

Utilizing laser spectrochemical analytical methods for assessing the ripening progress of tomato

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Abstract

To meet market demands and minimize losses, the tomato crop (*Solanum Lycopersicum* L.) requires a simple, rapid, and cost-effective method to distinguish between different maturity stages with high accuracy. This study aimed at evaluating two spectrochemical analytical techniques, namely laser-induced fluorescence (LIF) and laser-induced breakdown spectroscopy (LIBS), to discriminate three different maturity stages of tomato fruit ('Green/Breaker'; 'Turning/Pink'; and 'Light-red/Red'). The simple linear regression confirmed the obtained LIF results with chlorophyll content (mg/100 g), hue angle (h°), and firmness (kg/cm²) of the different maturity stages (measured by conventional methods). Furthermore, the findings showed that the peak intensities of LIF spectra decreased with the chlorophyll content depletion during ripening. Moreover, the data exposed a reasonably good association between LIF spectra and chlorophyll content with a regression coefficient of 0.85. On the other hand, firmness and skin hue have shown an excellent predictor for the spectra with a high regression coefficient of 0.94. For LIBS spectra of each maturity stage, the ratios of Ca's ionic-to-atomic spectral lines intensities have followed the same trend as conventionally measured firmness. The results demonstrated that LIF and LIBS are accurate, easy, and fast techniques used to define tomatoes' different ripening stages. Both methods are useable in situ without any prior laboratory work.

Keywords Tomatoes · Laser-Induced fluorescence · Laser-Induced Breakdown Spectroscopy · Maturity · Principal component analysis

Introduction

It is well-known that tomato (Solanum Lycopersicum L.) in the Solanaceae family is one of the most vegetable crops consumed worldwide [1]. Unfortunately, poor handling and management during storage contribute to tomato partial loss and damage. However, the significant factor influencing the nutritional contents, shelf life, and postharvest losses is the proper ripening of tomatoes during harvesting. Therefore, maturity assessment is vital to meet various demands (e.g., fresh or processed). It has been reported that early mature harvested tomatoes (mature green) had induced the highest weight loss percentage. Ripe green tomatoes, however, have achieved the most extended shelf life [2, 3], which makes them ideal for long-distance marketing.

On the other hand, fully-ripened fruits are convenient for fresh consumption [4] for excellent taste and flavor but shorter shelf life [5]. Consequently, accurate, rapid, and nondestructive maturity evaluation is essential in tomato production and relevant processing industries. Magnetic resonance imaging (MRI) was employed to differentiate the physiological changes of tomato fruit during ripening with an accuracy of 90% [6]. Raman spectroscopy was used to evaluate tomatoes' internal maturity [7]. Zhu et al. [8] utilized the optical absorption and scattering spectra at 500–950 nm wavelength to classify tomatoes' ripeness using hyperspectral imaging. Other groups [9, 10] have investigated the correlation between Mid-infrared (MIR) / Near-infrared (NIR) spectral data and variations in chemical constituents and cellular structure as ripening indicators. However, the quality prediction was excellent for some traits and showed a lower level of accuracy for the others.

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The equipment, the operator, and the operation process affect the precision of the ripening indices such as texture, soluble solids content, and titratable acidity. Moreover, the spectra are influenced by the ambient light, which affects the prediction accuracy of the internal maturity [11].

Tomato color is an essential external feature in evaluating fruit ripeness and optimal harvest time [12]. Of course, such visual assessment is non-invasive and nondestructive. In addition, chlorophyll degradation and carotenoid accumulation are mainly responsible for the changes in color from green to red during maturation [13]. Based on the exterior color of tomato fruit, the United States department of agriculture (USDA) established six ripening stages reflecting the human ability to distinguish ripeness: green, breaker, turning, pink, light red, and red [14]. Okiror et al. [5] suggested that color significantly correlates with physicochemical and nutritional characteristics, including total soluble solids, total titratable acidity, and protein content. Therefore, the tomatoes' attributes were evaluated to limit the quantitative loss and the deterioration of the qualitative characteristics based on the green to red ratio to determine the optimal picking time, varying from 50:50% to 75:25%, depending on the variety [2]. The traditional process is to select tomatoes based on the fruit surface color by experience; however, the human eye's decision is often deceptive because of the laborers' lack of experience and light conditions effects. Skolik et al. [15] reported that the tools available to study plant surfaces non-destructively are limited. Recently, laser-induced fluorescence (LIF) has been utilized to differentiate between plant types through the emitted fluorescence. LIF is characterized by high accuracy, rapid response, nondestructive and high discrimination for fluorescence monitoring. In this technique, the chlorophyll molecule absorbs the light photons at a specific wavelength (excitation process). The re-emitted energy is the fluorescence emission [18].

