A non-destructive method for monitoring the ripening of tomatoes based on their induction of chlorophyll fluorescence

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Abstract

Maturity is one of the most important factors associated with assessing the quality of tomatoes. The aim of this study is to develop a new device to measure the degree of ripeness of tomato fruits based on chlorophyll fluorescence. The results of this method, chlorophyll fluorescence, were compared with those of the widely used colorimeter for this purpose. Botanical variety of tomatoes “Alkazar” was used with different stages of maturity: green, breakers, turning, pink, light red, and red. The results indicated that specific parameters of the slow induction of chlorophyll fluorescence, such as the maximum chlorophyll fluorescence ($F_m$) and the coefficient of specific photosynthetic activity ($Rfd$), can be used to classify tomato fruits according to their maturity stage, as efficiently as the hue angle parameter of the color measurements. A correlation coefficient between the hue angle and the slow induction of chlorophyll fluorescence parameters was 0.96 with $F_m$, and 0.97 with $Rfd$. Using the hue angle or $F_m$, the fruits of all six-maturity stages were accurately classified. In conclusion, the developed device method is a non-destructive, innovative, convenient, and less time-consuming than the color-based method.

Introduction

Tomato (*Lycopersicon esculentum*) is one of the most important fruits in the world. It is a major vegetable crop in Egypt with a total production of 7297108 tons/year, making Egypt the fifth in the world in tomato production FAO (2017). Tomatoes contain lycopene, which plays a very important role in reducing the incidence of many diseases such as cardiovascular disease, cancer, heart disease, and osteoporosis. Moreover, Tomatoes are a rich source of vitamins A and C, potassium, folate, and vitamin K (Chang *et al*., 2006; Saad *et al*., 2016). Tomato maturity is one of the most important factors associated with assessing their quality. Assessment of tomato maturity is necessary for determining the timing of fruit harvest, optimizing storage conditions, forecasting of shelf life, export, etc. (Choi *et al*., 1995). Farmers and distributors use the manual sorting method to evaluate the maturity of tomatoes, which is time-consuming, and laborious. In addition, it depends on many
factors, including workers' experience and training, duration of tasks, and working environment (temperature, humidity, noise levels, and ergonomics of the workstation); Therefore, it is not an accurate method (Geyer and Perry, 1982). Color is one of the most valuable product attributes that are widely measured in post-harvest processing, and tomato color is the main factor in determining maturity (Arias et al., 2000; Pathare et al., 2013). USDA (1991) established criteria for classifying tomato fruits based on color, into six ripening stages, “green”, “breaker”, “turning”, “pink”, “light-red” and “red”. With this background, several researchers have measured fruit quality attributes and developed optical devices to measure them.

