Res 59, 658–669. doi:10.1002/mnfr.201400658 [PubMed: 25620547]

(28). De Jesús S (2005) Genetic Alteration of Plant Secondary Metabolism: Modification, Enhancement and Characterization of Pigments in Tomato (Lycopersicon Esculentum) Fruit, MS Thesis, The Ohio State University, Columbus, OH

(29). Orchard C (2014) Naturally Occurring Variation in the Promoter of the Chromoplast-Specific Cyc-B Gene in Tomato Can Be Used to Modulate Levels of ß-Carotene in Ripe Tomato Fruit, MS Thesis, The Ohio State University, Columbus, OH

(30). Sari E (2016) The Effects of CYC-B Introgressions on Cherry Tomato Fruit Quality, MS Thesis, The Ohio State University, Columbus, OH

(31). Stommel JR (2001) HortScience 36, 387–388

(32). Isaacson T, Ohad I, Beyer P, & Hirschberg J (2004) Plant Physiol. 136, 4246–55. doi:10.1104/pp.104.052092 [PubMed: 15557094]

(33). Kopec RE, Riedl KM, Harrison EH, Curley RW, Hruszkewycz DP, Clinton SK, & Schwartz SJ (2010) J. Agric. Food Chem 58, 3290–3296. doi:10.1021/jf100415z [PubMed: 20178389]

(34). Kopec RE, Cooperstone JL, Schweiggert RM, Young GS, Harrison EH, Francis DM, Clinton SK, & Schwartz SJ (2014) J. Nutr 144, 1158–1166. doi:10.3945/jn.113.187674 [PubMed: 24899156]

(35). Cooperstone JL, Francis DM, & Schwartz SJ (2016) Food Chem. 210, 466–472. doi:10.1016/J.FOODCHEM.2016.04.078 [PubMed: 27211672]

(36). The R Foundation, The R Project for Statistical Computing, http://www.R-project.org/

(37). Bates D, Mächler M, Bolker B, & Walker S (2015) J. Stat. Softw 67, 1–48. doi:10.18637/jss.v067.i01

(38). Mendiburu F (2009) Una Herramienta de Análisis Estadístico para la Investigación Agrícola, PhD Thesis, National Agrarian University, La Molina, Peru

(39). Lê S, Josse J, & Husson F (2008) J. Stat. Softw 25, 1–18. doi:10.18637/jss.v025.i01

(40). Rosso SW (1968) J. Ultrastruct. Res 25, 307–322. doi:10.1016/S0022-5320(68)80076-0 [PubMed: 5715767]

(41). Harris WM, & Spurr AR (1969) Am. J. Bot 56, 369–379. doi:10.1002/j.1537-2197.1969.tb07546.x

(42). Schweiggert RMM, & Carle R (2017) Crit. Rev. Food Sci. Nutr 57, 1807–1830. doi:10.1080/10408398.2015.1012756 [PubMed: 26115350]

(43). Sadler G, Davis J, & Dezman D (1990) J. Food Sci 55, 1460–1461. doi:10.1111/j.1365-2621.1990.tb03958.x

(44). Sérino S, Gomez L, Costagliola G, & Gautier H (2009) J. Agric. Food Chem 57, 8753–8760. doi:10.1021/jf902113n [PubMed: 19769393]

(45). Sander LC, Sharpless KE, Craft NE, & Wise SA (1994) Anal. Chem 66, 1667–1674. doi:10.1021/ac00082a012 [PubMed: 15943024]

(46). Arathi BP, Sowmya PRR, Vijay K, Dilshad P, Saikat B, Gopal V, & Lakshminarayana R (2015) Food Anal. Methods 8, 1962–1969. doi:10.1007/s12161-014-0083-5

(47). Abate-Pella D, Freund DM, Slovin JP, Hegeman AD, & Cohen JD (2017) J. Chromatogr. B 1067, 34–37. doi:10.1016/J.JCHROMB.2017.09.039

(48). Gupta P, Sreelakshmi Y, & Sharma R (2015) Plant Methods 11, 5. doi:10.1186/s13007-015-0051-0 [PubMed: 25688283]

