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RESEARCH ARTICLE

Non-destructive quality evaluation of intact tomato using VIS-NIR spectroscopy

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Abstract

The potential of visible/near-infrared (VIS/NIR) absorbance spectroscopy in determining the quality of intact tomato (*Lycopersicum esculentum*), at varying stage of maturity were evaluated in the wavelength range of 299 nm to 1100 nm. Prediction models were built between Vis/NIR spectra and the major fruit properties viz. total soluble solids (TSS), acidity (pH), titratable acidity (TA), and lycopene content by using partial least squares regression (PLS) method. Different pre-processing methods were applied to improve the predictability of the model for each parameter. The best prediction results were achieved using PLS model after orthogonal signal correction (OSC) data treatment at a wavelength range of 370–1040 nm for all tested parameters. The coefficient of determination (R^2) for majority of parameters were found to be higher than 0.82 except for TA (R^2 =0.77, RMSEP= 4.08), and lycopene (R^2 = 0.79, RMSEP = 4.94). The R^2 value for TSS and pH were found to be 0.85 and 0.82, respectively. For tomato samples good correlation was found between the quality properties (TSS, pH, TA, and lycopene content) parameters. The standard errors of calibration, prediction, biases and differences in them were low, which indicated that spectroscopy has the potential to predict quality of tomato non-destructively.

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Introduction

Tomato (*Lycopersicon esculentum Mill.*) is a major vegetable crop, incredibly popular round the globe and one of the most important vegetable crops of the world (**FAOSTAT 2014**). It is widely consumed both in fresh or processed form. There are many health benefits of eating tomatoes, as they are a good source of vitamins A and C. They reduce the risk of cancer, osteoporosis and cardiovascular disease, because of the antioxidant properties (**Bhowmik et al. 2012**). Carotenes present in tomatoes are important antioxidants (**Fanasca 2006**). These antioxidants are important to protect human body cells from the harmful effects of free radicals, molecules that form in the body through contact with oxygen. Carotenes and lycopene amount in tomatoes vary significantly depending on maturity stage, variety and environment (**Brandt et al. 2006**).

The most common parameters of tomato that affect the consumer acceptability are total soluble solid (TSS), acidity (pH), lycopene content and color (**Shao et al. 2007**). The TA and TSS are important components of flavour. Tomato fruits high in both acids and sugars have good flavour, while bland tomatoes have low acidity and tart tomatoes have low sugar content (**Yahia and Brecht 2012**). Consumer preference to any fruit is driven by external aspect and some of physiological parameters. Color measurements have been used as quality parameters and indicator of some inner constituents of the material (**Jha 2010**). The complexity of tomato color is due to the presence of a diverse carotenoid pigment system, their appearance being conditioned by pigment types and concentrations, and subject to both genetic and environmental regulation. The maturity of tomatoes is classified into six stages depending on the fruit color change from green to red. The change in fruit color during ripening is mainly related to chlorophyll

degradation, as well as synthesis of lycopene which is responsible for the red color, and other carotenoids, as chloroplasts are converted into chromoplasts (**Arias et al. 2000**; **Lopez and Gomez 2004**; **Radzevicius et al. 2008** and **Radzevicius et al. 2009**).

Most of traditional methods leading to a measurement of these qualitative properties of tomato fruit are time and effort consuming as well as destructivein nature. Moreover, they require sample preparation, costly instruments and chemicals (**Nikbakht et al. 2011**). Near infrared (NIR) spectroscopy is now gaining popularity as fast, user friendly, cheaper and accurate method for internal quality assessment and sorting of vegetables. **He et al. (2005)** demonstrated the application of the VIS/NIRS spectroscopy in spectral range of 400-2350 nm to be useful in assessing the quality characteristics of tomatoes. The change in the interaction spectra of green tomatoes have been used for predicting maturity levels in the wavelength range of 600–750 nm (**Tiwari et al. 2013**).

Chen (2008) mentioned the significance of spectral range of 450-1000nm for lycopene, and found excellent prediction results using PLS model for lycopene content (R^2 = 0.96 and RMSEP =2.15). However, the poor predictive for TA and TSS with R^2 value of 0.49 and 0.03 and RMSEP of 0.43 and 0.15, respectively. **Jha and Matsuoka (2004)** developed a PLS model to predict acid-Brix ratio in tomato juice using wavelength range of 1059.5 to 1124.8 nm. VIS-NIR spectroscopy coupled with PLSR models had shown the potential for in situ determination of optimal harvest time of tomatoes (**Yang 2011**).

