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Abstract

The USDA, ARS Plant Genetics Resources Unit conserves over six-thousand cultivated tomato (Solanum lycopericum L.) lines that are made publically available for breeding and research through the National Plant Germplasm System. An ongoing challenge is to characterize the collection for traits that are of interest to end-users. During 2008 and 2009 we adopted and optimized laboratory methods to efficiently estimate fruit nutritional traits vitamin C (ascorbic acid), titratable acids, °brix and lycopene. Results were highly reproducible and we have found significant differences among genotypes for the various traits. These data will be made available through the Germplasm Resources Information Network http://www.ars-grin.gov/gen . Here we present the protocols and work scheme for efficiently assaying hundreds of samples during a field season.
Optimized Work-flow for Assaying Tomato Fruit Quality: Vitamin C, Titratable Acids, Brix and Lycopene
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Abstract: The USDA, ARS Plant Genetics Resources Unit conserves over six thousand cultivated tomato (Solanum lycopersicum L) lines that are made publically available for breeding and research through the National Plant Germplasm System. An ongoing challenge is to characterize the collection for traits that are of interest to end-users. During 2008 and 2009 we adopted and optimized laboratory methods to efficiently estimate fruit nutritional traits vitamin C (ascorbic acid), titratable acids, "brix and lycopene. Results were highly reproducible and we have found significant differences among genotypes for the various traits. These data will be made available through the Germplasm Resources Information Network http://www.ars-grin.gov/geen . Here we present the protocols and work scheme for efficiently assaying hundreds of samples during a field season.

Materials & Methods: Pieces of one to ten fresh, washed fruits were homogenized in a commercial grade blender (A, B). Homogenate was poured into a 50 ml Falcon tube (C) and immediately frozen and stored. Homogenate was thawed 18-24 hr at 4°C, an aliquot was taken into a 5 cm petri dish for lycopene assay and 100 ul into a 1.5 ml tube for the vitamin C assay. Remainder was squeezed through double layers of cheese cloth, collecting the clear juice (D, E) fraction for the other two assays.

Lycopene:  
• Aliquot of homogenate (~10ml) transferred to 5 cm petri dish  
• Samples kept on ice in the dark until assayed. Readings taken using a Minolta Chroma Meter CR-300. Values were recorded for L*a*b* as the average of three measurements  
• Lycopene (ug/g) estimated using regression model based on the transformed a** value (Hyman et al. 2004)

Vitamin C assay:  
• 100 ul aliquot of thawed homogenate transferred to 1.5 ml eppendorf tube using large orifice pipette tip  
• 500 ul of freshly prepared 6% metaphosphoric acid added to the tube and vortexed  
• Centrifuged at 25,155 x g for 10 s at 4°C  
• 100 ul supernatant transferred to 1.5 ml tube containing 100 ul of freshly prepared 5% metaphosphoric acid to obtain a two-fold dilution. These were stored at -20°C  
• Cosmo Bio Co. Ltd. (Japan) kit (Prod. No. SML-RDIK02-EX) was used following the manufacturer’s protocol http://www.cosmobio.co.jp  
• Vitamin C (ug/ml) estimated using Tecan SpectraFluor (Tecan, Durham, NC) based on absorbance at 530 nm (reference 620 nm), using a standard curve of known concentrations.

Methods:

Homogenate poured into 50 ml Falcon tube (C) homogenized by vortexing (D) and strained through double layers of cheese cloth (E):

Refractometer

"Brix:  
• Model DR103L digital refractometer (QA Supplies, Norfolk, VA).  
• Droplets of strained, undiluted juice were added to window of refractometer via pipette tip.  
• Brix was calculated as a mean of three readings.

Titratable Acids:  
• Five-fold dilution was made by adding 40 ml of water to 10 ml strained homogenate  
• Used Brand Titrette® bottle-top digital buret  
• Recorded initial pH of sample  
• Titrated with 0.1 N NaOH to pH 7, then slowly titrated to pH 8.2  
• Percent citric acid was estimated based on ml NaOH using equation [14] of Sadler et al. (1998)

Literature Cited:  

Results and Discussion:  
• Two people working together on the same samples, but different assays can complete 24 samples per day including prep and cleanup.  
• Aliquots of homogenate were taken prior to start of all assays and placed in covered ice bucket for lycopene assay.  
• It takes ~ 20 min. per sample to assay titratable acids  
• The Vitamin C assay was separated into two stages. First extract Vitamin C, then store extracts at -20°C until 100 samples were ready to assay with the kit.

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