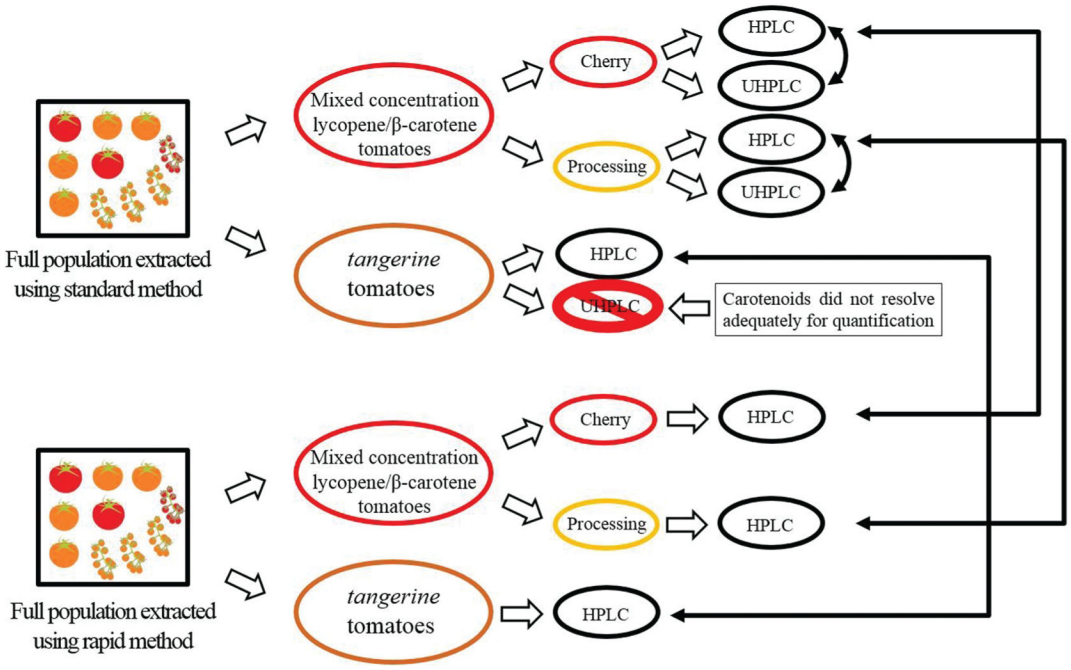

Figure 1.

Graphical representation of data analysis strategy. Linear models, detailed in the *Statistical Analysis* section, were used to determine if subpopulations should be analyzed separately because of inherent differences in carotenoid composition or concentrations. Arrows with solid black tails indicate comparisons made between extraction or analysis methods.