Clement et al. (2008) developed a regression model to predict tomato maturity stage (TMS) with RMSEP of 0.259 and R^2 of 0.93 by VIS/NIR spectroscopy. Thus the objective of this study was to assess the feasibility of determining quality parameters of intact tomato using VIS/NIR spectroscopy.

2. Materials and Methods

2.1. Tomato Samples

A total 143 samples of tomato (Naveen hybrid var.) were picked from CIPHET farm and brought to laboratory. Tomatoes free from any external injury/blemish were selected. All measurements, including spectral data collection and quality parameter determination (TSS, pH, TA and lycopene content) were carried out on the same day (day of harvesting). Tomato fruits were cleaned and equilibrated at room temperature (26–30 ˚C, RH 60–70%) approximately two hours before spectral acquisitions.

2.2. Optical Measurement

Spectra of tomatoes were acquired, in absorbance mode, using VIS/NIR spectroscopy (Avantes BV, Netherlands) in a spectral region of 299-1100 nm connected to 30 W halogen lamp and sample holder similar to **Jaiswal et al. (2012)** with 400 micron single optical fiber and spectra wiz software (version 3.3). A USB cable was used for the data transmission between the spectrophotometer and a portable computer. At an interval of every ten samples dark and reference spectra of a standard supplied with the equipment were acquired. The average of four spectra for each fruit (two at the distal area and two under equatorial zone in different fruit directions) were acquired and analysis.

2.3. Physicochemical analyses

After spectral measurements, each tomato fruit was cut into four equal parts and extracted juice from every part by using manual stainless steel squeezer, and the resultant tomato slurry was filtered through two layers of muslin cloth. The filtered tomato juice was used for carrying out physicochemical analyses.

2.3.1. Total soluble solids (TSS)

TSS was determined using portable digital refractometer (ERMA, Japan) with a scale of 0–32 ˚Brix (least count 0.2°Brix) at room temperature (~30 ˚C).

2.3.2. pH

The pH measurements were made using a digital pH meter (EcoTestr pH 2 Waterproof Pocket Tester) calibrated with pH 4.0 and 7.0 buffers.

2.3.3. Titratable acidity (TA)

Titratable acidity was determined according to the AOAC official method 942.15 (**AOAC 2000**). Five grams of tomato juice diluted in 25 ml of distilled water and titrated by 0.1N sodium hydroxide (NaOH) to pH 8.1. The titratable acidity was expressed as g citric acid/kg tomato, according to the following equation:

$$\text{Titratable acidity (g citric acid/kg of tomato)} = (V \times 0.1 \times 1000 \times 0.064)/m \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where: 0.1 is the normality of NaOH (N), 0.064 is the conversion factor for citric acid, V is the volume of NaOH required (mL) and m is the mass of tomato juice sample used (g).

2.3.4. Lycopene content

Fresh tomato juice was carefully weighed (4 ± 0.01 g) into a 200mL flask wrapped with aluminum foil to protect it from exposure to light. A 100ml mixture of hexane-acetone-ethanol, 2:1:1 (v/v %), was added to the flask and agitated continuously for 10 minutes on an orbital shaking incubator. Thereafter 15 ml of water was added followed by agitation for another 5 minutes. The solution was then left for separation into distinct polar and non-polar layers and filtered using filter paper (Whatman grade 42). Lycopene concentration was estimated by measuring the absorbance of the extract at 503 nm by UV/VIS Spectrophotometer (SHIMADZU, Japan, Model UV-1800) using hexane as a blank (**Ranveer et al. 2013**). Six independent measurements were done for individual fruit. The lycopene concentration was calculated using its specific extinction coefficient (E1%, 1 cm) of 3120 in hexane at 503 nm. The lycopene concentration was expressed as mg/kg fresh tomato, and calculated by the following formula:

$$\text{Lycopene (mg/kg fresh wt.)} = (A_{503} \times 537 \times 100 \times 0.55) / (4 \times 172) \quad \text{..... (2)}$$

$$= A_{503} \times 42.9 \quad \text{..... (3)}$$

Where: 537 g/mole is the molecular weight of lycopene, 100 ml is the volume of mixed solvent, 0.55 is the volume ratio of the upper layer to the mixed solvents, four grams are the weight of tomato added, and 172 mM^{-1} is the extinction coefficient for lycopene in hexane.

