Investigation of Raman chemical imaging for detection of lycopene changes in tomatoes during postharvest ripening

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A B S T R A C T

Lycopene is a major carotenoid in tomatoes and detecting changes in its content can be used to monitor the ripening of tomatoes. Raman chemical imaging is a new technique that shows promise for mapping constituents of interest in complex food matrices. In this study, a benchtop point-scan Raman chemical imaging system was developed to detect and visualize internal lycopene distribution during postharvest ripening of tomatoes. Tomato samples at different ripeness stages (i.e., green, breaker, turning, pink, light red, and red) were selected and cut open for imaging. Hyperspectral Raman images were acquired from fruit cross-sections in the wavenumber range of 200–2500 cm⁻¹ with a spatial resolution of 1 mm. A polynomial curve-fitting method was used to correct for the underlying fluorescence background in the original spectra. A hyperspectral image classification method was developed based on spectral information divergence to identify lycopene in the tomato cross-sections. Raman chemical images were created to visualize the spatial distribution of the lycopene for different ripeness stages. The system was also configured to test the feasibility of utilizing spatially offset Raman spectroscopy (SORS) technique for subsurface detection of a Teflon slab placed under samples of outer pericarp cut in 5-mm and 10-mm thick slices from green and breaker tomatoes. The results showed that the Raman spectrum of Teflon can be extracted from the SORS measurements of the pericarps placed over the Teflon, demonstrating the potential of the future development of a Raman-based nondestructive approach for subsurface detection of lycopene as an indicator of tomato maturity.

1. Introduction

The tomato is the second most produced vegetable in the US and one of the most widely consumed vegetables in the world. Tomatoes are harvested at different ripeness stages to satisfy different consumption requirements (e.g., fresh and processed). Based on the surface color, six ripeness stages (i.e., green, breaker, turning, pink, light red, and red) are defined for red-fleshed tomatoes (USDA, 1991). Also, four maturity stages (i.e., M-1–M-4) are defined for green tomatoes (Sargent and Moretti, 2004). Tomatoes harvested at the M-1 immature-green stage will not ripen to a quality suitable for consumption. M-3 and M-4 are mature-green stages, and fruit picked at these stages will ripen to high quality. Tomatoes harvested at the M-2 stage will ripen to moderate quality. Since these four stages are defined according to the internal fruit appearance (e.g., status of seeds and gel), it is challenging to accurately determine them without cutting open the fruit. Harvest at an inappropriate maturity stage is one of the major causes for postharvest losses in tomato production. Also, inconsistent harvest at varying maturity stages can discourage consumer consumption due to poor quality and potential food safety risks (e.g., greater occurrence of spoilage from the mixed presence of over-ripe and immature fruit). Accurate maturity evaluation is therefore critical for tomato production.

The tomato ripening process is associated with the loss of chlorophyll and the accumulation of carotenoids (e.g., lutein, β-carotene, and lycopene) in the fruit. The fruit colors of green, yellow/orange, and red are generally attributed to chlorophyll, lutein/β-carotene, and lycopene, respectively. As the major carotenoid, lycopene contributes more than 90% to the total pigmentation in full-ripe tomatoes, and a 500-fold increase occurs for the lycopene content during the tomato ripening (Heuvelink, 2005). Since lycopene is first generated within the interior of tomato fruit, the external color changes generally lag behind the internal composition changes, especially for tomatoes at the green stage. Hence surface appearance alone may not be an adequate indicator of the actual ripening status of the tomato fruit. Although tomato surface color is conventionally used as a maturity index, there is room for improvement by measuring internal fruit compositions. Lycopene content can provide...
complementary information to surface color. Methods that can non-
destructively achieve accurate measurement of lycopene content
are needed for assessing the internal maturity of tomatoes.

