

In situ screening of quality traits in tomato cultivars using the handheld 4200 FlexScan FTIR spectrometer

Application note

Food testing

Authors

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Abstract

Chemometrics was employed to develop models that utilize infrared spectral measurements to estimate the concentrations of key analytes in tomatoes that determine quality. Models constructed using the handheld Agilent 4200 FlexScan FTIR spectrometer gave results comparable to those obtained using a benchtop spectrometer. The 4200 FlexScan spectrometer thus provides the ability to accurately assess the quality of tomatoes in the field in order to assure the quality of the end food product.



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Introduction

Monitoring the quality of tomatoes (*Lycopersicon esculentum*) is important at all levels in the food industry. While appearance, texture and flavor are the important quality attributes of fresh-market tomatoes, the major quality components of processing tomatoes are soluble solids, pH, titratable acidity, viscosity and color.¹ Sugars and organic acids, responsible for the sweetness and tartness, are the major factors affecting consumer acceptability² and the sugar-acid ratio is vital to flavor acceptability.³ In the tomato industry, soluble solids dictate the factory yield: the higher the soluble solids, the lesser the quantity of tomato that is required to produce processed tomato products such as pulp, paste and concentrate⁴. Similarly, pH plays a vital part in microbiological safety and food spoilage. Generally, the pH of tomatoes has been reported to range from 3.9 to 4.9, or in standard cultivars, 4.0 to 4.75.

The development of tomato varieties with altered nutritional profiles requires efficient selection and scientific competence to measure metabolite contents in thousands of samples⁶. Field-based devices can streamline quality assurance and Fourier-transform infrared (FTIR) techniques combined with chemometrics offer tomato processors and breeders powerful tools for the rapid assessment of tomato quality attributes. Portable IR units enable the food manufacturer to quickly assess the quality of the product, allowing for timely correction measures during manufacture. Portable systems are simple to use and require minimal or no sample preparation. They reduce assay time and help to streamline the analytical procedure so that it is more applicable to higher sample throughput and automation, providing in-situ assessment of the sample's composition.

This application note evaluates the performance of the novel Agilent 4200 FlexScan portable infrared units against a benchtop IR spectrometer for the determination of tomato quality parameters (Brix, pH, titratable acidity, glucose, fructose and citric acid). Models were constructed on both spectrometers using partial least squares regression (PLSR) to predict these quality parameters, using reference data collected by traditional refractometer and chromatography

methods. The 4200 FlexScan demonstrated comparable performance to the benchtop spectrometer for the measurement of these key quality parameters, with the added advantage of delivering that information to food scientists in the field, providing increased efficiency and efficacy in assuring the quality of the end food product.

Experimental

Samples

A total of 80 tomato varieties (genotypes) was grown in Fremont, Ohio at the Ohio Agricultural Research and Development Center (OARDC), North Central Agricultural Experimental Station in 2009. The varieties represent landrace varieties from Central and South America, and vintage varieties from North America and Europe.

At least 12 representative fruit from each variety were cut into quarters and a quarter from each fruit was pooled and blended into juice.

Reference measurements

Soluble solid content was measured using an Abbe refractometer. The overall pH of diluted samples of puree were measured and the samples were titrated to pH 8.2 using 0.1N NaOH to determine titratable acidity. This was reported as % citric acid using the conversion factor of 0.064 Equivalent wt. (mg/mEq).

Sugar and acid contents were analyzed using high performance liquid chromatography. For extraction of sugars, tomato juice aliquots (~2 mL) were passed through a mixed-mode C18 and strong cation exchange SPE column to remove interfering acidic components from the solution. For extraction of acids, aliquots of tomato juice (2 mL) were mixed with 2% metaphosphoric acid and centrifuged.

Infrared measurements

Spectral data from all tomato juice samples were collected in parallel using (1) a dry air purged benchtop Excalibur Series 3100 Fourier-Transform infrared spectrometer (Varian, now Agilent Technologies, Santa Clara, CA) incorporating a KBr beamsplitter, DTGS detector, and three reflection ZnSe ATR accessory; (2) a handheld 4200 FlexScan FTIR spectrometer

(Agilent Technologies Inc., Danbury, CT), with a ZnSe beamsplitter, DTGS detector and single-reflection diamond ATR sampling interface. Both spectrometers were operated at 4 cm⁻¹ spectral resolution.

Aliquots (0.5 mL) from thawed tomato juice samples were centrifuged at 12,000 rpm for 5 minutes at 25 °C, and two drops were applied to the surface of the ATR crystal for spectral data collection. The absorbance spectra of the room temperature samples consist of 64 co-added interferograms at 4 cm⁻¹ resolution measured in the 4000 to 600 cm⁻¹ mid-IR wavelength range. The spectrum of each individual sample, which took less than 2 minutes to acquire, was corrected against a background spectrum of air. Duplicate independent measurements were taken on each sample and background spectra were collected after every sample to account for environmental variations. Between measurements, the ATR surface was cleaned with a 70% ethanol solution.