Besides, Laser-induced Breakdown Spectroscopy (LIBS) is a well-known spectrochemical elemental analysis technique. In LIBS nanosecond, picosecond, or femtosecond, laser pulses are focused on the sample surface to generate a plasma plume. The laser-induced plasma plume consists of collections and swirling electrons at extremely high temperatures (6000-60,000 K). As the plasma plume cools down, it gets rid of the previously absorbed laser energy in the form of emitted light photons. The emitted light is collected and analyzed spectroscopically. Qualitatively, the spectral lines in the obtained spectrum are the fingerprint emission lines of the elements in the plasma plume and consequently in the target material. Quantitatively, there is a linear relationship between the intensity of the spectral lines and the concentrations of the corresponding elements in stoichiometric ablation. In recent decades, LIBS has become an essential technique in different research fields, including agriculture and plant analysis, due to its simplicity, rapidity, and low cost [19, 20].

To our knowledge, only a few studies have discussed the LIF and LIBS spectra of the external surface of tomatoes to distinguish maturity stages. For example, Lai et al. [21] studied the LIF spectra of two different stages, unripe and over-ripe fruit, at 355 and 266 nm, respectively.

The present work's main objectives are to first evaluate LIF and LIBS as spectrochemical analytical techniques to differentiate between the various ripening stages of tomato fruit; second, to predict firmness, color, and chlorophyll content as maturity indices. The fluorescence spectra of external tomato surface were gained using LIF, then classified statistically, applying the principal component analysis method (PCA) for rapid and straightforward classification. The findings were then verified using the simple linear regression analysis with chlorophyll content, firmness, and hue angle (measured conventionally). Finally, LIBS was used as an elemental analysis technique to assess changes in various elements' concentrations and predict the firmness as a function of the ripening stage.

Materials and methods

Tomato samples

Greenhouse tomatoes were grown in Central Labs' experimental field of the Agricultural Climate, Agricultural Research Center, Giza, Egypt. The fruit was hand-picked free from defects at different maturity stages and transported to the laboratory within an hour. Tomatoes were cleaned with tap water dried at air temperature. According to Zhu et al. [8], the tomatoes were sorted based on training into three grades: 'Green/Breaker' (tomatoes with less than 10% of red color), 'Turning/Pink' (tomatoes with 10–60% of the red color) and 'Light-red/Red' (tomatoes with the red color more remarkable than 60%). For each grade, 60 fruit were randomly divided into two groups: one group was used to conduct LIF and LIBS measurements, while the other was used to perform the conventional estimation of the chlorophyll content, firmness, and hue angle.

Spectrochemical Analysis

LIF measurements

LIF analysis experimental arrangement is described according to Abdel-Salam and Harith [22]. The fruit samples were excited with a continuous wave (CW) diode-pumped solid-state (DPSS) laser [Changchun new industries optoelectronics Tech Co, Ltd. (CN)] at a wavelength of 405 nm with a low output power of an average of 100 mW [23]. The laser beam is delivered perpendicularly onto an area of 1cm² of the tomato pericarp via a special annular Y-type optical fiber that delivers the laser beam through a branch and collects the emitted fluorescent light through the other. The fluorescence is fed to the entrance slit of a compact spectrometer equipped with a CCD detector (USB2000 FLG, Ocean Optics, USA). The experiment was repeated separately on 30 samples for each maturity stage under the same conditions at room temperature to ensure the stability of the resulting fluorescence and the results' reproducibility. The obtained spectral signals were analyzed using SpectraSuit software (Ocean Optics, USA). The spectra were imported to Origin software (Origin Lab. Corp., USA, Version 8) for further processing and analysis.