Traditional methods for evaluating lycopene and other carote-
noids in tomatoes, such as high-performance liquid chromatogra-
phy (HPLC), require complicated procedures for sample prep-
aration, processing, and analysis. Hence they cannot satisfy the
requirements of rapid and nondestructive measurement, despite
the accurate results from the time-consuming process. Various
optical methods have been investigated for rapid, accurate, and
nondestructive evaluation of the quality and maturity of tomatoes.
These have included near-infrared spectroscopy (NIR) to assess sol-
uble solids content (Slaughter et al., 1996) and carotenoid content
(Pedro and Ferreira, 2005), surface color measurement to estimate
internal lycopene content (Arias et al., 2000), hyperspectral reflect-
tance imaging (Polder et al., 2004) and laser-induced fluorescence
spectroscopy (Lai et al., 2007) to quantify surface pigmentation,
absorption and scattering properties to evaluate tomato maturity
and firmness (Qin and Lu, 2008), and magnetic resonance imaging
(MRI) to assess internal structure changes (Musse et al., 2009)
and lycopene content (Cheng et al., 2010) during tomato ripening.
Most of these methods have involved the use of chemometric analysis or
multivariate modeling. Non-optical methods based on other phys-
ical principles have also been investigated, such electronic nose
technique for evaluating tomato maturity (Gómez et al., 2006)
and ultrasonic monitoring of intact tomatoes for firmness and su-
gar content (Mizrahi, 2007).

Raman techniques have recently been explored for sample-
destructive analysis of carotenoids in plant materials. Carotenoids
exhibit two strong Raman peaks in two separate spectral regions,
1100–1200 and 1400–1600 cm\(^{-1}\), due to the stretching vibrations
of the carbon–carbon bonds in the polyene chain (Withnall et al.,
2003). These spectral features can be used to identify and quantify
carotenoids in various plant tissues. Bhosale et al. (2004) used re-
sonance Raman spectroscopy to estimate carotenoid contents of se-
lected fruits and vegetables. The measurement results were found
to be correlated with those determined by the HPLC method.
Schulz et al. (2005) utilized a Fourier transform Raman spectrom-
ter to assess various carotenoids in a range of plant tissues, and
created 2-D Raman images to map the carotenoid contents of se-
lected plant samples such as leaves and flowers. Baranska et al.
(2006) compared FT-Raman, ATR-IR, and NIR to evaluate lycopene
and β-carotene contents in tomatoes; ATR-IR produced the best
prediction results, followed by FT-Raman which outperformed
NIR owing to the characteristic Raman peaks from the two types
of the carotenoids.

In an effort to noninvasively retrieve internal information from
diffusely scattering media, a new sensing technique called spatially
offset Raman spectroscopy (SORs) was proposed by Matousek et al.
(2005). This technique aims to acquire subsurface information by
collecting Raman scattering signals from different positions on
the sample surface that are laterally offset from the incident point
of the excitation laser. The Raman spectra collected in this manner
reveal different relative scattering intensities of the surface and
subsurface layers. Pure Raman spectra of the individual sample lay-
ers can be extracted by analyzing a series of spatially offset Raman
spectra. This technique provides a useful means for separating the
signals from the surface and internal layers, and has been success-
fully used for noninvasive measurement of human tissues, such as
in vivo evaluation of human bone (Matousek et al., 2006).

Raman chemical imaging is a novel technique that combines
Raman spectroscopy and digital imaging to map composition and
morphology of a target. This technique has great potential for tack-
ling challenging problems in the area of food quality and safety
evaluation. A Raman chemical imaging system was recently devel-
oped in our laboratory (Qin et al., 2010). The system is a versatile
platform that gives us more flexibility for experiment design than
that provided by commercially available systems. Utilizing this
system, new research was recently begun on qualitative evaluation
of tomato maturity. The long-term goal of this project is to provide
a Raman-based nondestructive sensing approach for monitoring
the internal maturity of tomatoes. For this goal, it is necessary to
determine what changes in internal lycopene distribution actually
occur within the tomatoes during the postharvest ripening. This
study aimed first to develop a Raman chemical imaging method
to visualize the spatial changes in lycopene distribution that occur
inside ripening tomatoes, and then to begin investigation into the
feasibility of spatially offset Raman spectroscopy to nondestruc-
tively and directly detect internal fruit attributes (e.g., lycopene).
Specific objectives were to:

