

Non-destructive method for monitoring tomato ripening based on chlorophyll fluorescence induction

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Abstract

Maturity is one of the most important factors in the assessment of tomato quality. The aim of this study is to develop a new device to measure the degree of tomato ripeness based on chlorophyll fluorescence. The results of this method in terms of chlorophyll fluorescence were compared with those from the most widely used colorimeter. The botanical variety of tomatoes 'Alkazar' was used at different stages of maturity: green, breakers, turning, pink, light red, and red. The results indicated that specific parameters of slow induction of chlorophyll fluorescence, such as maximum chlorophyll fluorescence (*F_m*) and the coefficient of specific photosynthetic activity (*R_{fd}*), can be used to classify tomatoes according to their maturity stage as efficiently as with the hue angle parameter of color measurements. The correlation coefficient between the hue angle and the slow induction of chlorophyll fluorescence parameters was 0.96 with *F_m*, and 0.97 with *R_{fd}*. Using the hue angle or *F_m*, tomatoes of all six-maturity stages were accurately classified. In conclusion, this new measurement method is a non-destructive, innovative and convenient approach, which is less time-consuming than the colour-based method.

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Introduction

Tomato (*Lycopersicon esculentum*) is one of the most important vegetable in the world. It is a major vegetable crop in Egypt with a total production of 7,297,108 tons/year, making Egypt the fifth tomato-producing country in the world (2017; www.fao.org/faostat/en/#data/QC). 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 in quality assessment. The assessment of tomato maturity is necessary to determine the timing of fruit harvest, to optimize storage conditions, to forecast 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, this is not an accurate method (Geyer and Perry, 1982). Colour is one of the most valuable product attributes that are widely measured in post-harvest processing. It is the main factor to determine maturity (Arias *et al.*, 2000; Pathare *et al.*, 2013). USDA (1991) criteria for classifying tomatoes based on colour into six ripening stages: *green*, *breaker*, *turning*, *pink*, *light-red* and *red*. Within this framework, several researchers have measured fruit quality attributes and developed optical devices to measure them.

Currently, the industrial players use several non-destructive techniques and tools that can capture external characteristics to describe the ripening of tomatoes. The colorimeter is one of the most popular instruments that measure colour using coordinate data in many colour systems. The most commonly used is the CIE colour space L*a*b*. The Hue angle is also a reliable ripening indicator, which correlates well with consumer perceptions (López Camelo and Gómez, 2004). In this study, a developed device based on the control of chlorophyll fluorescence induction 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 light. Chloroplasts can then perform photosynthesis to produce the 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 proplast resulting in the degradation of chlorophyll (Grass, 1991; Gould, 1992; Bramley, 2002). Chromoplasts also contain red or yellow carotenoids, such as lycopene (Thimann, 1980). Therefore, chlorophyll degradation with the accumulation of lycopene (a red pigment) turns the colour 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, chloroplasts were found to be one of the most sensitive membrane systems, and are similar to mitochondrial membranes in terms of sensitivity (Toivonen, 1992). Consequently, 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 for the assessment of ripeness of fruits and vegetables containing chloroplasts. A wide range of fruits and vegetables have shown changes in chlorophyll fluorescence, which was useful to predict the degree of ripeness in apples, mangoes, and tomatoes (DeEll and Toivonen, 2003). Lai *et al.* (2007) used fluorescence spectroscopy 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 chlorophyll fluorescence is a more accurate method than the 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 recorder. 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 tomatoes compared to the accuracy of colour indices. In addition, several indicators of fluorescence and colour were found to be 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, *i.e.* the use of chlorophyll fluorescence, were compared with those of the most widely used colorimeter for this purpose.

Materials and methods

Experimental design and set up

In our experimental study, we used the botanical tomato variety 'Alkazar' at 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 in winter. For each ripening stage, 25 fruits of the same size were harvested to measure skin colour and changes in chlorophyll fluorescence emission.

