Nondestructive Determination of Tomato Fruit Quality Parameters Using Raman Spectroscopy

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ABSTRACT

Tomato is a major fruit, as well as a major food science product. There is a need of determining the quality attributes of this fruit (nondestructively) due to the increasing demand of the in agro-industrially controlled areas. Most of the commonly employed techniques are time consuming and involve a considerable degree of manual work. Sample preparation, juice making, and laboratory tests are among the limitations. Raman spectroscopy was applied in this study to measure such important quality parameters of tomato as SSC, pH and color. A dispersive Raman instrument was employed and reference analyses were carried out to make calibration models regarding the spectral features and target attributes. Analysis of the spectra revealed that all the three characteristic bands of cartenoids, lycopene, and carotene, were significantly recognizable. Also there were several strong to medium bands recognized as related to carbohydrates. Principal Component Regression (PCR) and Partial Least Square (PLS) were selected as the multivariate calibration models. The prediction models proved to be robust resulting in a desirable mapping between the spectra and output attributes. The Root Mean Square Error of Predictions (RMSEP) through PLS and PCR for modeling the color index using the whole spectrum was obtained as 0.33 and 0.38, respectively. RMSEP for mapping the SSC using PLS and PCR models was resulted in respective figures of 0.30 and 0.38. PCA interpretation depicted that Raman spectra could make a favorable distinction among the samples based on their maturity stages. As a result, there is a great potential to use Raman spectroscopy in industrial approach and in line control.

Keywords: Nondestructive, Quality attributes, Raman spectroscopy, Tomato.

INTRODUCTION

Tomato (Lycopersicon esculentum L.) is a significant fruit in the global agricultural market due to its wide use in food industry both as fresh consumption and as extensive processed products. The fruit is mainly composed of water, soluble and insoluble solids, organic acids (mostly citric acid) and such micronutrients as carotenoids, and vitamins A and C (Pedro and Ferreira, 2007). Several quality criteria are considered for tomato, from which soluble solid content (SSC), acidity (pH) and color are the most important parameters affecting the consumers’ appreciation for selection (Shao et al., 2007). Most quantification techniques leading to a measurement of the qualitative attributes of tomato fruit are time and labor consuming in addition to their being destructive. Furthermore, there would be need for instruments and need for sample preparation for each trait determination. On the other hand, consumer interest to use...
sound and highly graded fruits together with strict quality standards approved globally, has forced industries and producers to find effective techniques for the evaluation of fruit quality. For practical reasons, the methods should be easily measurable, simple, rapid and precise. Although the best routine for such a task is sensory evaluation, it is more desirable to apply faster, simpler and cheaper techniques which satisfy the industrial control demands as well as being instrumentally facilitated.

Such a need has arisen a growing interest in the application of such spectroscopic screening methods as near-infrared (NIR) spectroscopy and Raman spectroscopy (Belie et al., 2003).

The Raman technique in particular, has a great potential for biochemical analysis on both the macroscopic and microscopic scale. It is a relatively specific spectroscopic technique that measures rocking, wagging, scissoring, and stretching fundamental vibrations of functional groups containing such bonds as C–C, C–O, C–H and O–H (Marquardt and Wold, 2004). Another major advantage of this technique is the capability to provide information about concentration, structure, and interaction of biochemical molecules within intact cells and tissues, nondestructively.

It is worthwhile to remember that water present in high concentrations in fruits, as in many food products, creates a severe drawback for IR method owing to the high IR light absorption coefficient, while having a weak Raman signal not interfering with the spectra of other components (Camerlingo et al., 2007). As a result, Raman spectroscopy has been recognized as a promising analytical tool within different areas of food science (Lewis and Edwards, 2001). Peaks in the IR and Raman spectra at specific frequency are characteristic of the specific functional groups within each of the components present in the fruit. The IR spectrum is obtained by a change in the molecular dipole moment during vibration, while the Raman spectrum is obtained through a change in polarizability during the vibration (McCreery, 2000). For instance, such polar functional groups as C=O and O–H stretching bear strong absorption in the IR spectrum, while the C=C stretching as a non-polar functional group, has strong Raman scattering. Therefore, IR and Raman spectroscopy can be complementary tools allowing extracting comprehensive information from the sample.

The main objective of this study is to evaluate Raman spectroscopy for the qualitative and quantitative analysis of tomato parameters and to develop a robust analytical procedure for determining, simultaneously and nondestructively, major parameters of tomato including Soluble Solids Content (SSC), acidity (pH) as well as fruit color using calibration models.