- use the Raman chemical imaging system to measure the Raman
  scattering signals from cut tomatoes at different ripeness stages;
- develop algorithms for fluorescence background correction and
  hyperspectral image classification for identifying lycopene in
tomatoes;
- visualize the lycopene generation patterns that develop inside
tomatoes during the postharvest ripening process by Raman
chemical imaging; and
- test the feasibility of nondestructive spatially offset Raman
  spectroscopy measurements for subsurface detection of a refer-
  ence Raman signal through outer pericarps from tomatoes.

2. Materials and methods

2.1. Raman chemical imaging system

A point-scan Raman chemical imaging system (Fig. 1) was devel-
oped for macro-scale imaging of samples such as tomatoes. A 16-bit
CCD camera with 1024 × 256 pixels (Newton DU920N-BR-DD, An-
dor Technology, South Windsor, CT, USA) was used to collect Raman
signals. A Raman imaging spectrometer (Raman Explorer 785,
Headwall Photonics, Fitchburg, MA, USA) was mounted to the cam-
era. The spectrometer accepts light through an input slit (5 mm
long × 100 μm wide), and it covers a Raman shift range of –98 to
3998 cm\(^{-1}\) (or a wavelength range of 779–1144 nm) with a spectral
resolution of 3.7 cm\(^{-1}\). A 785 nm laser module (I0785MM0350MF-
NL, Innovative Photonic Solutions, Monmouth Junction, NJ, USA)
served as the excitation source. The laser power at the sample
surface was 350 mW, which was measured by a handheld power
meter (NT54-018, Edmund Optics, Barrington, NJ, USA). A fiber
optic Raman probe (RPB, InPhotonics, Norwood, MA, USA) was used
to focus the laser and acquire Raman signals. A bifurcated fiber
bundle was used to deliver the laser light to the probe and transfer
the collected Raman signals to the imaging spectrometer. A two-
axis motorized positioning table (MAX4009W1-S4, Velmex,
Bloomfield, NY, USA) was used to move the samples in two perpen-
dicular directions (X and Y as illustrated in Fig. 1), with a displace-
ment resolution of 6.35 μm across a square area of 127 × 127 mm\(^2\).
The Raman probe, the positioning table, and the sample materials
were placed in a closed black box to avoid the influence of ambient
light.

These positions of the camera, which defines a rectangu-
lar area on the CCD to only include pixels illuminated by the incom-
ing light, was used for spectral data acquisition to ensure the best
possible signal-to-noise ratio. Spectral calibration was performed
using two Raman shift standards (i.e., polystyrene and naphtha-
lene) and a quadratic regression model. Based on the calibration re-
sult, the imaging system was found to cover a wavenumber range
of 200–2500 cm\(^{-1}\). System software was developed using LabVIEW
(National Instruments, Austin, TX, USA) to fulfill various functions
such as camera control, data acquisition, sample movement, and synchronization. The 3-D Raman image data were saved in the format of Band Interleaved by Pixel (BIP), which can be analyzed by commercial software packages such as ENVI (ITT Visual Information Solutions, Boulder, CO, USA). More detailed system description can be found in Qin et al. (2010).

To investigate the capacity of spatially offset Raman spectroscopy for subsurface detection, the bifurcated fiber bundle previously used both to illuminate and to collect Raman signals was configured only for signal collection, while a separate optical fiber and a laser focus unit were implemented for illumination, enabling offset measurements. The experimental setup is illustrated in Fig. 2. The tomato pericarp was placed on a Teflon slab, which was used as a subsurface reference material known to exhibit identifiable Raman peaks. The laser focus unit consisted of a fiber optic collimator (NT47-219, Edmund Optics), a 785 nm laser line bandpass filter (LL01-785-12.5, Semrock, Rochester, NY, USA), and a 50 mm focus lens (AC127-050-B-ML, Thorlabs, Newton, NJ, USA). The laser light was collimated, purified, and focused after going through these three optical components, respectively. The samples and the laser focus unit were placed on the positioning table to be moved along the X direction (see Fig. 1) as the Raman signals were acquired by the fixed Raman probe. As a result, a series of spectra with different source-detector distances were collected to form a set of spatially offset Raman spectra. The offset spectra were then analyzed to determine whether the Raman signals of the Teflon slab could be recovered.

