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Mathematical model for detecting tomato ripeness using chlorophyll fluorescence

M.A. Abdelhamid[1,*], S.A. Rawdhan[2], S.S. Shalaby[1] and M.F. Atia[1]

[1]Department of Agricultural Engineering, Faculty of Agriculture, Ain Shams University, 11241 Egypt
[2]Dept. of Agri. Mechanization and equip-Coll. Agri. engine. Sci. University of Baghdad, 47040 Iraq
[*]Correspondence: mahmoudabdelhamid@agr.asu.edu.eg

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Abstract. A precise assessment of tomato ripeness is crucial in the harvesting and marketing procedures. Chlorophyll fluorescence is being relied on as a harmless approach for tracking the maturity of tomatoes in postharvest research. In this study, mathematical model is proposed based on measuring the intensity of fast chlorophyll fluorescence of tomatoes depending on their degree of maturity. In the experimental study, four stages of tomato ripening (green, turning, pink, and red) for three varieties ('Alkazar', 'Lezginka', and 'Rosanchik') were used. The Fluorescence Intensity (FI) data over time were represented using a third-degree polynomial function and finding its first derivative curve. The FI parameter was obtained as the fluorescence level at the first inflection point on the fluorescence induction curve (at time t_I on the first derivative curve). According to the obtained mathematical models, the optimal time for monitoring the degree of ripeness of tomatoes was $t_I = 129 \pm 4$ ms. According to the results of experimental studies, there is a general trend, regardless of the variety used, that the FI decreases with tomato maturity. The FI may assist in sorting and grading processes for fresh vegetables and fruits. It can also be used as a system that can be integrated into harvest and post-harvest machinery for agricultural products.

Key words: tomato, model, maturity stage, fast chlorophyll fluorescence.

INTRODUCTION

Tomatoes are sensitive fruits that need to be transported swiftly to the market if they are mature or shipped if they have not reached one of the other maturing phases. As a result, determining the optimal ripeness is critical to maintaining its texture, aroma, and nutrient content. Tomato fruit sorting and postharvest maturing tracking according to fruit maturity levels at harvest are required to ensure the maximum possible quality and ability to sell the fully matured product (Kasampalis et al., 2020).Yet, large-scale measures are inapplicable when using destructive methods. The physical characteristics of tomatoes can be most frequently defined through the quality parameters, including degree of maturity (Vursavus & Kesilmis, 2016). Human labor is also highly used in fruit crop sorting (Guann., et al., 2022; Wang et al., 2022). In the past few decades, as urbanization has expanded and agricultural labor has become more and more scarce, laborious sorting and classification methods have become progressively costly and fail to meet market needs (Zhou et al., 2018; Bai et al., 2022; Rong et al., 2022; Yang et al., 2023). Recent years have seen attempts to control fruit quality using non-destructive technologies, particularly imaging techniques like thermal, infrared thermography, and microwave imaging; spectral analysis techniques like Raman and hyperspectral image analysis, fluorescent visualizing, and laser light backscatter imaging; nuclear magnetic techniques and other approaches (Avotins et al., 2020).

Tomato ripening includes multiple physiological processes, including the decomposition of chlorophyll and an increase in carotenoids, resulting in higher levels of beta-carotene and lycopene, which are the cause of tomatoes' antioxidant benefits. As tomatoes mature, their chlorophyll content reduces, but their carotenoid content rises until they reach full ripeness (Fraser et al., 2007). Tomatoes are among the world's most desirable fruits due to their wide range of uses in cuisine. Tomatoes are not just tasty, but their high carotene content is commonly recognized. Non-destructive testing procedures are becoming more popular in a variety of industries (Alsiņa et al., 2019). Tomato ripening consists of different changes in fruit color produced by biochemical processes in the fruit's tissues. During the early stages of ripening, the tomato fruit has a significant amount of the green pigment chlorophyll in cells called chloroplasts. During growth, the chloroplast of green tomatoes starts to separate into chromoplasts, which commence the breakdown of chlorophyll to tetrapyrroles, permitting the exposure or release of red pigments called carotenoids, which are also contained in the chloroplast (Wold et al., 2004; Klee & Giovanni, 2011; Saad et al., 2016). Therefore, a fluorescence method was used during different stages of tomato maturity.