Statistical analysis

Partial least squares regression (PLSR) analysis was used to correlate the infrared spectral results with those of the referee analysis methods. PLSR is a bilinear regression analysis method that decreases a huge number of variables into latent factors that are linear combinations of the spectral frequencies (X) used to ascertain the analyte's concentration (Y)⁷. This technique has the potential to estimate the component concentration, as well as the chemical properties of the spectra.⁸ This analytical approach offers a more information-rich data set of reduced dimensionality and eliminates data noise, resulting in more accurate and reproducible calibration models⁹.

Spectral data were exported from the spectrometers as .spc files and imported into a multivariate statistical program for partial least squares regression analysis in order to generate the calibration models. The calibration models correlated the spectra against the concentration of each tomato analyte (Brix, pH, titratable acids, glucose, fructose, and citric acid), which had been measured by the referee analysis method. Performance of these models was evaluated in terms of outlier diagnostics, standard error of cross-validation (SECV), and correlation coefficient (R). Distribution of

residuals and leverage were used for the evaluation of outliers. Any observation with atypical and large residual or leverage was re-analyzed and eliminated if it was considered a substantial outlier, and thereafter the model was recalculated. The calibration model that generated the best combination of minimum SECV, higher R and optimized numbers of latent factors was selected for each tomato analyte.

Results and discussion

The spectra of 160 (80 varieties, 2 replicate spectra per variety) juice samples appeared quite homogeneous upon visual inspection, and no outliers were identified. The major absorbance bands at approximately 3300 and 1635 cm⁻¹ (Figure 1A) arise from water in the samples and are consistent with those reported for other fruits and vegetables. The 1800–1000 cm⁻¹ fingerprint region exhibited peaks corresponding to the carbonyl stretching groups (1726 cm⁻¹), C=C stretching of ring vibration (1605 cm⁻¹, 1509 cm⁻¹), O-H deformation (1365 cm⁻¹) and C-OH stretching (1262 cm⁻¹, 1145 cm⁻¹, 1035 cm⁻¹) associated to sugars and acids.

Second derivative mathematical transformation of the spectra (Figure 1B) helps to resolve overlapping bands and eliminates the need for baseline correction, since the most significant offset and linear baseline errors are removed.¹⁰

PLSR analysis was used to generate models that are linear combinations of these spectral frequencies that correlate to the concentrations of analytes in the tomatoes that were determined using the reference analysis methods. Models were constructed using the data from both the benchtop and the 4200 FlexScan spectrometers. These models were then used to determine the concentrations of these analytes in samples from a total of 80 tomato varieties.

By using varieties representing the landrace and vintage 'core collection' used to study genetic variation in cultivated tomato under the Solanaceae Coordinated Agricultural Project, a large compositional range was obtained with varieties showing elevated sugar and organic acid contents (Table 1). In general, the values reported in this study are within those reported in the

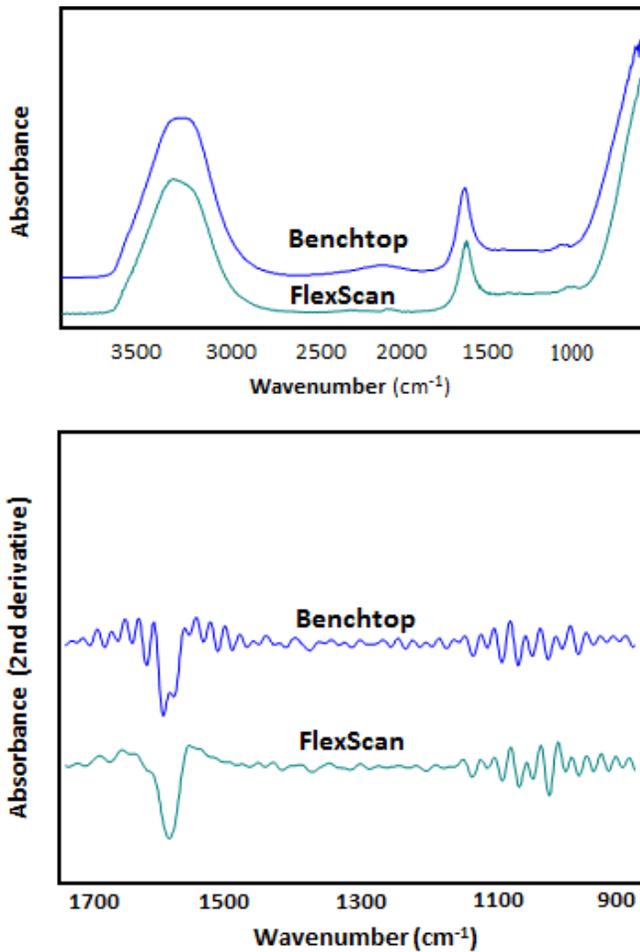


Figure 1. Attenuated total reflectance (ATR) infrared absorption spectrum of tomato sample (A) and its second derivative transformation (B) as shown on the benchtop Excalibur 3100 FTIR and portable Agilent 4200 FlexScan spectrometers

literature, taking into account that nutrient levels may be affected by variety, maturity, temperature and soil nutrients among others¹¹.