Fig. 1. Raman chemical imaging system for acquiring images from tomato samples.

Fig. 2. Diagram of experimental setup for spatially offset Raman spectroscopy measurement for tomato outer pericarp samples placed on Teflon slab.
Fig. 3. (a) Tomato fruit samples at different ripeness stages and (b) top view of tomato outer pericarps sliced from green and breaker samples.

Fig. 4. Reference Raman spectra of (a) lycopene and (b) Teflon.
2.2. Experimental samples and procedures

Tomatoes (‘sunbright’ cultivar) were handpicked from a local grower’s field in Beltsville, Maryland, in August 2010. All the samples were at the mature-green stage when picked, and they were kept at room temperature during the whole postharvest ripening process (i.e., from mature-green to full-ripe). Fruit samples at different ripeness stages (i.e., green, breaker, turning, pink, light red, and red) were selected and cut before imaging. There were three samples for each stage from green to pink, and six samples for the light red stage and the red stage, respectively. A total of 24 samples were tested in this study, and the photos of their cross sections are shown in Fig. 3a. During image acquisition, the sample was placed on a rubber cup fixed on the positioning table. The imaging system scanned the cross section of each tomato using a CCD exposure time of 0.2 s and a step size of 1 mm for both $X$ and $Y$ directions, resulting in a $100 \times 100 \times 1024$ hypercube covering a spatial area of $100 \times 100 \text{mm}^2$. Under these settings, it took approximately 2.5 h to finish the scan of each sample.

Outer pericarp samples (Fig. 3b) were cut from green and breaker tomatoes using a food slicer with adjustable thickness control (GS300, General Slicing, Weston, FL, USA). Two pericarps were prepared from the green tomatoes—one of maximum 5 mm thickness and one of maximum 10 mm thickness. Two pericarps were similarly prepared from the breaker tomatoes. The 5-mm and 10-mm thickness of the slices were chosen to be representative of the typical thickness of the outer pericarp of the tomato cultivar used in this study. Raman spectra were acquired in the offset range (Δx, Δy) of 0–5 mm with a step size of 0.2 mm, resulting in a total of 26 spectra for each pericarp/Teflon pairing. Dark current data were acquired with the laser off and a cap covering the probe, and subtracted from the original sample data. Only the corrected data were used for further analysis.

The Raman spectra of pure lycopene and the Teflon slab were also measured to provide reference spectra. The options for direct measurement of lycopene were limited by the small amounts in which pure lycopene is commercially available. Hence a lycopene solution was made by using chloroform, a common solvent for lycopene. One milligram of lycopene powder (L9879, Sigma–Aldrich, St. Louis, MO, USA) was dissolved in 1 ml of chloroform (650498, Sigma–Aldrich). The background Raman spectrum of pure chloroform was acquired, and the lycopene spectrum was obtained by subtracting the chloroform spectrum from that of the lycopene solution.

2.3. Raman spectral and image analysis

2.3.1. Fluorescence background correction

Autofluorescence of biological materials is a major challenge for Raman spectroscopy applications. Various mathematical methods have been developed for removing the underlying fluorescence baseline in Raman spectra, such as polynomial fitting, wavelet transformation, Fourier transformation, and derivatives (Schulze et al., 2005). Polynomial fitting method has been widely used because it is simple, fast, and effective. The basis of this method is that fluorescence spectra generally can be modeled by polynomial
functions of different degrees. In this study, a modified polynomial curve-fitting method (Lieber and Mahadevan-Jansen, 2003) was used to correct for the fluorescence background in the Raman spectra of the tomatoes. This method uses an iterative comparison approach to identify and prevent Raman peaks from being involved in the curve-fitting process, and it is effective for retaining Raman features and eliminating fluorescence background simultaneously. In each iteration, all the fitted data points with intensity values higher than those of the corresponding points in the original spectrum are assigned to the original values. The polynomial coefficients for the newly generated curve are determined in each cycle in a least squares manner. This process is repeated until convergence occurs for the number of data points affected in each iteration.