Fruit skin colour

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

$$\Delta E = \sqrt{(L^* - 50)^2 + (a^* - 60)^2 + (b^*)^2} \quad (1)$$

L^* refers to lightness, ranging from black = 0 to white =100,

while the hue angle value (H°) is defined based on a colour wheel with red-purple at an angle of 0° , yellow at 90° , bluish green at 180° and blue at 270° . The chroma (C^*) represents the colour 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 in changing the fluorescence intensity from the maximum to the stationary level. The vast majority of 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 costs, many of the components used are from the market and they are embedded with a small number of external connections. We conducted research to identify the best solutions for light excitation, taking into account power requirements. Figure 2 shows the components of the chlorophyll fluorescence device and some of the components are described in the following list.

- i) *LEDs*: As light intensities should be sufficient to saturate photochemistry, the choice of the light source is of particular interest to us. UV-light has a higher excitability on chlorophyll than IR or white light, thus enabling us to saturate the system using less power. This tends to shift slightly the emission spectrum, but for our purposes this is only advantageous, as the detection window for emission is also broader when UV excitation light is used (Lambers and Pons, 2008). Another reason for using UV LEDs is that excitation and emission can be easily separated with low-cost filters. In the case of IR excitation light, the filtering ability is more crucial and requires precision filters which we could not afford. In our device, a UV LED (470 nm) was used.
- ii) *Sensor*: chlorophyll fluorescence was detected using a camera (PK-836FN).
- iii) *Filter*: Two filters, one red and one green, were used in tandem in order to form a responsive curve appropriately and also to reduce the measurement of reflected UV light or any unwanted light.
- iv) *Microcontroller*: both UV LEDs and sensor are connected to the selected microcontroller (Arduino-nano). LEDs are programmed in order to turn on and off the light source or control its intensity (necessary to obtain the various parameters of chlorophyll fluorescence). The microcontroller is also responsible for acquiring the signal from the sensor, converting it into values and sending 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.
- v) *Software*: a simple program with a user interface was designed for receiving and processing data. This program receives signals and converts them into information.

The methodology for 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 towards the object (tomato). This light with different wavelength (from 650 to 820 nm) reflected from tomatoes crosses the filters. Subsequently, chlorophyll fluorescence is detected using a camera in which an electrical signal is sent through a USB port to the computer. Finally, the program installed in the computer analyses the signal levels from the amplitudes and spectral composition of the light flux emitted and reflected from the tomato and then displays the data. The fluorescence excitation wavelength was 470 ± 8 nm and its intensity on the fruit surface ranged from 3200 to 4700 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

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) with the naked eye depending on their colour. Each stage includes 25 fruits that were used to obtain the colorimeter and fluorescence values for that stage. Colour and chlorophyll fluorescence measurements were taken in the same spots for each fruit. A correlation analysis was performed between measurements of fluorescence and colour parameters. Based on the fluorescence values and correlation analysis, the slow induction of chlorophyll fluorescence method can be validated. The slow induction of chlorophyll fluorescence was measured in terms of 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:

$$R_{fd} = (F_m - F_s) / F_s \quad (2)$$

Statistical analysis

Data were analyzed in (SPSS, v. 20, USA). The correlation was calculated between the slow induction of chlorophyll fluorescence parameters and the colour of the same fruit pericarp, while the means were separated by Duncan's multiple range test ($P \leq 0.05$).

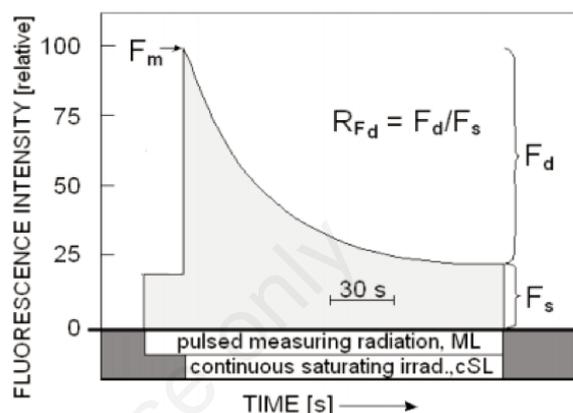


Figure 1. Induction curve of chlorophyll fluorescence, where: F_m is maximum chlorophyll fluorescence, F_s is stationary fluorescence (Lichtenthaler *et al.*, 2005).