2.4. Data analysis

Data analysis was performed using Unscrambler software (The Unscrambler X version 10.2, CAMO Software AS, Oslo, Norway). Partial least square regressions (PLS) were carried out to develop linear models of prediction between spectral data and the different fruit quality parameters determined through wet lab analyses. Several pre-processing techniques e.g., smoothing Savitzky-Golay, maximum normalization, range normalization, Quantile Normalization, Baseline Offset Correction (BOC), Standard Normal Variate (SNV), Orthogonal Signal Correction (OSC), and Multiplicative Scattering Correction (MSC) were applied to the original spectral data, to reduce the systematic noise and variation. Then, the samples were divided into calibration (110 samples) and validation (33 samples) sets by applying the random selection (**Costa and de Lima 2013**). Full cross validation procedures were used for calibration and prediction. The validation set was used to test the predictability of the PLS models. The efficiency of models were evaluated based on coefficient of determination value (R^2), the root mean square error of calibration (RMSEC) and root mean squared error of prediction (RMSEP) (**Jaiswal et al. 2012** and **Jha et al. 2014**).

3. Results and Discussion

Typical absorbance spectra of tomatoes in the wavelength range of 350-1050nm is presented in **Figure 1.** Each spectral curve represents the average four spectra of same sample.

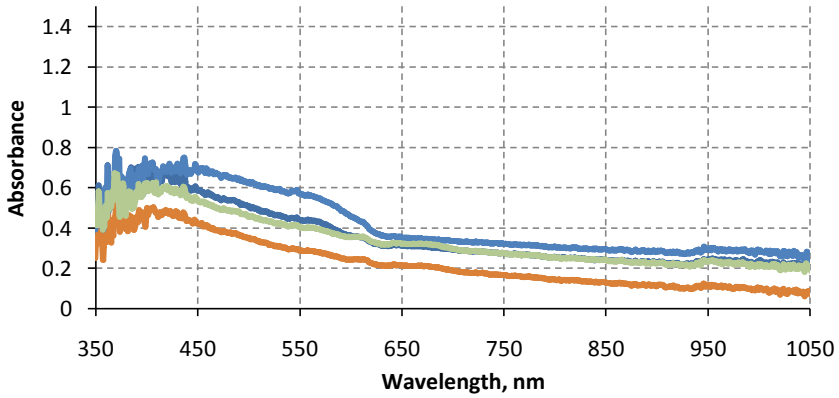

Figure 1. Original VIS/NIR average spectra curve of some samples of tomatoes at wavelength 350-1050nm.

The statistical details e.g ranges, means and standard deviations of all quality parameters of samples, are shown in **Table 1**. The coefficient of variation for TSS, lycopene content, TA, and pH of the prediction set were 0.12, 0.69, 0.17, and 0.07, respectively.

Table 1. Statistical details of the samples used in calibration and prediction sets.

Parameters	Calibration(110 samples),(440 spectra)					Prediction set (33 samples), (132 spectra)				
	Mean	Min.	Max.	SD[a]	CV[b]	Mean.	Min	Max.	SD[a]	CV[b]
TSS	4.61	3.08	6.83	0.60	0.13	4.76	3.93	5.9	0.56	0.12
Lycopene	18.90	0.51	40.22	10.53	0.56	17.22	0.52	41.45	11.00	0.69
TA	53.33	33.6	71.2	8.31	0.16	54.92	41.6	74.00	9.10	0.17
pH	3.69	3.2	4.2	0.23	0.06	3.64	3.3	4.2	0.25	0.07

Notes: SD[a], standard deviation; CV[b], coefficient of variation

Initially calibration models were developed on the whole range of spectra (299.484–1100.050) for predicting various qualities attributes of tomatoes non-destructively. Thereafter the numbers of wavelengths were sequentially minimized to select best performing group of wavelength so as to reduce the cost of instrument.