A first-order Savitzky-Golay filter was first used to minimize the high-frequency noise in the Raman spectra. After spectral smoothing, the aforementioned curve-fitting method with an eighth-order polynomial was applied to all the Raman spectra for fluorescence background removal. The fitted baseline at each hyperspectral pixel was then subtracted from the original spectrum to generate the Raman spectrum with a near-flat background. The fluorescence correction is an important step for Raman spectral analysis and classification. The fluorescence-free Raman spectra formed a basis for identification of lycopene in tomatoes using a spectral matching method. The same procedures were used to correct the spectra from the spatially offset measurements of tomato outer pericarps on the Teflon slab. The procedures described above were executed using in-house programs developed in MATLAB (MathWorks, Natick, MA, USA).

2.3.2. Image classification based on spectral information divergence

A hyperspectral image classification method based on spectral information divergence (SID) was developed to identify lycopene in tomatoes at different ripeness stages. SID is a spectral similarity metric that quantifies the discrepancy between two spectra by utilizing the relative entropy to account for the spectral information. The smaller the SID value, the smaller the difference between two hyperspectral pixels. SID has been successfully used in various

![Figure 6](image-url)

**Fig. 6.** Fluorescence background correction for Raman spectra of tomatoes: (a) locular tissues of red and breaker samples and (b) seeds of pink and green samples.
applications for hyperspectral image analysis and classification (van der Meer, 2006; Qin et al., 2009). Detailed information of the SID algorithm can be found in Chang (2000). The Raman spectrum of pure lycopene was used as a reference spectrum. Rule images, within which each pixel represents the SID value between the spectrum at the corresponding hyperspectral pixel and the reference spectrum of lycopene, were generated using the SID mapping function in ENVI. The SID mapping compressed the spectral dimension and maintained the spatial dimensions of the hyperspectral Raman images, which reduced the size of the original 3-D image data from 100 × 100 × 1024 (1024 bands) to 100 × 100 for each fruit sample. A simple thresholding method was then applied to the rule images to create Raman chemical images, in which lycopene pixels were segregated in the tested tomato samples.

Fig. 7. Original and corrected Raman images of tomatoes at selected ripeness stages.

Fig. 8. Fluorescence background correction for Raman images of tomatoes: (a) original and (b) corrected images at 1151 cm⁻¹.
3. Results and discussion

3.1. Raman spectra of lycopene, Teflon, and tomatoes

Raman features of the lycopene solution, the pure chloroform, and the pure lycopene in the wavenumber range of 200–2500 cm\(^{-1}\) are shown in Fig. 4a. The spectrum of the lycopene was obtained as the difference spectrum between the lycopene solution and the pure chloroform. As shown in the figure, Raman peaks of the chloroform and the lycopene generally occupied two distinct spectral regions. Four Raman peaks of the chloroform were observed in the spectral region of 200–800 cm\(^{-1}\). Only one small peak (around 1200 cm\(^{-1}\)) was located beyond 800 cm\(^{-1}\). On the other hand, major Raman features of the lycopene were found in the spectral region of 800–1700 cm\(^{-1}\). Four prominent Raman peaks were identified in this region. The lycopene showed its highest Raman intensity at 1151 cm\(^{-1}\). The intensity at 1513 cm\(^{-1}\) was slightly lower than that at 1151 cm\(^{-1}\). The other two peaks (i.e., 1001 cm\(^{-1}\) and 1282 cm\(^{-1}\)) showed relatively low intensities. Beyond 1700 cm\(^{-1}\), no notable Raman scattering signals were observed for either chloroform or lycopene. Since the main Raman features of the lycopene are in the spectral region of 800–1700 cm\(^{-1}\), this region was selected when using the reference spectrum of the lycopene and the hyperspectral data of the tomatoes for the SID-based image classification. Fig. 4b shows the reference spectrum of Teflon. Seven Raman peaks were identified in the spectral region of 200–1400 cm\(^{-1}\), and the corresponding wavenumbers are marked in the figure.