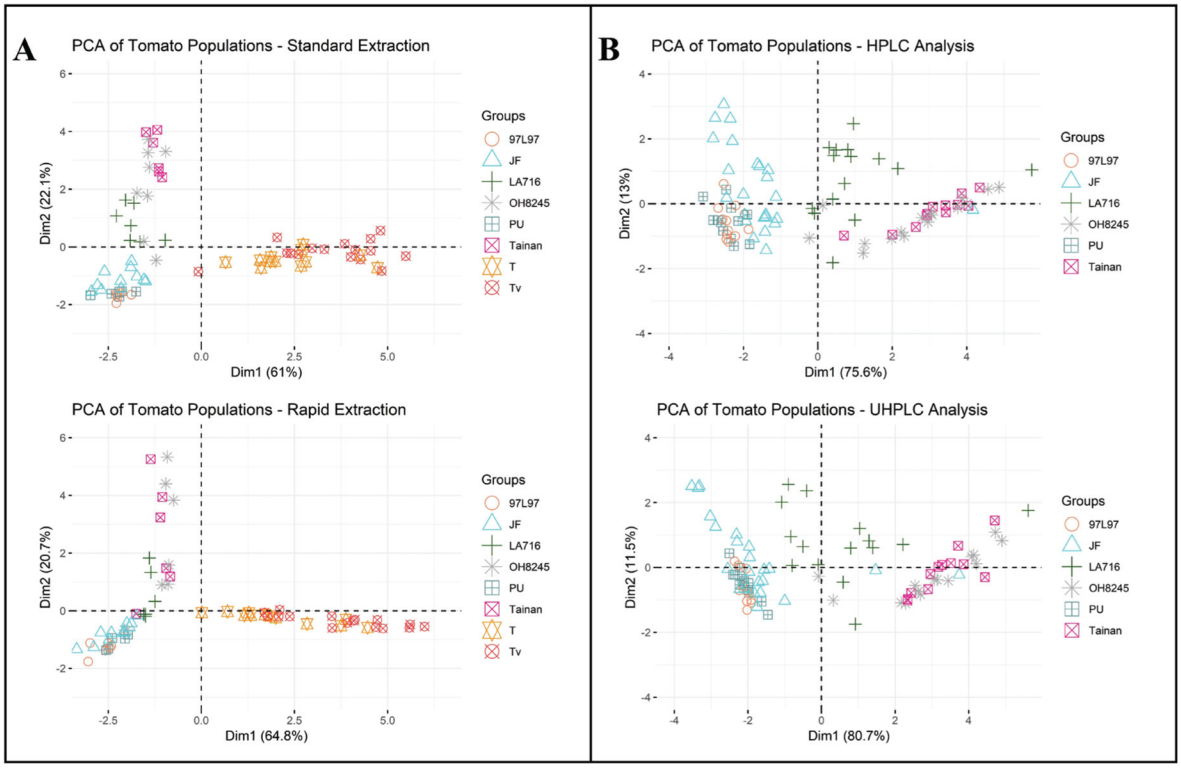

Figure 2.

PCA of (A) tomatoes extracted using the standard or rapid method and (B) tomatoes analyzed by HPLC-DAD or UHPLC-DAD. Individuals with alleles of *tangerine* were not included in B because of an inability to resolve ζ-carotene, neurosporene, and tetra-*cis*-lycopene by UHPLC-DAD. Subpopulation clustering was similar regardless of extraction or analysis method.

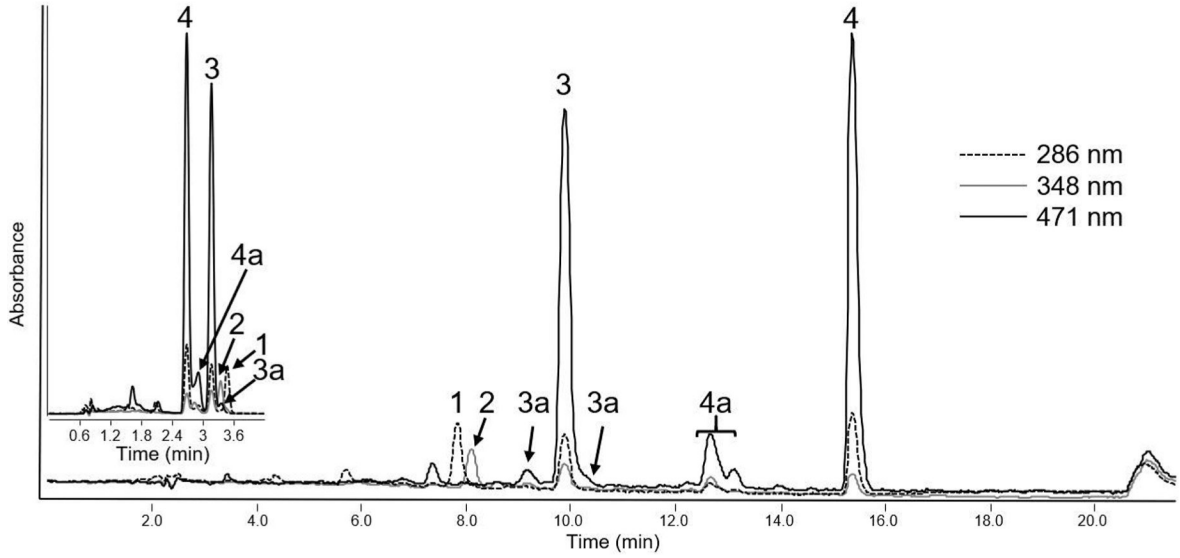

Figure 3.

Chromatograms of tomatoes carrying an allele of *Beta* (LA716) generated by HPLC-DAD and UHPLC-DAD (inset and scaled for difference in run time). Carotenoids quantified included (1) Phytoene; (2) Phytofluene; (3) β -carotene, (3a) β-carotene isomers; (4) all-trans lycopene, and (4a) *cis*-lycopene isomers. Traces indicate DAD wavelengths 286, 348, and 471 nm.