Currently, the industrial field uses several non-destructive techniques and tools that can capture the external characteristics of fruits to describe the ripening of tomatoes. The colorimeter instrument is one of the most popular instruments that measure color using coordinate data in many color systems, with the CIE color space L* a* b* being the most preferred. In addition, the Hue angle is a reliable ripening indicator, which correlates well with consumer perceptions (López Camelo and Gómez, 2004). In this study, a developed device based on controlling the induction of chlorophyll fluorescence was designed to assess tomato ripening. Chlorophyll is one of the most important pigments found in all plant tissues that contain chloroplasts. The complete differentiation of plastids into the chloroplasts containing high levels of chlorophyll enables the plant tissues to absorb the light. Chloroplasts can then perform photosynthesis to produce energy required for growth and other vital processes. However, as the fruit reaches its mature green degree, the chloroplasts transform again into chromoplast or other types of protoplast resulting in the degradation of the chlorophyll (Bramley, 2002; Gould, 1992; and Grass, 1991). Chromoplasts also contain red or yellow carotenoids such as lycopene (Thimann, 1980). Thus, degradation of chlorophyll with the accumulation of lycopene (a red pigment) turns the color of tomato fruit into red. Chlorophyll fluorescence emissions can enable us to detect any slight changes in chlorophyll concentration in plant tissues before visible morphological symptoms appear; this can also be used in post-harvest fruit operations (Smillie, 1987). This technique makes the early detection of chlorophyll degradation achievable. In post-harvest
physiological studies, it was found that chloroplasts are one of the most sensitive membrane systems, and are similar in sensitivity to mitochondrial membranes (Toivonen, 1992). Thus, chlorophyll fluorescence changes can be potentially the most sensitive measure of membrane changes or disturbances in the plant cell. This fact allows postharvest researchers to obtain useful information about assessing the degree of ripeness of fruits and vegetables containing chloroplasts. A wide range of fruits and vegetables have shown changes in the fluorescence of chlorophyll which was useful for predicting the degree of ripeness such as apples, mangoes, and tomatoes (DeEll and Toivonen, 2003). (Lai et al., 2007) used a fluorescence spectroscopy method to evaluate tomato maturity in the laboratory, while (Lechaudel et al., 2010) assessed the degree of ripeness of mango regardless of the fruit growth conditions using chlorophyll fluorescence, and showed that the chlorophyll fluorescence method is more accurate than daily degree method for ripeness assessment. (Betemps et al., 2012) assessed the ripeness and some features of apple quality using a hand-held multiparametric fluorescence recordings. (Hoffmann et al., 2015) used the chlorophyll fluorescence method to evaluate the ripeness of tomatoes in pre- and post-harvest processes and validated the fluorescence recordings against established non-invasive optical methods based on reflection and remittance. (Kim et al., 2019) measured carotenoid content in tomato fruits and then explored the accuracy of fluorescence indices as a predictor of carotenoid content in tomato fruit compared to the accuracy of color indices. In addition, it was revealed that several indicators of fluorescence and color were extremely correlated. This study aims to develop a new device to measure the degree of ripeness of tomato fruits based on chlorophyll fluorescence. In addition, the results of this method, chlorophyll fluorescence, were compared with those of the widely used colorimeter for this purpose.

Materials and methods

Experimental design and set up

In our experimental study, we used botanical tomato variety “Alkazar” with six ripening stages (green, breakers, turning, pink, light red, and red) based on the USDA standard classification of
tomato maturity USDA (1991). Samples of tomatoes were obtained from a greenhouse at the Russian State Agricultural Academy named after K. A. Timiryazev, Russia – winter season. For each ripening stage, 25 fruits of the same size were harvested to measure skin color and changes in fluorescence emission of chlorophyll.

**Fruit skin color**

Tomato Skin color was measured at different ripening stages at two diametrically opposite spots at the fruit's equator using a colorimeter (Minolta Chromameter 400, Japan). The colorimeter was calibrated using the manufacturer's standard whiteboard. In the L*, a*, b* color space the color changes were measured. The hue angle \[ (H^o = 180 + \tan^{-1}(b*/a*)) \] and chroma values \[ C^* = (a^*2 + b^*2)^{1/2} \] were calculated from the values of a* and b*. Meanwhile, the color difference (ΔE) was determined in according to the following equation (López Camelo and Gómez, 2004).

\[
ΔE = \sqrt{(L^* - 50)^2 + (a^* - 60)^2 + (b^*)^2}
\]  

L* refers to lightness, ranging from black = 0 to white = 100, while the hue angle value (H°) is defined as a color wheel, with red-purple color at an angle of 0°, yellow color at 90°, bluish-green color at 180° and blue color at 270°. Chroma (C*) represents color saturation, and varies from dull (low values) to vivid (high values). Care was taken to measure the same blossom spot on tomato fruits, so that skin discoloration could be monitored and correlated with other measurements such as chlorophyll fluorescence.

**Measurements of chlorophyll fluorescence**

Hans Kautsky discovered the induction curve (Lichtenthaler et al., 2005) of chlorophyll fluorescence (Figure 1), also called the Kautsky curve. This curve represents the temporary changes in the level of intensity (emission) of the chlorophyll fluorescence reflected by a photosynthetic object. Usually, it
consists of two phases: the fast phase (less than a second) which includes the reactions of the light phase of photosynthesis. The second phase tends to be slow and takes a few minutes. Slow induction of chlorophyll fluorescence consists of changing the fluorescence intensity from the maximum to the stationary level. The vast majority of the fluorescence under normal conditions is due to the chlorophyll a photosystem II.