Fig. 5 shows representative Raman spectra measured for areas of the outer pericarp, locular tissue, and seed parts of tomatoes at green, breaker, pink, and red ripeness stages, with example parts labeled in the cross-section photo of a breaker-stage fruit. All the spectra shared in common a broad peak around 350 cm\(^{-1}\), the result of autofluorescence from the laser–tomato interaction. This background signal showed the greatest intensity for seed spectra and the lowest intensity for outer pericarp spectra. Among spectra for the same fruit part, the background intensities varied randomly. The development of peaks at two wavenumbers corresponding to those associated with the Raman signal of pure lycopene, 1151 and 1513 cm\(^{-1}\) (see Fig. 4a), was clearly observed in the spectra for outer pericarp and locular tissue (Fig. 5a and b) at the more mature ripeness stages. Neither outer pericarp nor locular tissue spectra show Raman peaks of lycopene for green samples, suggesting no lycopene presence in the fruit at the green ripeness stage. A small Raman peak was observed at 1525 cm\(^{-1}\) for the green locular tissue, which is likely due to lutein, another carotenoid found in tomatoes (Schulz et al., 2005). The Raman peaks did not appear for the outer pericarp at the breaker stage, but were observed for some locular tissue at the breaker stage. This observation can be associated with the development of lycopene that occurs first within the inside of fruit and later in the outer pericarp. Owing to the increasing lycopene content in the more mature samples, the intensities of the Raman peaks generally increased in the locular tissue and outer pericarp as the tomatoes ripened. Due to the lack of lycopene, the seed spectra did not exhibit peaks at any stage of ripeness (Fig. 5c). Note that although the Raman spectra of only four representative tomato samples are shown in Fig. 5, the discussion above is based on the results observed for all the tested samples.

![Fig. 9: Identification of lycopene in tomatoes: (a) rule images from SID mapping and (b) Raman chemical images of lycopene.](image-url)
3.2. Fluorescence background correction for Raman spectra and images

Fig. 6 shows example results of correcting the Raman spectra for the background fluorescence, using two locular tissue spectra (red and breaker stages) from Fig. 5b and two seed spectra (pink and green stages) from Fig. 5c. The performance of the modified polynomial curve-fitting method is demonstrated by using these spectra, which include examples with and without the lycopene peaks. Original, fitted, and corrected spectra of the locular tissues are plotted in Fig. 6a. As shown in the figure, the fitting method produced a good fit for the background autofluorescence at both high and low intensity levels. Removal of the fluorescence baseline enhanced the Raman peaks as can be seen in the corrected spectra. It can be clearly observed that the Raman intensities of the red sample were higher than those of the breaker sample. Four Raman peaks were identified in the corrected spectra, occurring at the same wavenumbers as those for peaks of pure lycopene (i.e., 1001, 1151, 1282, and 1513 cm\(^{-1}\)). Another small peak was also observed around 960 cm\(^{-1}\), probably attributable to the shoulder of the Raman peak at 1001 cm\(^{-1}\) (see Fig. 4a). Overall, the corrected spectra with a flat baseline exhibited patterns similar to the reference spectrum of pure lycopene, which formed the basis for identifying lycopene in tomatoes by spectral information divergence. For comparison, the corrected seed spectra in Fig. 6b are almost entirely flat without any notable spectral features, no matter what the fluorescence intensity level was. The background correction for the full set of tomato spectra produced similarly effective results across all ripeness stages.