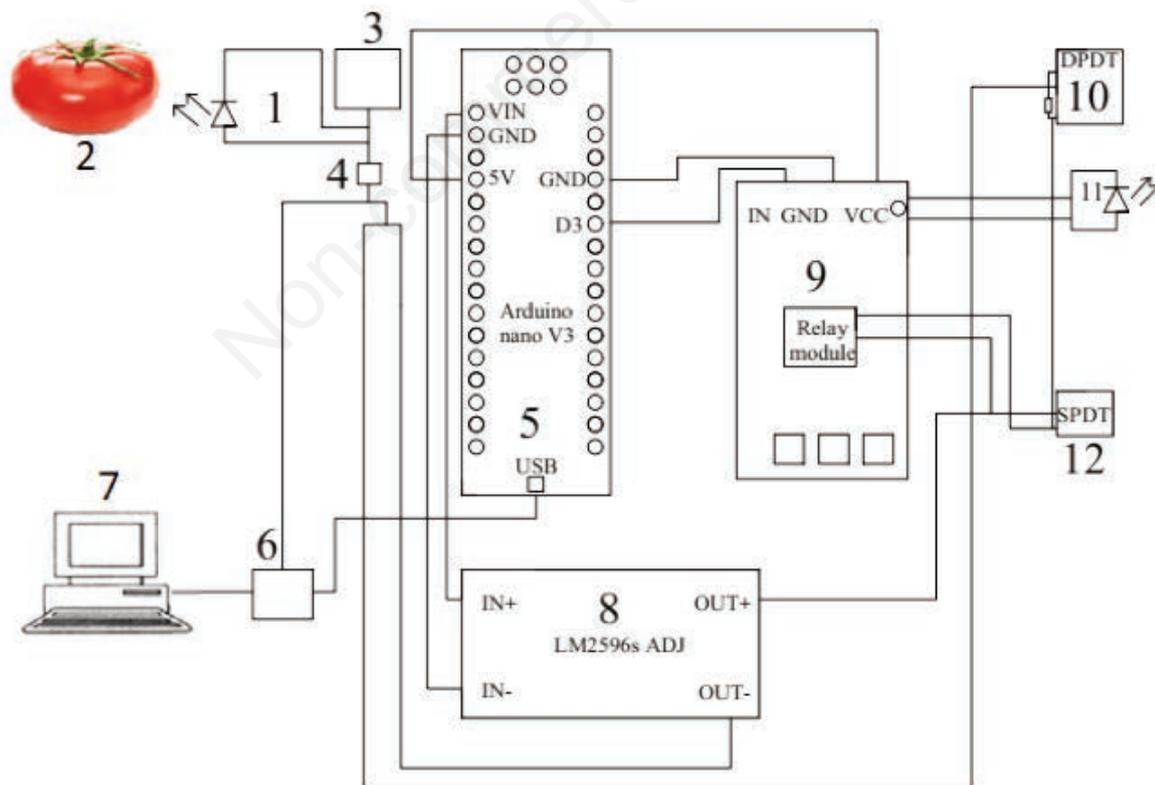


Figure 2. Schematic diagram of a chlorophyll fluorescence device: 1-Led, 2 - object of research (tomato), 3-light filter (KC19), 4- camera (PK-836FN), 5-microcontroller (Arduino-nano), 6-USB port, 7-computer, 8- LM259s, 9- Relay module, 10- switch DPDT, 11-led, 12-switch SPDT.