Table 1.

Carotenoid concentration in tomatoes grown in multiple locations as a function of background, *Beta* allele, and extraction method

Background	Method	*B*-allele	Sample size	Phytoene	Phytofluene	All-*trans* lycopene	*cis*-Lycopene	Total lycopene[a]	All-*trans* (β-carotene	*cis*-β-carotene	Total β-carotene
Cherry, mg/100 g fresh weight ± SD	Standard	97L97	6	0.46 ± 0.14^{b}	0.12 ± 0.05^{c}	0.06 ± 0.02^{c}	0.03 ± 0.03^{c}	0.09 ± 0.04^{c}	3.46 ± 0.87^{d}	0.66 ± 0.09^{d}	4.11 ± 0.81^{d}
		JF	4	0.63 ± 0.26^{b}	0.22 ± 0.08^{b}	1.02 ± 0.40^{b}	0.29 ± 0.1l^{bd}	1.32 ± 0.51^{b}	3.83 ± 1.85^{d}	0.65 ± 0.18^{a}	4.47 ± 2.02^{a}
		PU	6	0.44 ± 0.08^{b}	0.13 ± 0.03^{bc}	0.19 ± 0.21^{c}	0.07 ± 0.07^{bd}	0.26 ± 0.27^{c}	3.46 ± 1.03^{d}	0.74 ± 0.25^{d}	4.19 ± 1.12^{d}
		Tainan	6	2.10 ± 0.24^{d}	0.87 ± 0.13^{d}	8.92 ± 1.73^{d}	1.01 ± 0.10^{d}	9.93 ± 1.83^{d}	0.77 ± 0.17^{b}	0.28 ± 0.07^{b}	1.05 ± 0.21^{b}
	Rapid	97L97	6	0.51 ± 0.09^{b}	0.13 ± 0.04^{b}	0.15 ± 0.19^{c}	0.06 ± 0.09^{d}	0.20 ± 0.28^{b}	4.53 ± 1.51^{d}	0.63 ± 0.17^{d}	5.16 ± 1.43^{d}
		JF	4	0.56 ± 0.15^{b}	0.15 ± 0.05^{b}	0.34 ± 0.14^{b}	0.10 ± 0.05^{a}	0.44 ± 0.18^{b}	2.86 ± 0.89^{a}	0.47 ± 0.09^{a}	3.33 ± 0.97^{d}
		PU	6	0.61 ± 0.40^{b}	0.10 ± 0.04^{b}	0.08 ± 0.07^{c}	0.04 ± 0.03^{a}	0.12 ± 0.10^{b}	3.43 ± 1.14^{a}	0.81 ± 0.50^{a}	4.24 ± 1.56^{d}
		Tainan	6	1.58 ± 0.35^{d}	0.61 ± 0.17^{d}	2.43 ± 1.66^{d}	0.23 ± 0.05^{d}	2.65 ± 1.69^{d}	1.25 ± 1.14^{b}	0.16 ± 0.06^{b}	1.41 ± 1.19^{b}
Processing, mg/100 g fresh weight ± SD	Standard	JF	8	0.52 ± 0.18^{b}	0.20 ± 0.08^{b}	1.26 ± 0.59^{b}	0.34 ± 0.15^{b}	1.59 ± 0.72^{b}	4.92 ± 2.09^{d}	0.46 ± 0.18^{d}	5.38 ± 2.18^{d}
		LA716	7	1.89 ± 0.60^{d}	0.60 ± 0.26^{d}	4.05 ± 1.07^{d}	0.84 ± 0.28^{d}	4.89 ± 1.32^{d}	3.32 ± 1.35^{d}	0.29 ± 0.12^{d}	3.61 ± 1.44^{d}
		OH8245	8	1.62 ± 0.75^{d}	0.62 ± 0.25^{d}	6.53 ± 2.74^{d}	0.80 ± 0.37^{d}	7.33 ± 3.03^{d}	1.12 ± 0.96^{b}	0.29 ± 0.18^{d}	1.41 ± 0.85^{b}
	Rapid	JF	8	0.51 ± 0.13^{b}	0.16 ± 0.05^{b}	0.35 ± 0.47^{b}	0.15 ± 0.06^{b}	0.51 ± 0.22^{b}	4.91 ± 1.81^{d}	0.39 ± 0.15^{d}	5.30 ± 1.85^{d}
		LA716	7	1.94 ± 0.61^{d}	0.59 ± 0.19^{d}	0.94 ± 0.71^{d}	0.28 ± 0.16^{d}	1.21 ± 0.88^{d}	3.34 ± 0.49^{d}	0.20 ± 0.04^{d}	3.54 ± 0.51^{d}
		OH8245	8	1.60 ± 0.86^{d}	0.53 ± 0.28^{d}	1.97 ± 1.24^{d}	0.22 ± 0.11^{bd}	2.18 ± 1.89^{d}	1.42 ± 1.42^{b}	0.15 ± 0.12^{d}	1.57 ± 1.51^{b}