MATERIALS AND METHODS

One hundred samples of tomato fruits, PS variety, were collected in September from Karaj, Iran greenhouses. These samples presented a reasonable range of variation for building suitable calibration models for the properties of interest. As tomato fruits are quite sensitive to environmental conditions, the instrumental reference measurements were performed immediately after conducting spectroscopy tests on samples. The whole fruit was subjected to Raman spectroscopy measurements.

Instrumental Reference Measurements

Brix values (Soluble Solids Content, SSC) were assessed in the juice of the samples using a temperature compensated refractometer (DR-A1, Atago, Tokyo, Japan). The results were averaged. Refractometric index was used as an indicator of sugar content of the fruits. The pH of the juice samples was determined using the pH meter (Metrohm, 827 pH Lab, UK). Tomato ripening involves a number of physiological processes that include the visible breakdown of chlorophyll
accompanied by a build-up of carotenoids, with massive accumulation of such antioxidant components as lycopene and β-carotene (Lai et al., 2007; Baranska et al., 2006). The spectra revealed strong bands associated with carotenoids. Previous analysis using liquid chromatography showed that the main carotenoids present in tomato are mainly lycopene and carotenes (Bender and Bender, 1999). Besides representing the color of fruits, carotenes are precursors of vitamin A. Thus it would be of a great importance to probe them in fruits. A HunterLab instrument (ColorFlex 45/0, Reston, VA, USA) was employed to analyze the color of juices. This instrument expresses color in numerical terms along the $L^*$, $a^*$ and $b^*$ axes (from white to black, green to red and blue to yellow, respectively). Camelo and Gomez (2004) have done extensive experiments on tomato to determine the best mathematical representation of colorimetric components for the tomato ripening. They have concluded that $a^*/b^*$ relationship could be used as an objective ripening index in practical purposes giving a realistic perception of consumer perception (Camelo and Gómez, 2004). As a consequence, the ratio $a^*/b^*$ was selected as a grouping index in the analyses and fruits marked as U, R and O representing the three ripening stages: unripe, ripe and over-ripe, respectively. Additional experiments were carried out through Liquid Chromatography tests on tomato to determine the sugars in the fruit. Sugars (fructose and glucose) were determined using a HPLC (WATERS 600, E USA) equipped with a Bondapack™ C18 (250 mm×4.6 mm) column as a separator and a Waters refractive index detector. The mobile phase was acetonitrile:water (75:25), at 1 ml/min. Sigma–Aldrich standards were used for building calibration curves. Tomato juices were centrifuged at 1,000 rpm using SIGMA 4 K 15 refrigerated centrifuge and twice filtered to be completely clarified and injected at the injection volume of 20 µl.

Spectroscopic Measurements

FT-Raman spectra on the whole fruits were obtained using the Nicolet Almega Dispersive Raman (Thermo Scientific, USA) spectrometer consisting of a laser, operating at a maximum power of 100 mW. The laser output power of 30 mW, used for analysis was low enough to prevent possible laser induced sample damage, yet provided a high signal to noise ratio. Data were collected at 2 cm$^{-1}$ resolution with 16 scans. Spectra were obtained in the Raman shift range between 400 and 4,000 cm$^{-1}$. The system was operated using OMNIC software (Version 5.1, Madison, WI).

Data Analysis

Pre-treatment routine data was applied to enhance the spectra. A four-order polynomial was fitted to the signals to remove the background and obtain the desired peaks. Moreover, 1-norm normalization was performed on the spectra, resulting in a uniform quantitative procedure. Savitzky-Golay 2$^{nd}$ order-polynomial and moving average (segment size of 5) algorithms carried out done to take the first derivative and smoothen the signals. Due to the narrow vibrational bands indicative of specific molecular vibrations, one must expect spectral overlap between chemical components. Yet, several multivariate methods for correlation between Raman spectra and chemical quality attributes are proposed, from which Principal Component Analysis (PCA) and Partial Least Square (PLS) were selected out in this study. Furthermore, analyses with Multiple Linear Regression (MLR) did not yield any desirable results, thus it was not recommended due to the collinearity between adjacent wavelengths (Gemperline, 2006). Also, the spectra variation was analyzed by Principal Component Analysis (PCA) to eliminate the defective spectra outliers. Scores, loadings and explained variance were studied for the first 10
Principal Components (PC) (Marquardt and Wold, 2004).