Since the benchtop spectrometer used a three-reflection ATR, it would follow that it would have higher sensitivity than the 4200 FlexScan handheld instrument equipped with a single reflection ATR. In spite of that and although the benchtop instrument provided the best quality parameter predictive models in general, the handheld 4200 FlexScan system generated very comparable correlation values (R) and SECVs (Table 1). The 4200 FlexScan handheld spectrometer can therefore provide reliable estimations of Brix, glucose, fructose, pH, titratable acids and citric acids in tomato samples

Table 1. Predictive models for determining tomato qualities by FTIR using a benchtop and the Agilent 4200 FlexScan spectrometers

Tomato analyte	Spectrometer	Concentration/pH range	SECV	R
Brix	Benchtop	4.40–7.50	0.36	0.79
	FlexScan		0.39	0.75
Fructose	Benchtop	1.37–5.70 g/100 g	0.32	0.87
	FlexScan		0.43	0.79
Glucose	Benchtop	1.03–5.75 g/100 g	0.43	0.89
	FlexScan		0.49	0.86
pH	Benchtop	3.90–4.76	0.06	0.92
	FlexScan		0.06	0.91
Titratable acids	Benchtop	0.27–0.88	0.05	0.91
	FlexScan		0.05	0.87
Citric acid	Benchtop	256.37–2015.93 mg/100 g	136.50	0.90
	FlexScan		123.40	0.92

in the field. This information enables optimization of the quality of the end food product, since it can be obtained before harvest and processing.

The estimated analyte contents measured by the ATR-IR spectroscopy showed some data scattering along the regression line (Figure 2), when compared to those determined by the reference methods (refractometer, pH meter, or HPLC), as the R value ranged from 0.75–0.92 (Table 1). However, the Brix measurements had a $\pm 0.1^\circ$ accuracy, which were further complicated by rounding errors, and coefficients of variability (%CV) for HPLC analysis were $\sim 20\%$, which could limit the precision of the PLSR prediction.