In this study, a new, low-cost, and simple device was developed to calculate the parameters related to chlorophyll fluorescence. In order to reduce cost, many of the components used are complete packages or embedded that only require a small amount of external components. We researched for the best choices for light excitation, taking into account the power requirements. Figure 2 shows the components of the chlorophyll fluorescence device and some of the components are described as follows.

1) LEDs: As the light intensities should be sufficient to saturate the photochemistry, the choice of light source is of particular interest to us. UV-light has a higher excitability on chlorophyll than IR or white light, yielding the ability of saturating the system using less power. This does tend to shift the emission spectrum somewhat, but for our purposes, it is only advantageous, as the detection window for emission is also broader when we use UV excitation light (Lambers et al., 2008). Another reason to use UV LEDs is the fact that excitation and emission can be easily separated using low-cost filters. In the event of IR excitation light, the filtering ability is more crucial, requiring precision filters that we cannot afford. In our device, a UV LED (470 nm) was used.

2) Sensor: the chlorophyll fluorescence was detected using a camera (PK-836FN).

3) Filter: Two filters, one red and the other green, were used in tandem in order to form a responsive curve appropriately. In addition, to reduce the measuring of the reflected UV light or any unwanted light.

4) Microcontroller: both UV LEDs and sensor are connected to the used microcontroller (Arduino-nano). LEDs are programmed in order to turn on and off the light source or control its intensity (necessary for obtaining the various parameters of chlorophyll fluorescence). The microcontroller is
also responsible for acquiring the signal from the sensor and converting it into values. Then, send it via USB-hub to a computer for processing and analysis. The microcontroller is powered directly from the USB-hub, and the operational voltage of the whole device is 3.3V, as this is the reference voltage of the microcontroller. As such, the LED also runs at 3.3V.

5) Software: for receiving and processing data, a simple program with a user interface is designed. This program receives signals and converts it into information.

The methodology involving the determination of chlorophyll fluorescence is shown in Figure 3. The microcontroller unit (based on "Arduino") with its installed program generates a control signal for an LED that directly emits a light with a wavelength of 470 nm to the object (tomato). Light reflected from tomatoes of different wavelength (from 650 to 820 nm) passes through the filters. After that, the chlorophyll fluorescence is detected using a camera in which an electrical signal is formed through a USB port to the computer. Finally, according to the program installed on the computer, it analyzes the signal levels from the amplitudes and spectral composition of the light flux emitted and reflected from the tomato and then the data is displayed. The fluorescence excitation wavelength was 470 ± 8 nm and its intensity on the fruit surface ranged from 3200 to 4700 µmol. m⁻².s⁻¹.

**Evaluation of chlorophyll fluorescence as a tool to monitor fruit maturation after harvest**

In this study, 150 fruits were used and classified into six ripening stages (green, breakers, turning, pink, light red, and red) by naked eyes depending on their color. Each stage includes 25 fruits that were used to obtain the colorimeter and fluorescence values for that stage. Color and chlorophyll fluorescence measurements were taken at the same spots for each fruit. Correlation analysis was performed between measurements of fluorescence and color parameters. Based on the fluorescence values and correlation analysis, the slow induction of chlorophyll fluorescence method can be validated.

Slow induction of chlorophyll fluorescence was measured such as the maximum chlorophyll fluorescence ($F_m$) and the stationary level of chlorophyll fluorescence ($F_s$). Additionally, the ratio of
the specific photosynthetic activity was calculated according to the following equation:

\[ R_{fd} = \frac{(Fm - Fs)}{Fs} \quad (2) \]

**Statistical analysis**

Correlation was performed between the slow induction of chlorophyll fluorescence parameters and the color of the same fruit pericarp, while means were separated by Duncan’s multiple range test (p ≤ 0.05). Fruits with different maturity stages were applied using a discriminant analysis (SPSS, v. 20, USA).