In order to reduce systematic noise and variation in the spectra, the original spectra were transformed by Savitzky-Golay Smoothing (SGS), maximum normalization, range normalization, Quantile Normalization, Baseline Offset Correction (BOC), Standard Normal Variate (SNV), and Multiplicative Scattering Correction (MSC) techniques. The best prediction results were achieved using PLS model after OSC data treatment at a wavelength range of 370–1040 nm for all tested parameters. Orthogonal signal correction (OSC) is a pre-processing technique used for removing the information unrelated to the target variables based on constrained PLS model and principal component analysis (**Niazi and Azizi 2008**). OSC is a suitable pre-processing method for PLSs calibration of mixtures without loss of the prediction capacity using a spectrophotometric method. **Wold et al. (1998)** reported that best results were obtained with orthogonal signal correction (OSC) and gave substantial improvements of NIR spectra. While **Bohac et al. (2002)** mentioned that one of OSC components is optimal for the signal correction and reduces the X variance by about 40%, this provide PLS models with a better predictive ability. In all parameters MSC was applied effectively to reduce the offset originally present in the spectra.

Table 2. Results obtained for TSS and lycopene using the PLS models for all samples based on the absorbance spectra (370-1040 nm).

	Pre-processing method	Calibration			No. of components	Full-cross validation		
		RMSEC	R^2	Bias		RMSEP	R^2	Bias
TSS (°Brix)	None	0.20	0.89	-0.00		0.25	0.82	-0.0041
	SGS	0.22	0.86	-0.00	7	0.27	0.79	-0.0065
	Maximum Normalization	0.25	0.83	-0.00	7	0.32	0.72	-0.0038
	Range Normalization	0.22	0.86	-0.00	7	0.29	0.76	-0.0047
	Quantile Normalization	0.20	0.89	-0.00	7	0.24	0.83	0.0046
	BOC	0.21	0.87	-0.00	7	0.26	0.80	0.0043
	SNV	0.23	0.85	-0.00	7	0.29	0.76	0.01
	MSC	0.23	0.85	0.00	6	0.29	0.76	0.0088
	OSC	0.02	0.99	-0.00	6	0.02	0.99	-0.00
Lycopene (mg/kg)	None	4.30	0.84	0.00	2	4.94	0.79	-0.086
	SGS	4.39	0.83	0.00	5	4.96	0.78	-0.047
	Maximum Normalization	5.39	0.74	0.00	5	5.98	0.69	0.018
	Range Normalization	5.01	0.78	0.00	4	5.28	0.75	0.016
	Quantile Normalization	4.26	0.84	0.00	3	4.87	0.79	-0.0837
	BOC	4.56	0.81	0.00	5	5.32	0.75	-0.175
	SNV	4.65	0.81	0.00	4	4.89	0.79	-0.0207
	MSC	4.65	0.81	0.00	2	4.89	0.79	-0.0251
	OSC	0.88	0.99	0.00	2	0.91	0.99	0.0086

3.1. Total soluble solids (TSS)

The coefficient of determination (R^2) value for calibration and validation of TSS using PLS was found to be higher in the wavelength range 370-1040 nm. The R^2 values were found to be 0.89 for calibration and 0.92 for validation

(**Table 2**). The pre-processing of spectra, such as SGS, Normalization, BOC, MSC, OSC were employed for improvement of calibration model. However, none of them produced better calibration models except the PLS model developed after OSC processing (R^2 = 0.99).

The PLS regression model was used for measuring the model's ability in TSS prediction for 33 samples (prediction set). The developed model was found to be more suitable for TSS prediction. Scatter plots of the model developed based on data in the wavelength of 370-1040 nm has been shown in **Figure 2, A**. It showed highly adequate in the correlation between the measured TSS values of intact tomato along with prediction. The PLS regression model curve was indicated R^2= 0.895, RMSEP = 0.18 and Bias = -0.002 for prediction set samples were used in TSS prediction. The results closely resemble as in the study of (**He et al. 2005**; **Pedro and Ferreira 2007**; **Xie et al. 2008**; **Sirisomboon et al. 2012**; **Zhang et al. 2013**; and **Ecarnot et al. 2013**).