[a]Total = The sum of *cis* isomers and all-*trans* configurations for a given carotenoid.

*bcd*Values with different letters within an extraction method are statistically different as determined by a Tukey's HSD test (α = 0.05).

Table 2.

Carotenoid concentration in processing tomatoes grown in multiple locations as a function of *tangerine* allele and extraction method

Method	*t* Allele	Sample size	Phytoene	Phytofluene	ζ-Carotene	Neurosporene	Tetra-*cis*-lycopene	All-*trans* lycopene	Other *cis*-lycopene[a]	Total lycopene[b]
Standard, mg/100 g fresh weight ± SD	*t*	12	5.85 ± 2.64[c]	1.86 ± 0.87[c]	3.58 ± 1.99[d]	0.91 ± 0.65[d]	3.32 ± 1.22[c]	0.06 ± 0.02[c]	0.67 ± 0.22[c]	4.79 ± 1.78[d]
	t_v	16	8.16 ± 2.59[d]	2.72 ± 0.83[d]	5.04 ± 2.03[d]	1.26 ± 0.48[d]	4.56 ± 1.41[d]	0.08 ± 0.03[d]	0.98 ± 0.26[d]	5.34 ± 1.83[d]
Rapid, mg/100 g fresh weight ± SD	*t*	12	4.71 ± 2.38[c]	1.49 ± 0.75[c]	2.53 ± 1.58[c]	0.87 ± 0.49[c]	2.14 ± 0.83[c]	0.05 ± 0.04[c]	0.37 ± 0.13[c]	2.63 ± 0.96[c]
	t_v	16	7.14 ± 2.56[d]	2.37 ± 0.83[d]	3.82 ± 1.53[d]	1.31 ± 0.49[d]	2.98 ± 0.85[d]	0.09 ± 0.05[d]	0.64 ± 0.21[d]	3.71 ± 1.07[d]

[a] Other *cis* lycopene = Indicates the sum of all *cis*-lycopene isomers excluding tetra-*cis*-lycopene.

[b] Total = The sum of *cis* isomers and all-*trans* configurations for a given carotenoid.

[cd] Values with different letters within an extraction method are statistically different as determined by a Tukey's HSD test (α = 0.05).

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Table 3.

Proportion of variance explained by genetics, environment, and methodology for all carotenoids measured

Method	Phytoene	Phytofluene	ζ-Carotene	Neurosporene	Tetra-*cis* lycopene	All-*trans* lycopene	Other-*cis*-lycopene[a]	Total lycopene[b]	All-*trans*-β carotene	*cis*-β-carotene	Total β-carotene
Extraction, %											
G[c]	68.2	61.5	65.7	68.9	73.8	33.8	28.0	33.2	72.7	96.7	75.8
G:EX[d]	0.0	0.0	0.7	0.0	5.0	36.0	19.0	36.3	0.0	0.04	0.0
G:L[e]	0.0	1.6	2.7	0.9	0.5	7.5	4.6	7.7	3.4	0.7	2.9
EX	0.2	0.6	0.9	0.0	1.3	15.6	28.3	16.2	0.0	0.0	0.0
L	0.0	0.0	0.9	0.7	0.3	0.6	4.9	0.8	0.0	0.0	0.0
Residual	31.6	25.4	29.0	29.6	19.1	6.5	15.3	5.8	23.9	2.48	21.4
Analysis, %											
G	60.9	31.1	NA[f]	NA	NA	82.1	10.3	81.0	54.1	40.4	35.7
G:AN[g]	0.0	3.5	NA	NA	NA	0.0	5.2	0.0	0.0	4.6	0.0
G:L	2.2	2.7	NA	NA	NA	1.4	2.8	1.7	5.5	3.5	4.4
AN	0.0	11.5	NA	NA	NA	0.0	1.0	0.0	1.2	14.9	1.9
L	3.2	2.9	NA	NA	NA	0.6	0.1	0.8	0.0	0.4	0.0
BLK(L)[h]	7.0	29.9	NA	NA	NA	4.7	61.2	3.5	19.2	12.1	43.5
Residual	26.7	18.5	NA	NA	NA	11.2	19.4	13.0	20.1	24.0	14.3