**Results and discussion**

**Evaluation of chlorophyll fluorescence as a tool for sorting fruits based on their maturity stage**

Colorimeter and slow induction of chlorophyll fluorescence parameters were measured throughout the tomato-ripening period. It has been observed that different indices of maturity have different values. Figure 4 shows that chroma did not change significantly in the early ripening stages, as it would increase later as the tomato changed from pink to light red, then decling into the red stage. Chroma is not a good indicator of a tomato's ripeness mainly because it expresses the purity or saturation of a single color (different colors may have the same color values). However, the maturity indices (Fm and Rfd) showed a significant difference between different ripening stages.

The calculated maturity indexes indicated that ΔE and a*/b* were essentially expressing the same pattern as Fm and Rfd (Figures 5 and 6). In all maturity stages, all these parameters were significantly different between the visually different stages. a*/b* increased with a higher percentage of red color. a*/b* parameter has a negative relationship with both Fm and Rfd while ΔE has a positive relationship with both of them. Figure 6 shows that the color changes during tomato ripening were a result of changes in the value of ΔE that related to chlorophyll degradation and lycopene synthesis. The ΔE value gradually decreases with maturity as chlorophyll breaks down. Means of ΔE differ significantly
between different maturity stages.

The results of the maximum induction of chlorophyll fluorescence ($F_m$) and the coefficient of specific photosynthetic activity ($R_{fd}$) of tomatoes are shown in Figure 5. A specific pattern of maximum fluorescence of chlorophyll ($F_m$) and the coefficient of specific photosynthetic activity ($R_{fd}$) was observed, since both are gradually decrease with maturity. Thus, the green maturity stage is characterized by high values of maximum fluorescence of chlorophyll and the coefficient of specific photosynthetic activity ($R_{fd}$). On the contrary, full maturity stage has low values of both maximum fluorescence and $R_{fd}$. However, (Kim et al., 2014) did not observe any changes in the levels of hydroxycinamic acids (vanillic acids, chlorogenic, and caffeic) and alkaloids (dehydroand α-tomatine) at different ripening stages. While, terpenoids including carotenoids (lycopene, β-carotene, and lutein) exhibited a significant increase with maturation. Chlorophyll fluorescence emissions decrease with maturity due to the breakdown of chlorophyll. When the chloroplasts transform to chromoplasts, the chlorophyll is broken down and accompanied by a carotenoid pool that begins in the middle, and then diffuses into the fruit (Bramley, 2002; Hobson and Grierson, 1993). This decrease in chlorophyll content is best indicated by the declining rate in $F_m$ and $R_{fd}$.

Measurements of the slow induction of chlorophyll fluorescence parameters and skin color as hue angle ($H^o$) were measured during the tomato ripening process (Figure 7). Fruits of all six maturity stages can be accurately classified using the hue angle. Interestingly, the discriminant analysis demonstrated very accurate classification of harvested tomatoes at the six different ripening stages, and also by using the slow induction of chlorophyll fluorescence parameters (Figure 7). In this regard, the maximum of chlorophyll fluorescence $F_m$ can be used to assess tomato ripeness. Tomato fruits (Gedamu, 2008; Seifert et al., 2014) were also distinguished by their ability to photosynthesize due to the presence of both types of chlorophyll. However, they also observed that during maturation and fruit ripening, there was a significant decrease in chlorophyll $a$ and $b$ with a relatively clear decrease in the chlorophyll type $a$. Similar trends were obtained by (Greer, 2005), in which measurements of color in terms of hue angle ($H^o$) decrease over time throughout the growing season of apples. The
decrease in the chlorophyll content in fruit tissues can be indirectly assessed depending on the
decrease in the fluorescence levels that these tissues reflect when the fruit ripens. Determination of
ripeness based on measurements of the hue angle color has been shown to be a reliable and non-
destructive tool in tomatoes (López Camelo and Gómez, 2004). Also, slow induction of chlorophyll
fluorescence parameters provides easy-to-use and low-cost techniques for monitoring tomato
ripening.