3.2. Lycopene content

Lycopene could be predicted in the wavelength range 370-1040 nm using PLS with R^2 values calibration and validation of 0.84 and 0.79, respectively without any data processing. The performance of model was significantly improved by application of OSC pre-processing (R^2 =0.99). Rest of the techniques did not produce better calibration model when the PLS regression model was applied to predict lycopene content for 33 prediction samples, the prediction results (**Figure 2, B**) showed the R^2 of 0.80, RMSEP of 4.83 and Bias of 1.27. This result was superior with **Baranska et al. (2006)** for estimating lycopene in tomato fruits (R^2 =0.85 and SEP =91.19) using NIR spectroscopy.

Table 3. Results obtained for TA and pH using the PLS models for all samples based on the absorbance spectra (370-1040 nm).

	Pre-processing method	Calibration			No. Of components	Full-cross validation		
		RMSEC	R^2	Bias		RMSEP	R^2	Bias
TA (g/kg)	None	3.45	0.83	0.00	7	4.08	0.77	0.0102
	SGS	3.54	0.82	0.00	7	4.34	0.74	0.0901
	Maximum Normalization	3.90	0.79	0.00	7	5.24	0.62	-0.0263
	Range Normalization	3.70	0.81	0.00	7	4.89	0.67	-0.0211
	Quantile Normalization	3.46	0.83	0.00	7	4.06	0.77	-0.0347
	BOC	3.70	0.81	0.00	6	4.32	0.74	-0.0169
	SNV	3.91	0.79	0.00	5	4.62	0.71	0.0416
	MSC	3.93	0.78	0.00	5	4.61	0.71	0.0417
	OSC	0.88	0.98	0.00	2	0.90	0.98	-0.0087
Acidity (pH)	None	0.087	0.86	0.00	5	0.10	0.82	-0.0139
	SGS	0.088	0.86	0.00	5	0.10	0.83	-0.0009
	Maximum Normalization	0.10	0.80	0.00	5	0.12	0.73	0.0014
	Range Normalization	0.09	0.84	0.00	6	0.11	0.77	0.0009
	Quantile Normalization	0.087	0.86	0.00	5	0.10	0.81	-0.0013
	BOC	0.09	0.85	0.00	5	0.11	0.80	0.0009
	SNV	0.10	0.82	0.00	4	0.11	0.76	0.002
	MSC	0.1	0.82	0.00	4	0.12	0.76	0.0019
	OSC	0.025	0.98	0.00	7	0.037	0.98	0.0013

3.3. Titratable acidity (TA)

The best performing wavelength range for developing calibration model using PLS for prediction of TA was found to be 370-1040 nm (**Table 3**).The R^2 values calibration and validation of 0.83 and 0.77, respectively without any data processing. It was observed that any treatment given to the base data did not improve the accuracy of prediction except the PLS model developed after OSC processing (R^2 = 0.98) as shown in **Table 3**. The probable reason for the comparatively lower R^2 value for TA is the covalent bond between carbon and oxygen in the acid functional group (–COOH), having lower absorbance as compared to C-H and O-H bonds (**Cayuela 2008**; **chen 2008** and **Flores et al. 2012**).

For using prediction set samples, the PLS prediction results for TA is presented in the scatter plots shown in (**Figure 2, C**), the ordinate and abscissa axes represent the predicted and measured fitted values. The correlation between them was 0.78, and the RMSEP was 4.41.

3.4. Acidity (pH)

Similar to other parameters pH was also best predicted in the wavelength range of 370-1040 nm with R^2 values calibration and validation of 0.86 and 0.82, respectively without any data processing. Application of OSC improved the predictability of the model (0.98). Rest of the methods did not have any influence on the accuracy of the model. Predicted results of a PLS model for the prediction set samples present in scatter plots shown in (**Figure 2, D**), which are RMSEP = 0.1, R^2 = 0.82, and bias = 0.002. **He et al. (2005)** and **Shao et al. (2007)** obtained the results with SEP=0.096, r = 0.83, and RMSEP = 0.251 and r = 0.83 both of which were superior to the results in this research.