[a] Other *cis* lycopene = Indicates the sum of all *cis*-lycopene isomers excluding tetra-*cis*-lycopene.

[b] Total = The sum of *cis* isomers and all-*trans* configurations for a given carotenoid.

[c] G = Genotype.

[d] G:EX = Genotype by extraction method.

[e] G:L = Genotype by location.

[f] NA = Not applicable.

[g] G:AN = Genotype by analysis method.

[h] BLK(L) = Block within location.

Table 4.

Regression analysis for both extraction and analysis methods

Comparison	Phytoene	Phytofluene	ζ-Carotene	Neurosporene	Tetra-*cis*-lycopene	All-*trans* lycopene	Other *cis*-lycopene[a]	Total lycopene[b]	All-trans-β carotene	*cis*-β Carotene	Total-β carotene
Extraction methods											
PCC[c]	0.937[d]	0.937[d]	0.853[e]	0.724[f]	0.604[e]	0.735[d]	0.672[d]	0.708[d]	0.775[d]	0.336[f]	0.713[d]
Model[g]	1.063x + 0.15	1,055x + 0.09	1.13x + 0.56	0.818x + 0.15	0.995x + 1.29	2.1x + 0.82	1.11x + 0.3	2.108x + 0.81	0.81x + 0.42	0.26x + 0.36	0.636x + 1.12
Analysis methods											
PCC	0.948[d]	0.906[d]	NA[h]	NA	NA	0.985[d]	0.777[d]	0.984[d]	0.961[d]	0.874[d]	0.946[d]
Model	1,083x − 0.04	1.129x − 0.19				0.93x + 0.04	1.31x + 0.2	0.974x + 0.16	0.858x + 0.04	0.653x + 0.12	0.828x + 0.12

[a]Other *cis* lycopene = Indicates the sum of all *cis*-lycopene isomers excluding tetra-*cis*-lycopene.

[b]Total = The sum of *cis* isomers and all-*trans* configurations for a given carotenoid.

[c]PCC = Pearson Correlation Coefficient (r).

[d]Statistically significant at $P \leq 0.001$.

[e]Statistically significant at $P \leq 0.01$.

[f]Statistically significant at $P \leq 0.05$.

[g]Model = Linear model to convert carotenoid values from a rapid extraction or UHPLC-DAD method to values obtained using standard methods.

[h]NA = Not applicable.

Table 5.

Carotenoid concentration in tomatoes grown in multiple locations as a function of background, *Beta* allele, and analysis method