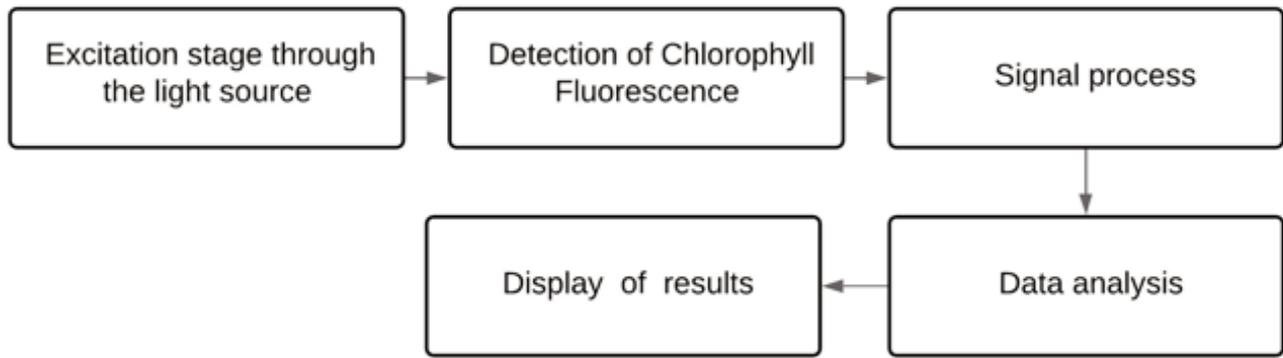


Figure 3. Stages of the chlorophyll fluorescence measuring process.

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 was observed that different maturity indices had different values. Figure 4 shows that the chroma did not change significantly in the early ripening stages, but it increased later as the tomato changed from pink to light red, then declined in the red stage. The chroma is not a good indicator of a tomato ripeness mainly because it expresses the purity or saturation of a single colour (different colours may have the same colour values). However, the maturity indices (*Fm* and *Rfd*) showed a significant difference between different ripening stages.

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

Figure 5 reports the results of the maximum induction of chlorophyll fluorescence (*Fm*) and the coefficient of specific photosynthetic activity (*Rfd*) of tomatoes. A specific pattern was observed for maximum chlorophyll fluorescence (*Fm*) and the coefficient of specific photosynthetic activity (*Rfd*), since both gradually decreased with maturity. Therefore, the green maturity stage was characterized by high values of maximum chlorophyll fluorescence and the coefficient of specific photosynthetic activity (*Rfd*). On the contrary, the full maturity stage had low values of both maximum fluorescence and *Rfd*. However, Kim *et al.* (2014) did not observe any changes in the levels of hydroxycinnamic acids (vanillic acids, chlorogenic acids, and caffeic acids) and alkaloids (dehydroand α -tomatine) at different ripening stages. Conversely, terpenoids including carotenoids (lycopene, β -carotene, and lutein) exhibited a significant increase with maturation. Chlorophyll fluorescence emissions decreased with maturity due to the breakdown of chlorophyll. When the chloroplasts transformed to chromoplasts, the chlorophyll was broken down and accompanied by a carotenoid pool that began in the middle, and then diffuses into the fruit (Bramley, 2002; Hobson and Grierson, 1993). This decrease

in chlorophyll content was best indicated by the declining rate in *Fm* and *Rfd*.

Measurements of the slow induction of chlorophyll fluorescence parameters and skin colour as hue angle (H°) were measured during the tomato ripening process (Figure 7). Fruits from all six maturity stages could be accurately classified using the hue angle. Interestingly, the discriminant analysis demonstrated a 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

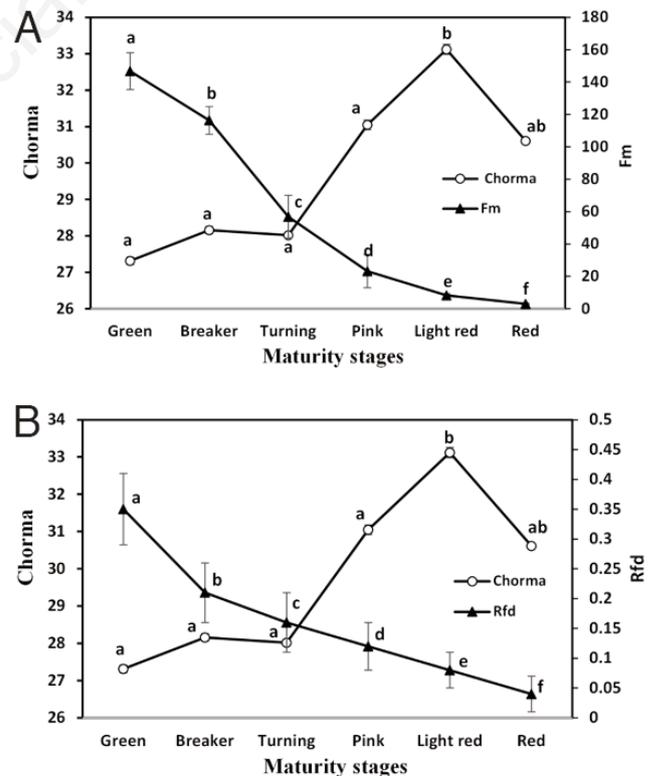


Figure 4. Changes in the slow induction of chlorophyll fluorescence parameters and Chroma according to the degree of maturity of 'Alkazar' tomatoes. Each bar represents the average value of a specific maturity stage associated with the standard deviation. The average values with different letters for each parameter differ significantly at $P \leq 0.05$, $n=25$.