The data were divided into training and validation sets. Twenty percent of the dataset were specified for the validation stage and the remaining samples used for training procedure. The quality of the calibration model was evaluated by the correlation coefficient ($r$) between the predicted and the measured parameters, the Root Mean Square Error of Calibration (RMSEC) and the Root Mean Square Error of Prediction (RMSEP). RMSEP and RMSEC were calculated for different numbers of components being included in the model, which allowed for the selection of the optimal number (i.e. giving the lowest RMSEP) for the final prediction model. RMSEC and RMSEP are defined as (Nas et al., 2004).

\[
RMSEC = \left( \frac{1}{I_c} \sum_{i=1}^{I_c} \left( \hat{y}_i - y_i \right)^2 \right)^{1/2}
\]

\[
RMSEP = \left( \frac{1}{I_p} \sum_{j=1}^{I_p} \left( \hat{y}_j - y_j \right)^2 \right)^{1/2}
\]

where $\hat{y}_i$ is the predicted value of each observation and $y_i$ the measured value; $I_c$ is the observation number in the calibration set and $I_p$ the observation number in the validation set.

Data analyses were performed using the software Unscrambler ver. 9.7 (Camo AS, Oslo, Norway). The pre-processing routine was written in Matlab code (The MathWorks Inc., Natick, MA).

**RESULTS AND DISCUSSION**

Interpretation of Raman Spectra

A dominant characteristic of Raman spectra is strongly enhanced bands due to the skeletal $v_1$ and $v_3$ vibrations. Moreover, the wavenumber of the $v_1$ band decreases with the extent of the conjugation length of the main polyene chain due to electro-phonon coupling as shown in Figure 1 (Withnall et al., 2003).

$N$ denotes the number of conjugated carbon-carbon double bonds in the main chain. Figure 2 shows a typical Raman spectra of tomato fruit in a red ripe stage. As seen in the figure, there are some sharp and strong peaks in the region 1,004-1,530 cm$^{-1}$. As reported previously, these bands are assigned to stretching C-C or C=C bonds which compose the structure of carotenoids lycopene, $\beta$-carotene and $\gamma$-carotene (Schulz and Baranska, 2007). In tomato fruit, the bands at 1,510 cm$^{-1}$, 1,156 cm$^{-1}$ are related to stretching vibrations of C=C and stretching vibrations of C-C bonds.

![Figure 1. Relationship between the $v_1$ wavenumber location and the number of double bonds in the polyconjugated main chain of carotenoids (Withnall et al., 2003).](image-url)

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Figure 2. Raman spectrum of a red ripe tomato fruit at the region 900-1,600 cm\(^{-1}\), having several peaks related to stretching vibrations of C-C and C=C groups.

Moreover, the bands appeared at 1,524 cm\(^{-1}\), 1,157 cm\(^{-1}\) can be assigned to stretching \(\nu(C=C)\) and stretching \(\nu(C-C)\) of \(\beta\)-carotene, respectively (Schulz \textit{et al.}, 2005). Additionally, in-plane rocking mode of CH\(_3\) groups attached to the polyene chain and coupled with C-C bands can be observed as a peak of medium intensity in the 1,000-1,020 cm\(^{-1}\) region which is obvious in Figure 2. According to Figure 1 and considering the number of conjugated bonds in carotene and lycopene, it can be concluded that the peak observed at 1,520 cm\(^{-1}\) in Figure 2 implies the predominance of carotenes (9-conjugated) rather than lycopene (11-conjugated). However, it can be seen that the band at 1,520 (or 1,510) is asymmetric and there appears a shoulder related to \((\beta\)-carotene or lycopene).

Region 400-1,500 cm\(^{-1}\) of Raman spectra contains several peaks which are related to carbohydrates shown in Figure 3. The spectrum in this figure is a first derivative (2\textsuperscript{nd} order polynomial) of normalized Raman spectrum done by Savitzky-Golay algorithm. As seen clearly in Figure 3, derivation makes the observation of bands easier, since some bands which are hidden by wider bands, are now clearly visible. Although, these bands in this region are not predominant because of strong bands of carotenoids, but one can use them on calibration models to correlate with the sugars in tomato.

The next region that should be discussed
regarding the Raman spectroscopic features in tomato, is 2,000-3,000 cm\(^{-1}\) region (Figure 4), in which there appear several peaks assigned to stretching vibrations of C-H. This band is highly correlated with carbohydrates due to their molecular structure. According to liquid chromatography experiments carried out to determine the exact type of sugars in the samples in this research, it was revealed that a medium tomato (100 g) fruit contains 3-4 g of carbohydrates, mostly as fructose and glucose. Therefore, the region shown in Figure 4 should be related to the vibrations of molecular bonds linked to fructose and glucose molecular composition.