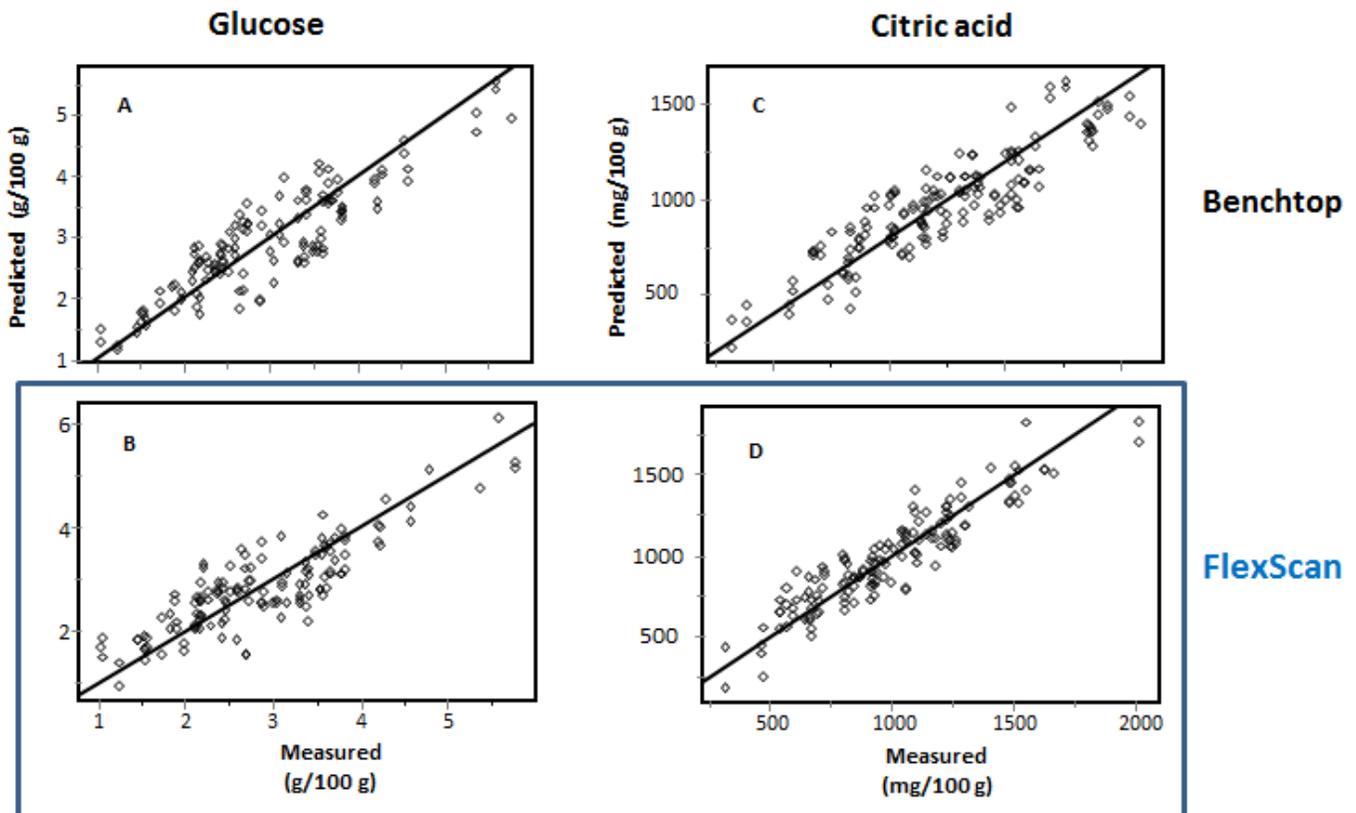


Figure 2. PLS regression plots of glucose (A and B) and citric acid (C and D) on the benchtop Excalibur 3100 FTIR (A and C) and 4200 FlexScan (B and D) spectrometers. The regression plots showed a good correlation per analyte across spectrometers.

Conclusions

Screening of quality parameters in tomatoes has been accomplished through the use of the handheld Agilent 4200 FlexScan FTIR spectrometer in the mid-infrared spectral region. A simple, quick and reliable technique was developed for the determination of key analytes and the results were comparable to those obtained with a benchtop unit. The ease of use, convenience and ruggedness offered by the handheld infrared spectrometer make it an ideal tool for food scientists and technicians to obtain vital information in the field. This tool can play a vital role in crop and process improvement studies, thus improving the quality assurance of the end food product.

References

1. Moraru, C., Logendra, L., Lee, T. C. & Janes, H. (2004). Characteristics of 10 processing tomato cultivars grown hydroponically for the NASA Advanced Life Support (ALS) Program. *J. Food Compos. Anal.* 17, 141–154.
2. Baldwin, E. A., Goodner, K. & Plotto, A. (2008). Interaction of volatiles, sugars, and acids on perception of tomato aroma and flavor descriptors. *Journal of Food Science*, 73, S294–S307.
3. Petroturza M. & Telekyvamosy, G. (1989). 3. Sensory evaluations. *Study on Taste Substances of Tomato*. *Nahrung*, 33, 387–394.
4. Gould, W. A. (1992). Tomato juice manufacture. *Tomato Production, Processing & Technology*. Gould W., ed., Baltimore, CTI Publications.

5. Sapers, G. M., Phillips, J. G. & Stoner, A. K. (1977). Tomato acidity and safety of home canned tomatoes. *Hortscience*, *12*, 204–208.
6. De Nardo, T., Shiroma-Kian, C., Halim, Y., Francis, D. & Rodriguez-Saona, L. E. (2009). Rapid and simultaneous determination of lycopene and beta-carotene contents in tomato juice by infrared spectroscopy. *J. Agric. Food Chem.* *57*, 1105–1112.
7. Martens, H. & Naes, T. (1989). Models for calibration. Chapter 3 in *Multivariate Calibration*. H. Martens & T. Naes, ed. John Wiley & Sons Ltd., London, UK.
8. Haaland, D. & Thomas, E. V. (1988). Partial least-squares methods for spectral analyses. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. *Anal. Chem.*, *60*, (11), 1193–1202.
9. Wold, S. (1999). Multivariate calibration — Direct and indirect regression methodology — Discussion and comments. *Scand. J. Stat.* *26*, 204–205.
10. Kohler, A., Bertrand, D., Martens, H., Hannesson, K., Kirschner, C. & Ofstad, R. (2007). Multivariate image analysis of a set of FTIR microspectroscopy images of aged bovine muscle tissue combining image and design information. *Anal. Bioanal. Chem.* *389*, 1143–1153.
11. Jones, R. A. & Scott, S. J. (1983). Improvement of tomato flavor by genetically increasing sugar and acid contents. *Euphytica*. *32*, 845–855.

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