LIBS Arrangement

A typical LIBS experimental setup used in the present work is described according to [24]. A Q-switched Nd: YAG laser (BRIO, Quantel, France), operating at its fundamental wavelength of 1064 nm, with pulse energy of 50 mJ, pulse duration 5 ns, and a repetition rate of 20 Hz, is used to induce the plasma onto the fruit sample's surface through a planoconvex lens of 10 cm focal length. The collected plasma emission is then fed to the entrance slit of an echelle spectrometer (Mechelle 7500, Multichannel, Sweden) via an optical fiber. A Gateable ICCD camera (DiCAM-Pro, PCO, computer optics-Germany), coupled to the spectrometer, was used to detect the dispersed light. The ICCD was triggered optically, at a typical delay time of 1500 ns and gate width 2500 ns for measurements performed in air at atmospheric pressure. The measured spectrum represents the average of 5 spectra taken at five different positions (10 shots at each spot) onto the sample's surface. The obtained spectra have been analyzed using the LIBS⁺⁺ software [25].

Conventional measurements

Skin Hue

The superficial color (Hue) of the tomatoes was evaluated by measuring parameters a^* (redness-greenness) and b^* (yellowness-blueness) with a chromameter (Konica Minolta, Model CR-400, Japan) according to CIE $L^*a^*b^*$ color space. The measurements were performed on each fruit's equator in four positions approximately 90° apart, and the average of the four readings was considered. Hue angle (°h = arctangent [b*/a*]) was calculated [26]. Fifteen tomatoes were measured for each maturity stage.

Firmness

Firmness was measured using a digital penetrometer with a 10 mm diameter flat end plunger (ST 308-made in Italy). Each fruit was aligned from stem to stylar end on a stationary steel plate. The plunger was pressed into the equatorial zone of the flesh perpendicular to the stem-stylar axis with steady downward pressure until 6.5 mm depth. Hence, the reading in the penetrometer dial stopped [27]. Each fruit was measured in three positions on the equatorial zone, and the measurements were expressed in kg/cm². The average readings at the end were considered.

Chlorophyll content

After conducting the surface color and firmness measurements, chlorophyll A and chlorophyll B contents were determined spectrophotometrically according to [28]; briefly, 0.5 g of the sample was mixed with acetone and homogenized in a mortar. MgCO3 was added before homogenization to avoid chlorophyll pheophytinization. The contents of the mortar and pestle were quantitatively transferred to the filter after the mixtures were filtered. The mortar and pestle were then rinsed multiple times with acetone. The rest of the filter was washed with acetone until it was entirely white. Then, the filtrate was diluted to a total amount of 25 ml with acetone. The absorbance of prepared mixtures was recorded at 662, 644 nm using acetone as blank, and pigment content was calculated. Three replicates were performed for each maturity stage, and then the means of total chlorophyll were obtained and expressed in mg/100 g.

Statistical analysis

Principal component analysis (PCA) has been applied as a multivariate analytical method [23] to discriminate between the obtained LIF spectra of the tomatoes in each of the three maturity stages using Origin Pro-2017 software (OriginLab Corporation, MA, USA). One-way analysis of variance (ANOVA) was performed to statistically analyze conventional methods data using the Excel program (Microsoft Office Professional Plus 2010). All measurements were made in triplicate. Statistical significance test (HSD) at a 5% significance level. Finally, the gained peaks of LIF spectra for all tomatoes' maturity stages were used to predict the chlorophyll content, hue angle, and firmness through simple linear regression [29] using the Excel program.

Results and discussion

LIF measurements

Figure 1 shows the measured fluorescence spectra of the three maturity stages of tomato fruit. Typical fluorescence spectra have been gained in the range of 650–775 nm, including the characteristic emission of chlorophyll fluorescence at the red to the near-infrared band, according to [23]. For example, the excitation laser beam of 405 nm wavelength induced two fluorescence peaks near 685 and 740 nm. This is in line with the findings of Yang et al. [16], who used 556 and 355 nm lasers as excitation sources to distinguish different plant types and achieved the same emission peaks. Furthermore, when employing a 266 nm laser as an excitation source for some regions of the outer surface of an unripe tomato, [21] recognized a fluorescence emission band in the red area 670–730 nm (chlorophyll) with a peak around 680 nm.