chlorophyll fluorescence F_m could be used to assess tomato ripeness. Tomato fruits (Gedamu, 2008; Seifert *et al.*, 2014) were also distinguished based on their ability to photosynthesize due to the presence of both types of chlorophyll. However, these authors also observed that during maturation and fruit ripening, there was a significant decrease in chlorophyll a and b with a relatively clear decrease in chlorophyll type a. Similar trends were obtained by Greer (2005), in which measurements of colour in terms of hue angle (H°) decreased 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. The determination of ripeness based on measurements of the hue angle color has proven a reliable and non-destructive tool in tomatoes (López Camelo and Gómez, 2004). Also, slow induction of chlorophyll fluorescence parameters provided easy-to-use and low-cost techniques for monitoring tomato ripening.

Correlations among variables

The correlation analysis between the different measured variables can help formulate possible relationships between them (Table 1). The correlation between the slow induction of chlorophyll fluorescence parameters, F_m and Rfd , was very strongly positive (0.99), and there was also a very strong positive correlation between the slow induction of chlorophyll fluorescence parameters and (hue and ΔE). On the other hand, the correlation coefficients between F_m 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 coeffi-

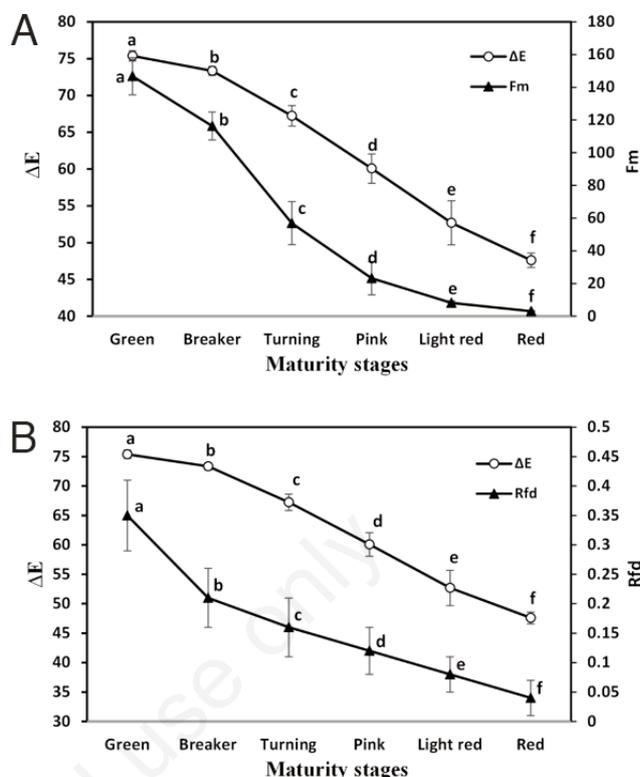


Figure 6. Changes in the slow induction of chlorophyll fluorescence parameters and ΔE according to the degree of maturity of 'Alkazsar' tomatoes, $n=25$.