The polynomial fitting method was applied to all hyperspectral pixels to remove the fluorescence background. Representative corrected images are shown in Fig. 7: original and corrected single-band images of four tomato samples at ripeness stages of green, turning, pink, and red are shown at four wavenumbers corresponding to lycopene peaks. The original Raman images were dominated by the high intensities of the strong fluorescence from the tomato seeds and adjacent areas. After background correction, the influence of the seed fluorescence was largely diminished. The locular tissue and the outer pericarp areas became major features in the tomato images. The brightness of the fruit cross sections in the corrected images generally revealed the changes in lycopene content that occur during the postharvest ripening process. Since more lycopene would generate more Raman scattering signals, the image brightness at each peak wavenumber increased as the tomatoes ripened. The red tomato had the highest brightness, while the green tomato had the lowest, appearing almost completely dark at all four peak wavenumbers. For each fruit sample, the image brightness also agreed with the Raman intensity levels of the lycopene at the four peak positions (see Figs. 4a and 6a). Among the selected samples, the brightest one occurred for the red tomato.
at 1151 cm\(^{-1}\), which is the wavenumber of the highest Raman intensity of the lycopene. The background-corrected results for all 24 tomato samples at 1151 cm\(^{-1}\) are shown in Fig. 8. The overall image brightness patterns were consistent with the findings discussed above.

3.3. Identification of lycopene in tomatoes

Spectral information divergence mapping was performed on the corrected hyperspectral images of all the tomato samples using the Raman spectrum of the lycopene (Fig. 4a) as the reference. The resultant SID rule images are shown in Fig. 9a. The intensity of each pixel in the rule images was the SID value between the lycopene reference spectrum and the spectrum extracted from the corresponding hyperspectral pixel. For example, SID values between the lycopene reference spectrum and the corrected Raman spectra shown in Fig. 6 were 0.06 (locular tissue of the red tomato), 0.11 (locular tissue of the breaker tomato), 0.71 (seed of the pink sample), and 0.75 (seed of the green sample), respectively. Due to the smaller spectral differences with the reference, the SID values of the locular tissues from the red and the breaker samples were much lower than those of the seeds from the pink and the green samples. Generally the seeds and the pericarps (e.g., outer wall, radial wall, and inner wall) of the tomatoes at different ripeness stages appeared brighter than the locular tissues because of their larger SID values in the rule images. Also, the green tomatoes appeared brighter than the red tomatoes for the same reason. A steadily decreasing brightness pattern can be observed in the rule images with the increasing lycopene content in the tomatoes during the postharvest ripening process.

Finally, a simple thresholding method was applied to the SID rule images to isolate lycopene from other parts of the tomatoes, and the results are shown in Fig. 9b. Pixels with intensity values larger than the SID threshold (0.48 was used in this study) were classified as the tomato background, and they formed white round areas in the classification images. Dots and blocks inside the white areas represented the lycopene separated from the background. The lycopene patterns illustrated in the binary images revealed the mechanism of the lycopene generation during the postharvest development of the tomatoes. No lycopene was found in the green tomatoes. Starting from the breaker stage, the lycopene content gradually increased as the tomatoes ripened, and reached the highest level in the red tomatoes. The remaining white regions in the red samples represented lycopene-free areas, which could generally be attributed to the seeds and the pericarp walls (e.g., outer, radial, and inner). The lycopene first became visible at the breaker stage from the tissues in the locular cavities, which is immediately under the outer pericarp. As shown in the figure, the outer pericarp generally did not have any lycopene before the turning ripeness stage. These findings create a possibility of developing a nonde-

Fig. 11. Original (a and b) and corrected (c and d) spatially offset Raman spectra of breaker tomato pericarps on Teflon slab. The spectra are vertically shifted for clarity.
structive optical sensing method to detect subsurface lycopene within green-surfaced tomatoes. Such a method could be used to monitor the internal maturity status of the tomatoes. The lycopene classification images in Fig. 9b can be regarded as Raman chemical images since they create a visualization map based on Raman scattering signals to reflect quantity and spatial distribution of a specific chemical of interest within a complex food matrix.