It has been reported that Photosystems II and Photosystems I are protein complexes that are responsible for the photosynthetic activity of the plant, and the fluorescence peak at 685 nm is attributed to chlorophyll-a of Photosystems II. In comparison, broadband from 700 to 750 nm is due to an energy transfer from Photosystem II to Photosystems I (Saito et al., 1998; Pedrós et al., 2008)[30, 31]. Furthermore, according to [32], changes in the excitation wavelengths resulted in different fluorescence spectral shapes, as the chlorophyll fluorescence may be re-absorbed in its way towards the plant surface. For example, in a previous study on sweet orange leaves, a diode laser light at 405 nm has induced fluorescence emissions in two distinct bands: a blue-green emission in range 450-630 nm, and a red to near-infrared in the range of 650-800 nm. On the other hand, the 561 nm diode laser only resulted in chlorophyll fluorescence emission in the red to the near-infrared region [23].

The obtained data revealed that the emission intensity of fluorescence near 740 nm was too limited to be used as a valid signature of chlorophyll fluorescence, which may be due to the insufficient chlorophyll for re-absorbing the light from Photosystem II (685 nm) to Photosystem I (740 nm). This means that the fluorescence emission is due to the chlorophyll content and not the activity of the photosystems. According to [30], the organic constituents and the chlorophyll absorption inside the tissue influenced the shapes of LIF spectra and peaks intensities since the peak intensity at 740 nm was about twice that at 685 nm on the upper surface of the plant leaves. However, on the lower surface of the leaves, the intensity of the two fluorescence bands did not vary and was nearly the same. Ripened Tomato fruit exhibited diminished fluorescence intensity at 685 and 740 nm



Fig. 1 LIF Spectra of tomatoes samples for the three maturity stages

(see Fig. 1). Moreover, the peak at 740 nm has disappeared in the 'Light-red/Red' stage. According to Obledo-Vázquez and Cervantes-Martínez [17], the considerable decline in fluorescence could be relevant to the decreased photosynthetic activity depletion of chlorophyll content during ripening.

Principal component analysis (PCA)

PCA is an unsupervised statistical technique that reduces the dimensionality of an enormous data set into small data sets without losing much information. The obtained variables, called principal components, are evaluated in linear sets of the primary variables [33]. Yang et al. [16] have been successfully applied PCA to distinguish fluorescence spectra of six plant types. In general, this multivariate statistical technique can be exploited to identify similarities and dissimilarities in the measured data. In this study, the PCA method was chosen for such analysis to discriminate between the tomatoes' three maturity stages; 'Green/ Breaker', 'Turning/ Pink', and 'Light red /Red' using LIF spectra. In Fig. 2, PCA has been performed to the whole spectral range at (200-780 nm). Clusters of 30 spectra for each of the three maturity stages resulted in 83.4% of the total variance, with PC1 = 67.2% and PC2 = 16.2% indicating better discrimination of the maturity stages. According to Lu et al. [11], the physiological variations in tomato fruit are more evident in the visible range of 664-680 nm, and the differences in spectra are related to the changes in the fruit's physicochemical properties. These results demonstrate the potential of PCA for classifying the maturity stages.

Conventional methods

Chlorophyll content (mg/100 g), hue angle (h°), and firmness (kg/cm²) for the different maturity stages have been



Fig. 2 The principal component plot for discriminating tomatoes' maturity stages at the whole spectral range (200–780 nm)

measured to validate the spectroscopically obtained results. The simple linear regression between the peaks of LIF spectra and each characteristic parameter is expressed by the regression coefficient (R^2) and the obtained linear equation y = bx + a, where y is the predicted value, x is the actual value of the characteristic parameter. The actual values corresponding to the predicted values are shown in Fig. 3 (A), (B), and (C). The Y-axis represents the actual value (measured using conventional methods), and the X-axis represents the predicted value (calculated using the linear regression equation). The findings revealed that characteristic parameters in tomato fruit altered throughout ripening and depended on the maturity stage (Table 1).