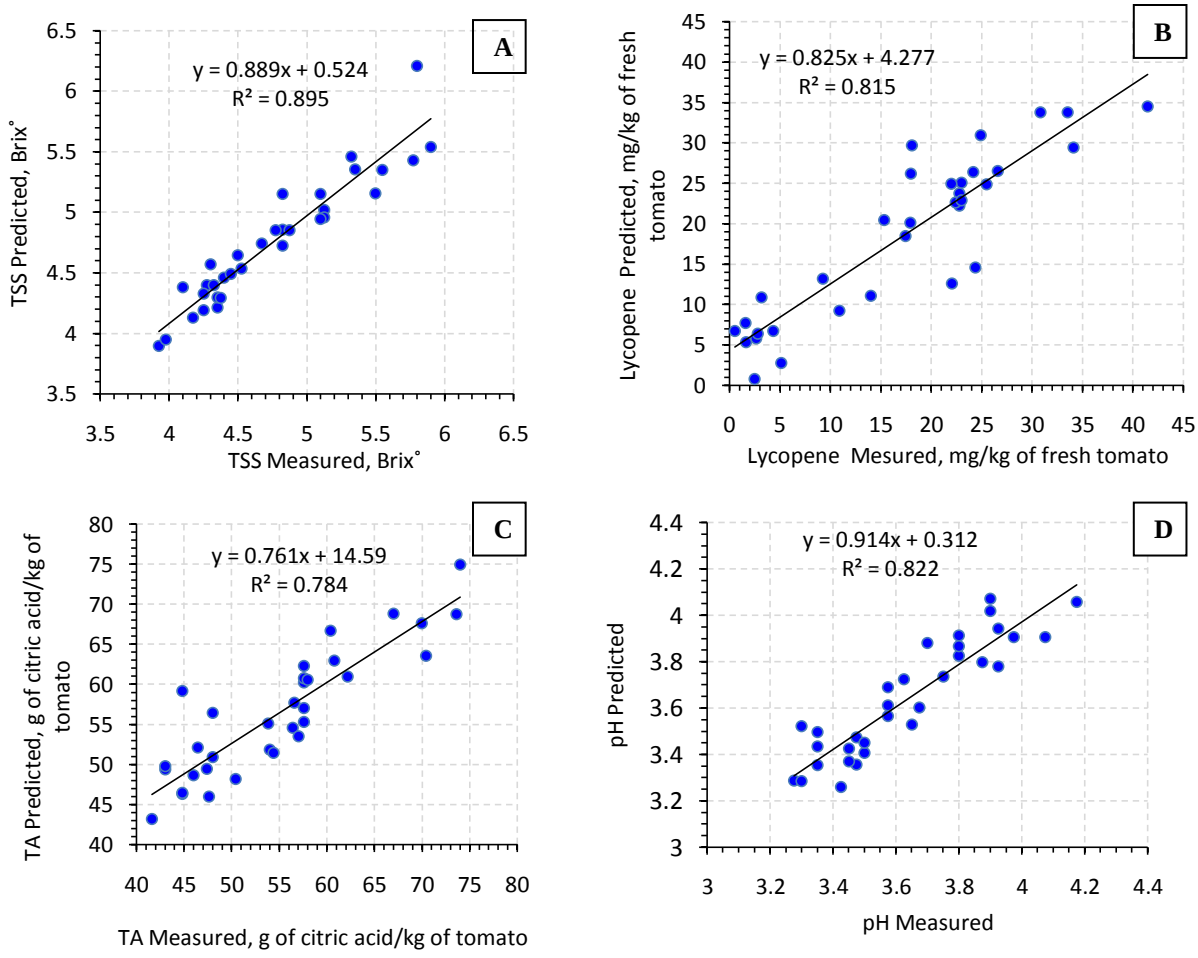

Figure 2. Predicted concentration vs. reference measured concentration of the properties of the prediction set for the optimal PLS models: **A.** TSS (Brix˚), **B.** Lycopene content (mg/kg fresh wt.), **C.** TA (g citric acid/kg of tomato) and **D.** Acidity (pH).

4. Conclusion

In this study, the VIS/NIR spectroscopy was used as rapid and non-destructive method to estimate the TSS, lycopene content, TA, and pH of intact tomato. It can be concluded that VIS/NIR is a very promising technique for the non-destructive quantification of important parameters in tomato as well as other fresh produces. It must be highlighted that the results obtained from the analysis of intact tomatoes, without any preliminary sample preparation, could be used in combination with VIS/NIR technology for online control tomato sorting.

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