Background	Method	*B* allele	Sample size	Phytoene	Phytofluene	All-*trans* lycopene	Other *cis*-lycopene[a]	Total lycopene[y]	All-*trans* β-carotene	*cis*-β-carotene	Total β-carotene
Cherry, mg/100 g fresh weight ± SD	HPLC-DAD	97L97	12	0.49 ± 0.16^{cd}	0.13 ± 0.05^{d}	0.12 ± 0.21^{d}	0.05 ± 0.07^{d}	0.17 ± 0.28^{d}	3.51 ± 0.66^{e}	0.63 ± 0.05^{e}	4.15 ± 0.65^{e}
		JF	8	0.66 ± 0.18^{c}	0.21 ± 0.07^{c}	0.95 ± 0.38^{c}	0.28 ± 0.08^{ec}	1.23 ± 0.46^{c}	3.59 ± 1.25^{e}	0.56 ± 0.07^{e}	4.15 ± 1.38^{e}
		PU	12	0.43 ± 0.08^{d}	0.12 ± 0.03^{d}	0.21 ± 0.22^{d}	0.07 ± 0.06^{cd}	0.27 ± 0.28^{d}	3.57 ± 0.75^{e}	0.66 ± 0.02^{e}	4.22 ± 0.78^{e}
		Tainan	12	2.01 ± 0.47^{e}	0.80 ± 0.21^{e}	8.71 ± 2.02^{e}	0.95 ± 0.24^{e}	9.66 ± 2.23^{e}	0.91 ± 0.36^{c}	0.25 ± 0.21^{c}	1.16 ± 0.32^{c}
	UHPLC-DAD	97L97	12	0.47 ± 0.14^{cd}	0.32 ± 0.05^{c}	0.08 ± 0.03^{d}	0.06 ± 0.04^{c}	0.14 ± 0.05^{d}	4.44 ± 0.74^{e}	0.41 ± 0.06^{e}	4.84 ± 0.76^{e}
		JF	8	0.70 ± 0.38^{c}	0.37 ± 0.14^{c}	1.41 ± 1.60^{c}	0.12 ± 0.16^{c}	1.53 ± 1.76^{c}	3.75 ± 0.40^{e}	0.46 ± 0.16^{e}	4.21 ± 0.53^{e}
		PU	12	0.42 ± 0.09^{d}	0.33 ± 0.03^{c}	0.22 ± 0.24^{d}	0.08 ± 0.02^{c}	0.30 ± 0.24^{d}	4.42 ± 0.77^{e}	0.45 ± 0.08^{e}	4.87 ± 0.83^{e}
		Tainan	12	1.87 ± 0.30^{e}	0.79 ± 0.20^{e}	9.13 ± 2.02^{e}	0.60 ± 0.26^{e}	9.73 ± 2.13^{e}	0.98 ± 0.22^{c}	0.07 ± 0.02c	1.04 ± 0.24^{c}
Processing, mg/100 g fresh weight ± SD	HPLC-DAD	JF	16	0.65 ± 0.36^{c}	0.25 ± 0.15^{c}	2.14 ± 2.49^{d}	0.45 ± 0.28^{d}	2.59 ± 2.77^{d}	4.88 ± 2.08^{e}	0.38 ± 0.16^{e}	5.25 ± 2.04^{e}
		LA716	15	1.88 ± 0.66^{e}	0.62 ± 0.25^{e}	4.33 ± 2.54^{c}	0.82 ± 0.29^{c}	5.16 ± 2.78^{c}	3.23 ± 1.29^{e}	0.26 ± 0.12^{e}	3.48 ± 1.38^{e}
		OH8245	16	1.77 ± 0.61^{e}	0.68 ± 0.23^{e}	7.81 ± 2.86^{e}	0.94 ± 0.34^{e}	8.75 ± 3.15^{e}	0.92 ± 0.69^{c}	0.27 ± 0.14^{e}	1.19 ± 0.63^{c}
	UHPLC-DAD	JF	16	0.66 ± 0.66^{c}	0.38 ± 0.14^{c}	2.09 ± 2.48^{d}	0.15 ± 0.12^{c}	2.24 ± 2.59^{d}	5.59 ± 2.28^{e}	0.52 ± 0.24^{e}	6.12 ± 2.47^{e}
		LA716	15	1.70 ± 0.48^{e}	0.73 ± 0.20^{e}	4.59 ± 2.68^{c}	0.28 ± 0.26^{c}	4.88 ± 2.83^{c}	3.47 ± 1.31^{e}	0.38 ± 0.32^{e}	3.84 ± 1.51^{e}
		OH8245	16	1.78 ± 0.47^{e}	0.76 ± 0.18^{e}	8.57 ± 3.00^{e}	0.50 ± 0.17^{e}	9.07 ± 3.13^{e}	1.04 ± 0.55^{c}	0.08 ± 0.11^{c}	1.11 ± 0.63^{c}

[a]Other *cis* lycopene = Indicates the sum of all *cis*-lycopene isomers excluding tetra-*cis*-lycopene.

[b]Total = The sum of *cis* isomers and all-*trans* configurations for a given carotenoid.

[cde]Values with different letters within an analysis method are statistically different as determined by a Tukey's HSD test (α = 0.05).