The chlorophyll content measured using the conventional method has been significantly decreased from the early stages to the end of ripening (P > 0.05, HSD = 1.8). It is well-known that chloroplast plastids are responsible for chlorophyll synthesis in plant cells. The conversion from chloroplasts to chromoplasts, the plastids responsible for carotenoid accumulation, induce chlorophyll degradation throughout ripening and storage [34, 35]. The results exhibited a good correlation for the chlorophyll content with an \mathbb{R}^2 value of 0.85. The predicted values were calculated from the linear regression equation y = 0.0031x + 6.315. According to the data shown in Fig. 3 (A) and Tables 1, the highest actual and predicted chlorophyll values of 15.52 and 15.94 mg/100 g, respectively, were established in the 'Green/Breaker' stage. However, the lowest actual and predicted chlorophyll values of 5.55 and 7.01 mg/100 g were established in the 'Light-red/Red' stages. However, the predicted value showed the least coefficient of variation, reaching 6.02%. Even though the differences between actual and predicted values were not statistically significant (P = 0.985), the decrease in R² value could be due to the inaccuracy of chemically measuring chlorophyll content.

As shown in Tables 1, the flesh firmness measured using conventional technique significantly decreased (P > 0.05, HSD = 0.41) with advanced maturity phases from 'Green/Breaker' stage to 'Light-red/Red' stage, which is in agreement with [Radzevičius et al.; Huang et al.] [29, 36]. Overall, the firmness has significantly decreased by 40.32 and 64.64% for the 'Turning/Pink' and 'Light-red/Red' stages, respectively, compared to the 'Green/Breaker' stage. According to [8], the firmness reduction during ripening is due to the cell wall depolymerization and the middle lamel-la's increased solubility.

The results showed that the firmness prediction has a high regression coefficient of 0.94 (Fig. 3 (B)) without a

Maturity Stage	Descriptive Statistics	Firmness kg/cm ²		Hue angle (h°)		Total chlorophyll mg/100 g	
		Actual	Predicted	Actual	Predicted	Actual	Predicted
Green/Breaker	Mean	2.49	2.47	99.82	97.82	13.12	13.05
	Standard error	0.09	0.10	2.46	2.48	0.29	0.43
	Minimum	1.80	1.87	79.84	81.84	11.42	10.32
	Maximum	3.30	3.65	116.32	115.27	15.52	15.94
	Coefficient of variation %	18.20	19.92	10.18	10.46	9.19	13.66
Turning/Pink	Mean	1.48	1.43	73.39	73.28	10.73	10.11
	Standard error	0.03	0.04	1.05	1.87	0.27	0.36
	Minimum	1.1	1.17	67.19	63.58	8.98	8.41
	Maximum	1.7	1.79	82.33	89.38	12.67	13.66
	Coefficient of variation %	11.16	13.16	6.41	11.42	11.53	16.18
Light Red/Red	Mean	0.88	0.97	54.33	55.72	6.21	7.7
	Standard error	0.03	0.01	1.18	0.71	0.17	0.15
	Minimum	0.7	0.92	47.56	53.33	5.55	7.01
	Maximum	1.1	1.10	63.34	63.32	7.11	8.37
	Coefficient of variation %	14.57	6.62	9.77	5.72	8.53	6.02
HSD _{0.05}		0.41	0.44	9.42	10.43	1.8	2.47

Table 1 Descriptive statistics of actual and predicted values of firmness, hue, and total chlorophyll of tomatoes at different maturity stages



Fig. 3 The simple linear regression of the actual values versus the predicted values using LIF spectra for (A) chlorophyll content, (B) firmness, and (C) skin hue (h°) of tomatoes

significant difference between the actual and the predicted value (P=0.952). In addition, the Green/Breaker stage has the highest coefficient of variation of both actual and predicted values, reaching 18.2 and 19.92%, respectively.

Throughout ripening, the actual firmness values ranged from 3.3 to $0.7.\text{kg/cm}^2$. However, the regression equation y = 0.0006x + 0.8044 exhibited the predicted values ranging from 3.65 to 0.92 kg/cm², indicating that LIF spectra and the conventional method both showed that tomatoes had lost the firmness during the ripening period.