Correlations among variables

Correlation analysis between the different measured variables can help formulate the possible
relationships between them (Table 1). The correlation between the slow induction of chlorophyll
fluorescence parameters, $Fm$ and $Rfd$, was very strong positive (0.99), and there are also a very strong
positive correlation between the slow induction of chlorophyll fluorescence parameters and (hue and
$\Delta E$). On the other hand, the correlation coefficients between $Fm$ with both chroma and $a^*/b^*$ were -
0.85 and -0.95, respectively. Also, a negative correlation was observed between $Rfd$ with both chroma
and $a^*/b^*$. Moreover, the correlation coefficients between hue and both $Fm$ and $Rfd$ were 0.96 and
0.97, respectively. Skin color parameters (hue angle parameter), as well as photosynthetic method
(slow induction of chlorophyll fluorescence parameters), are significantly correlated to tomato
maturity. According to (Pék et al., 2010), it is known that the ripening stage of tomato fruits is closely
related to the external fruit color. In this study, the $Rfd$ and $Fm$ parameters provide an alternative,
non-destructive, objective and highly accurate tool that better describe tomato ripening.

Several recent studies have shown that excitation-based chlorophyll fluorescence indices using
different wavelengths can determine fruit quality in grapes (Agati et al., 2013; Cerovic et al., 2009;
Ghozlen et al., 2010), tomato (Hoffmann et al., 2015; Seifert et al., 2014), Jujube (Lu et al., 2012),
and apples (Betemps et al., 2012; Seifert et al., 2014; Huybrechts et al., 2002). Therefore,
fluorescence measurements can be considered as an important tool in evaluating the overall quality
of a fruit and its ripeness at different stages. In our experiments, the $Fm$, and $Rfd$ indicators provided
appropriate information about ripening progress and how to sort tomato fruits as well as in post-harvest processes.

Conclusions

In this study, a device to measure chlorophyll fluorescence was developed to estimate the degree of ripeness of fruits. The choice of LEDs and sensors was important to the design of the device, where simplicity and power requirements took precedence, as long as they were within functional parameters. The use of the slow induction of chlorophyll fluorescence parameters allow tomato separation by ripening more objectively and accurately resulting in a more homogeneous separation. This method can be adapted and implemented as a fast, low-cost, non-destructive method capable of monitoring the post-harvest ripening process in tomato fruits, and tomatoes can be classified according to the ripening stage at least with the same efficiency and reliability as the color of the hue angle. The system relates to agricultural machinery and can be combined with a conveyor belt in harvesters for sorting tomatoes. In addition, it can be used in post-harvest operations.

References


Bramley P. M. 2002. Regulation of carotenoid formation during tomato fruit ripening and


Table 1. Correlation coefficients among the different variables measured during the experiment.

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<tr>
<th></th>
<th>Fm</th>
<th>Rfd</th>
<th>a*/b*</th>
<th>Chroma</th>
<th>Hue</th>
<th>∆E</th>
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<tr>
<td>Rfd</td>
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<tr>
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<tr>
<td>∆E</td>
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<td>-0.84</td>
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Figure 1. Induction curve of chlorophyll fluorescence, where: \( Fm \) - maximum chlorophyll fluorescence, \( Fs \) - stationary fluorescence (Lichtenthaler et al., 2005).
Figure 2. Schematic diagram of a chlorophyll fluorescence device: 1-Led, 2 - object of research (tomato), 3-light filter (KC19), 4- camera (PK-836FN), 5-microcontroller (Arduino-nano), 6- USB port, 7-computer, 8- LM259s, 9- Relay module, 10- switch DPDT, 11-led, 12- switch SPDT.

Figure 3. Stages of the process of measuring chlorophyll fluorescence.
Figure 4. Changes in the slow induction of chlorophyll fluorescence parameters and Chroma with the degree of maturity of “Alkazar” tomatoes. Each bar represents the average value of a specific maturity stage associated with the standard deviation. The average values with different letters for each parameter differ significantly at $p \leq 0.05$, $n=25$. 
Figure 5. Changes in the slow induction of chlorophyll fluorescence parameters and $a^*/b^*$ with the degree of maturity of tomatoes of the “Alkazar” variety, $n=25$. 
Figure 6. Changes in the slow induction of chlorophyll fluorescence parameters and $\Delta E$ with the degree of maturity of “Alkazar” tomatoes, $n=25$. 
Figure 7. Changes in the slow induction of chlorophyll fluorescence parameters and the hue angle ($H^*$) with the degree of maturity of “Alkazar” tomatoes, n=25.