**Developing Calibration Models**

Samples were analyzed by Principal Component Analysis (PCA) to identify whether the spectra were able to sort the samples based on the maturity stage or not. The index which defines the maturity was selected as the ratio of \(a^*/b^*\) as measured by HunterLab colorimeter. There appeared large differences in the wavenumbers below 600 cm\(^{-1}\) and after 3,200 cm\(^{-1}\). As a result there wasn’t any correlation between the spectral features at this range and chemical parameters. Furthermore, few bands are found in spectral libraries related to this domain, thus it was omitted in the calibration models and the remaining region containing valuable information (as mentioned before) was used for the analysis of tomato quality. Figure 5 presents PCA results run by 5 PCs and Cross Validation process. All the spectra were area normalized and mean centered. The input spectra were the first derivative of spectra (Savitzky-Golay algorithm, 2\(^{nd}\) order polynomial) and smoothed by a moving average window (with the segment size of 5). The 800-1,700 cm\(^{-1}\) range was selected as a representative of cartenoid bands while the second range had several bands representing carbohydrates. An interesting observation from Figure 5a is that the Raman spectra have been able to detect the maturity stage based on \(a^*/b^*\) index. The samples are grouped as ‘U’, ‘R’ and ‘O’ indicating the three maturity stages of the fruit. It is demonstrated by Figure 5a that the PCA has been able to separate the ‘U’ samples at the range 800-1,700 cm\(^{-1}\) but the other two categories were not well grouped. This may originate from the fact that the color of fruit at the later stages, is not subjected to meaningful changes. Tomato color undergoes some changes at different maturity stages but this change is more significant when it gets red from pink. This is why the range 800-1,700 cm\(^{-1}\) has detected the ‘U’ fruit (pink color) from the others and PC1 has shown to be a proper indicator of this score.

Additional analyses by PCA were carried out at the region 2,000-3,000 cm\(^{-1}\) (Figure 4).
Determine Tomato Quality by Raman Spectroscopy

Figure 5. Classification of samples based on maturity stage at two different regions: (a) 800-1700 and (b) 2000-3000 cm\(^{-1}\).

5b). Figure 5b shows a scatter plot of the 1\(^{st}\) versus 2\(^{nd}\) PC scores. The plot exhibits the clearest separation of the spectra according to the samples circled by three groups. This was a valuable step to build calibration models based on two regions.

Different calibration models were designed based on MLR, PLS and PCR. MLR models did not provide significant correlation between the spectral features output parameters. The models could successfully predict the color indices and SSC parameters, but the prediction of pH was not desirable. Therefore, the correlation coefficients expressed in Tables 1 and 2 are denoted to SSC and color index. Hence the models could predict both factors simultaneously. After several trials to select the best model through PLS and PCR, the results were summarized in Table 1 and 2.

Three ranges were employed for the inputs in the calibration models: 800-1,700 cm\(^{-1}\), 2,000-3,000 cm\(^{-1}\), and 800-3,000 cm\(^{-1}\). The selection of these ranges refers to PCA results in which one could make clear separation of fruits by their maturity stage. As detailed in Tables 1 and 2, PLS presented a better performance than PCR. Moreover,
Table 1. Results of PLS and PCR calibration and validation to estimate the color of tomato indexed by a*/b* ratio.

<table>
<thead>
<tr>
<th>Spectral Range</th>
<th>Model</th>
<th>Number of components</th>
<th>Calibration</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>r RMSEC</td>
<td>r RMSEP</td>
</tr>
<tr>
<td>800-1700 cm⁻¹</td>
<td>PLS2</td>
<td>6</td>
<td>0.92 0.31</td>
<td>0.84 0.30</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>7</td>
<td>0.88 0.34</td>
<td>0.77 0.37</td>
</tr>
<tr>
<td>2000-3000 cm⁻¹</td>
<td>PLS2</td>
<td>7</td>
<td>0.89 0.33</td>
<td>0.73 0.37</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>6</td>
<td>0.84 0.30</td>
<td>0.69 0.38</td>
</tr>
<tr>
<td>800-3000 cm⁻¹</td>
<td>PLS2</td>
<td>7</td>
<td>0.87 0.26</td>
<td>0.85 0.33</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>7</td>
<td>0.87 0.27</td>
<td>0.82 0.38</td>
</tr>
</tbody>
</table>

Table 2. Results of PLS and PCR calibration and validation to estimate SSC of tomato.