Hue angle (h°) represents the color (red, orange, yellow, green, etc.). According to Mcguire [37], the color wheel subtends 360°, with red-purple at an angle of 0°, yellow at 90°, bluish-green at 180°, and blue at 270°. Therefore, the lower hue values indicate more red color development during the ripening of tomato fruit. An excellent prediction of tomatoes' skin hue was obtained with a high regression coefficient of 0.94 (Fig. 3(C)) and a regression equation of y=0.0213x+49.17. The results showed that actual and predicted values were similar, with no significant differences between both approaches (P=0.961). The 'Green/Breaker' stage is responsible for the highest actual value of hue angle of 116.32°, corresponding to a predictive value of 115.27°, while the lowest actual value of 47.56° corresponds to a predictive value of 53.33° noticed in the 'Light-red/Red' stage.

Moreover, the coefficient of variation of the actual values varied in a small range from 6.41% up to 10.18%. In comparison, the variation coefficient of the predicted values varied a little more from 5.72% up to 11.42%. According to Noh and Lu [38], the gradual loss of greenness resulting from carotenoid accumulation may explain the good correlation between chlorophyll fluorescence and skin hue. The authors found an excellent prediction between fluorescence measurement and the skin color of the apple with a correlation coefficient (R^2) of 0.93. In contrast, the correlation coefficient between the fluorescence and fruit firmness did not exceed 0.75.

LIBS measurements

The LIBS technique has been used to follow up the elements' composition in the tomatoes for each maturity stage via the spectroscopic analysis of the laser-induced plasma of the investigated tomato samples. In the spectra of the studied tomato samples, Fig. 4, it is noticeable that the intensities of the cyanide bands, calcium I, II lines, and magnesium lines are the highest in the 'Light red/Red' maturity stage, followed by 'Turning/Pink' then the 'Green/Breaker'. Such results agree with [39], who stated that the minerals content increases during the growth and maturation of tomato fruit. According to [40], calcium, magnesium, and sodium are significant elements in tomato's minerals content. [41] have found that calcium content ranged from 19 to 32 mg/100 g, whereas magnesium content was 12–20 mg/100 g, and sodium level has varied from 9–15 mg/100 g.

In a previously published work, it has been demonstrated that there is a linear relationship between the ratios of ionic to atomic spectral lines intensities and the sample's surface hardness in the LIBS spectrum of a solid target. This means that the harder the surface material is, the greater is the ionic species in the plasma volume [42]. Therefore, the ratio of ionic to atomic intensities has been estimated for the LIBS spectra of the investigated tomato samples. Figure 5 shows a bar graph of the ratios of Ca (II) 317.9 nm/Ca (I) 422.5 nm in the spectra of the tomato's three maturity stages. The spectroscopic estimation of the surface hardness has been shown to have the same trend of the conventionally measured firmness, as shown in Fig. 5. The results show that the tomato is harder in the 'Green/Breaker' maturity stage than in the other stages.



Fig. 4 CN, Na, Ca, and Mg spectral emission lines in the LIBS spectra for all tomatoes' maturity stages



Fig. 5 Surface hardness test [Ca (II) 317.9 nm/Ca (I) 422.6 nm] and firmness for all tomatoes' maturity stages. Error bars show the standard deviation

Conclusions

In the present work, it has been shown that the differences in chlorophyll fluorescence intensity revealed by LIF spectra are strongly associated with firmness and skin color. Such correlation provides a simple tool to predict the ripening stage of tomatoes and any other fruit that vary in skin color with maturation. In the present work, it has been demonstrated that using laser-induced fluorescence (LIF) combined with principal component analysis (PCA) and laser-induced breakdown spectroscopy (LIBS) integrate three techniques in a single system for fast and reliable estimation of ripening characteristics for the tomato. Laser-induced breakdown spectroscopy (LIBS) is a feasible spectrochemical analytical technique for detecting minor and major elements as well as predicting firmness in different maturity stages of tomatoes.

The overall results demonstrated the feasibility of utilizing LIF and LIBS techniques in a compact mobile or portable system to perform in situ fast and accurate characterization of different fruit and plants. However, further research is needed to predict other quality traits and improve chlorophyll content prediction.

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Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

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Conflict of interest The authors declare no competing interests.

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