3.4. Spatially offset Raman measurements of tomato outer pericarps on Teflon slab

Two sets of original spatially offset Raman spectra of the 5-mm and 10-mm green pericarps on the Teflon slab are shown in Fig. 10a and b, respectively. The spectra at eight offset positions in the range of 0–5 mm were selected to demonstrate the general patterns observed in the raw data. The Raman peaks of Teflon were clearly observed for all the spectra, from zero offset to 5 mm offset. The tomato pericarp baseline shows a pronounced slope for the clear offset spectra (upper most spectrum in both Fig. 10a and b), along with several spectral shoulders (e.g., 851, 1083, 1440, and 1618 cm⁻¹). The contribution from the pericarp layer gradually decreases as the offset between the laser’s incident point and the detection probe was increased—the tomato pericarp baseline is much less sloped for the 5-mm offset spectra. After subtracting the fluorescence background, the corrected spectra (Fig. 10c and d) clearly show that the subsurface Raman signals from the Teflon slab were effectively recovered using the offset of 5 mm for both the 5-mm and 10-mm thickness green pericarps, as can be seen by comparison with Fig. 4b. With zero offset (equivalent to using the previous configuration of bifurcated probe for both illumination and signal collection), information from the sample surface and the subsurface were more mixed. When the offset was increased, the relative Raman intensity from the top layer was largely reduced because the pericarp scattering signals dropped more than those of the Teflon slab.

Similar results were obtained for the 5-mm and 10-mm breaker pericarps on the Teflon slab, as shown in Fig. 11. A small Raman peak at 1525 cm⁻¹ was observed for the tomato pericarps, which may be attributable to lutein in the green locular tissue attached inside the pericarps. This Raman peak, along with other scattering and fluorescence signals from the upper layer of the pericarps, was greatly diminished with the increasing offset distance. As a result, the Raman chemical information of the Teflon slab was effectively obtained from under the outer pericarps. The corrected spectra of the 10-mm pericarp (Fig. 11d) appeared noisier than those of the 5-mm pericarp (Fig. 11c), demonstrating that the thicker surface layer will introduce more undesired signals for the subsurface evaluation. The above results suggested that it is possible to extract subsurface chemical information under tomato outer pericarps by spatially offset Raman spectroscopy measurement. Considering the strong penetration ability of the laser and the specificity and sensitivity of the Raman scattering signals to the carotenoids in the tomatoes (e.g., lycopene and lutein), a SORS-based nondestructive approach is currently under investigation in our laboratory for detection of internal lycopene as an indicator of tomato maturity.

4. Conclusion

Lycopene content is a good maturity index for tomatoes since it changes significantly during the fruit ripening process. Assessment of lycopene changes thus can be used to evaluate ripeness of the tomatoes. In this study, a laboratory-based Raman chemical imaging system was utilized to acquire hyperspectral macro-scale Raman images from cross-sections of cut tomatoes. Autofluorescence of varying intensity levels was observed for different parts of the tomatoes. A modified polynomial curve-fitting method effectively removed the underlying fluorescence background regardless of intensity level. Hyperspectral Raman imaging coupled with a spectral information divergence based Raman classification method was shown to provide a useful means for detecting and visualizing the lycopene distribution for the tomatoes at different maturity stages (i.e., green, breaker, turning, pink, light red, and red). Raman chemical images illustrated the internal lycopene development patterns during the postharvest ripening process. The breaker tomatoes showed no lycopene in the outer pericarp but did show the initial appearance of lycopene in the locular tissues. The lycopene content gradually increased as the tomatoes ripened, and reached the highest level at the red stage. The spatially offset Raman spectroscopy technique is capable of extracting subsurface Raman chemical information from under the tomato outer pericarps. The methods and findings of this study form a basis for developing a Raman-based nondestructive method to detect internal lycopene as a maturity indicator for tomatoes. Utilizing the strong penetration ability of the laser and the specificity and sensitivity of the Raman scattering signals to carotenoids in the tomatoes, testing of a nondestructive sensing approach based on SORS is planned as the next step of this research project.

References