<table>
<thead>
<tr>
<th>Spectral Range</th>
<th>Model</th>
<th>Number of components</th>
<th>Calibration</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>r RMSEC</td>
<td>r RMSEP</td>
</tr>
<tr>
<td>800-1700 cm⁻¹</td>
<td>PLS2</td>
<td>6</td>
<td>0.78 0.28</td>
<td>0.77 0.29</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>6</td>
<td>0.80 0.35</td>
<td>0.73 0.40</td>
</tr>
<tr>
<td>2000-3000 cm⁻¹</td>
<td>PLS2</td>
<td>7</td>
<td>0.81 0.34</td>
<td>0.69 0.37</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>6</td>
<td>0.77 0.24</td>
<td>0.66 0.41</td>
</tr>
<tr>
<td>800-3000 cm⁻¹</td>
<td>PLS2</td>
<td>7</td>
<td>0.84 0.31</td>
<td>0.79 0.30</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>7</td>
<td>0.80 0.32</td>
<td>0.78 0.38</td>
</tr>
</tbody>
</table>

PLS runs faster than PCR with the over-fitting less probable. Range selection was done for increasing the selectivity of spectra together with decreasing errors. As shown in Tables 1 and 2, the range selection will not be necessary for the Raman spectra since the r coefficient and errors did not decrease as result of range reduction. Therefore, the whole spectrum at the range 800-3,000 cm⁻¹ can be used for the calibration of models.

CONCLUSIONS

The results indicate that it was possible to use Raman spectroscopy as a useful analytical method and as an effective technique for non-destructive evaluation of tomato fruit quality attributes. Both the external property (color) and the internal properties (SSC and acidity) were detected in this study. The obtained spectra demonstrated several medium and strong bands which can be assigned to chemical groups related to carotenoids and carbohydrates existing in a typical fruit. Fructose and glucose compose the main sugars and carbohydrates in tomato fruits used in this research. Based on the spectral information, multivariate calibration models could provide a mapping between the bands and qualitative properties. By means of PCA, it was shown that the spectra could be employed to distinguish the stages of maturity in samples. Moreover, the calibration was done through PLS method with favorable results being obtained. The Raman spectrum was first divided into two sections and the regions of the less importance eliminated in the models. The color index of tomato was modeled with the correlation coefficients of 0.85 and 0.82 through PLS and PCR models, respectively. The RMSEP of color modeling was 0.33 for PLS and 0.38 for PCR. PLS and PCR models could provide a desirable fitting between the input variables and SSC of as fruit based on the correlation coefficients of 0.79 and 0.78, respectively. The RMSEP for PLS and PCR models for prediction of SSC were obtained to be 0.30 and 0.38, respectively. Thus, PLS model is proposed for both its accuracy and fastness.

Further research is planned to implement Raman spectroscopy as a detection technique in line processes or control applications.
REFERENCES


اندازه‌گیری غیر‌مغزب پارامترهای کیفی میوه گردو توسط طیف‌سنجی رامان

چکیده

میوه گردو فرگی محصول مهمی در کشاورزی به شمار می‌رود و با برای اندازه‌گیری برای تعیین خصوصیات کیفی این محصول به‌صورت غیر مغزب در حووزه‌های مرتفع با کاربرد هستن کنترل صنعتی وجود دارد. بسیاری از روشهای رایج زمانی بوده و نیازمند تیروی کاری زیادی است. به همین دلیل در این تحقیق از طیف‌سنجی رامان به منظور اندازه‌گیری خصوصیات کیفی مهم مانند 

$\text{pH}$، $\text{SSC}$ و تکیه هشمند، ر. مکل‌فر و ب. قبادیان

$\text{PCA}$، $\text{PLS}$ و $\text{PCR}$

در سال‌های اخیر به عنوان روش‌های اصلی به جای تجزیه مولکول‌های اشیایی (PCA) و تجزیه PLS به عنوان روش‌های کاربردی ترقی‌درجه‌ای انتحاب شد. مدل‌های پیش‌بینی بسیار باید عمل کرده و نگاشت قابل قبولی بین طیف‌های و وزنه‌گری مورد نظر برقرار نمودند. خطا‌های مربوط به مقایسه طیف‌های $\text{PCA}$ و $\text{PLS}$ به ترتیب $32$، $38$ بسته‌ای به ترتیب برای مدل‌های $\text{SSC}$ و $\text{PCR}$ به ترتیب $32$، $38$ حاصل شد. تفسیر نتایج حاصل از تجزیه مولکول‌های اصلی نشان داد که طیف‌های رامان توام استند بر مبنای مرحله سردی‌گی مابین نمودنها تا میزان برقرار کننده. بر همین اساس، طیف‌سنجی رامان چشم‌پیار بالایی در کاربردهای کنترلی در دست میوه می‌تواند داشته باشد.