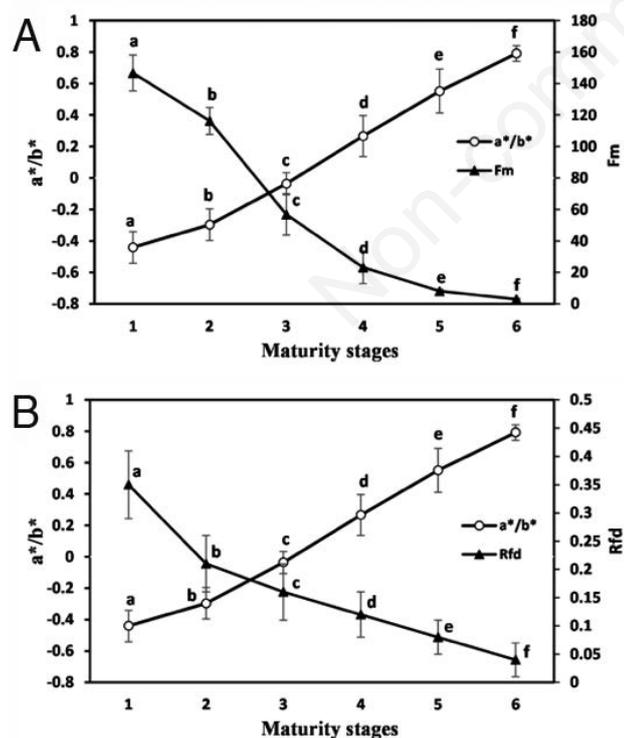


Figure 5. Changes in the slow induction of chlorophyll fluorescence parameters and a^*/b^* according to the degree of maturity of tomatoes of the 'Alkazsar' variety, $n=25$.

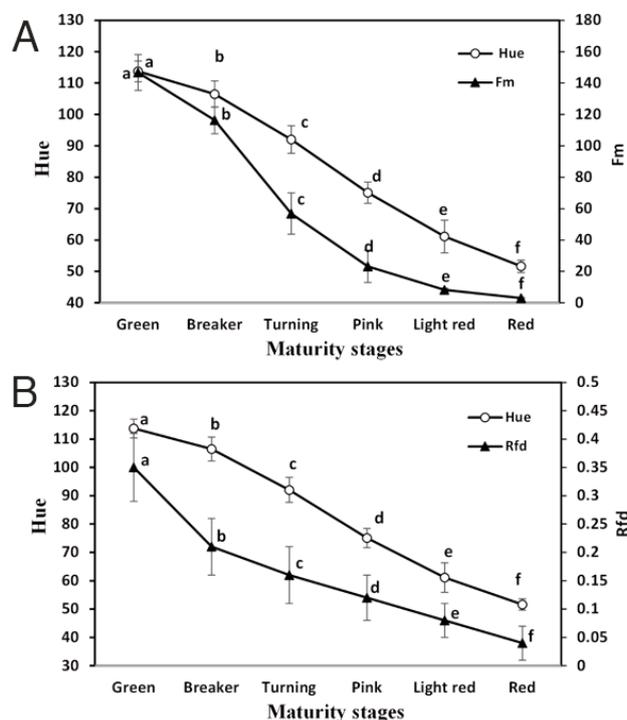


Figure 7. Changes in the slow induction of chlorophyll fluorescence parameters and the hue angle (H_0) according to the degree of maturity of 'Alkazsar' tomatoes, $n=25$.

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

	<i>Fm</i>	<i>Rfd</i>	<i>a*/b*</i>	Chroma	Hue	ΔE
<i>Fm</i>	1					
<i>Rfd</i>	0.99	1				
<i>a*/b*</i>	-0.95	-0.96	1			
Chroma	-0.85	-0.84	0.83	1		
Hue	0.96	0.97	-0.99	-0.85	1	
ΔE	0.94	0.95	-0.99	-0.84	0.99	1

coefficients between hue and both *Fm* and *Rfd* were 0.96 and 0.97, respectively. Skin color parameters (hue angle parameter) as well as the photosynthetic method (slow induction of chlorophyll fluorescence parameters) were 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 provided an alternative, non-destructive, objective and highly accurate tool to 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 (Seifert *et al.*, 2014; Hoffmann *et al.*, 2015; Abdelhamid *et al.*, 2020), 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 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 the 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 fruit ripeness. The choice of LEDs and sensors was important for the design of the device, in which simplicity and power requirements were privileged, provided they were within functional parameters. The use of the slow induction of chlorophyll fluorescence parameters allows tomato separation based on ripening in a more objective and accurate manner, thus leading to 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 as efficiently and reliably as based on the colour of the hue angle. This system can be installed on agricultural machinery and can be combined with a conveyor belt in harvesters for sorting tomatoes. In addition, it can also be used in post-harvest operations.

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