The fluorescence of chlorophyll corresponds strongly with the amount of light taken up by cell pigments. The majority of the energy absorbed by light is used for photosynthesis, while one portion disappears as heat, and only a small amount is released as fluorescent light shortly after the charged electron goes to its fundamental state (Maxwell & Johnson, 2000). Among the postharvest techniques accessible, chlorophyll fluorescence has been examined as a useful tool for evaluating the maturation and aging of green cells, used in both green leaves and many pigment-containing fruits (Song et al., 1997). Chlorophyll fluorescence in mature or aged fruit is affected by chlorophyll concentration and chlorophyll's activities. The decrease in fluorescence of chlorophyll throughout the banana and maturation was correlated with chlorophyll concentration and chloroplast ability loss. Maturity is characterized by a rise in cell membrane decomposition (Smillie, 1978), which results in chloroplast aging, a process in which cells degrade their physical integrity, decreasing photosynthetic activity, and, as a result, modifying fluorescence characteristics (Sanxter, 1992; Tucker, 1993).

After illumination, the fluorescence of chlorophyll starts and maintains a characteristic pattern known as the 'Kautsky curve', fluorescence transient, OJIP transient, or OJIP-test. This curve contains four separate phases (O, J, I, and P) and depends on the thylakoid membrane 'energy flow' hypothesis (Strasser et al., 2000). Furthermore, the OJIP method includes variables related to quantum yields, energy changes, and essential values determined and measured. The OJIP test has already been utilized to examine a variety of environmental conditions, including dehydration and chilling challenges, along with basic temperature impacts, nutritional shortages, and toxic metal loads (Kalaji et al., 2016). Changes in chlorophyll fluorescence can be used to detect changes in photosynthesis. Fig. 1 shows the kinetics of chlorophyll fluorescence in all photosynthetic content which shows an increase in heterogeneity over time and is characterized by four typical steps: the first rise from the origin (O) at 20 s or 50 s, followed by an intermediate state at 2 ms (J step), then a second slower rise involving a second intermediate step at 30 ms (I step), followed by the P step, where maximum fluorescence occurs (Liu et al., 2021).

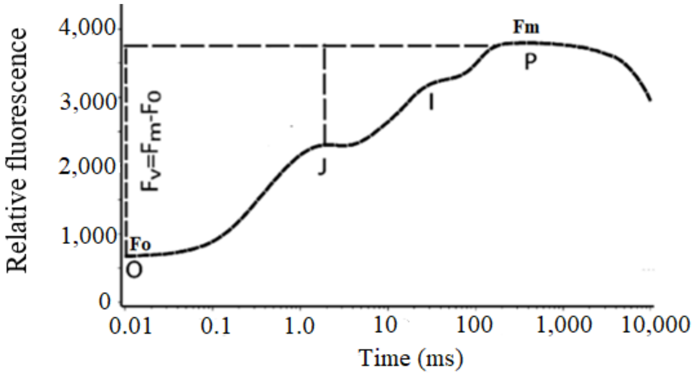


Figure 1. The standard Kautsky curve. Fo represents the initial degree of fluorescence upon illumination. Fm stands for maximum fluorescence, which is the highest level of fluorescence achieved following illumination. Fv stands for variable fluorescence, and it is calculated by subtracting the Fo value from the Fm value (Kargar et al., 2019).

Chlorophyll fluorescence technology is a valuable tool for non-destructive analysis to classify tomato fruit maturity stages (Kasampalis et al., 2020; Abdelhamid et al., 2021). According to the literature, maximum chlorophyll fluorescence (Fm) has been utilized as a non-destructive technique for measuring fruit ripening in postharvest research. In this study aims to propose mathematical model based on chlorophyll fluorescence measurement that can distinguish and classify tomato fruits.

MATERIALS AND METHODS

Tomato samples

For this research, three tomato varieties were chosen: (i) ‘Alkazar’, (ii) ‘Lezginka’, and (iii) ‘Rosanchik’. Four phases of maturation were used for each variety (green, turning, pink, and red). 100 samples of each variety were chosen at random, 25 for each degree of ripeness. This study was conducted on defect-free tomatoes. Samples of tomatoes were obtained from the greenhouse at the Russian State Agricultural Academy named after K. A. Timiryazev, Russia and they were taken to the laboratory for fluorescence testing after being picked. Within half an hour, all fruits were brought to the laboratory in open boxes of cartons for assessments.

Measurements of chlorophyll fluorescence

The fluorescence emitted for each sample was monitored separately at the four distinct maturity stages of all three varieties under study in order to determine the level of maturity of tomatoes based on chlorophyll fluorescence induction. Fluorescence was recorded at two diametrically opposed locations at the fruit's equator at various ripening phases. A silicon photodiode sensor and a blue light emitting diode (5 W) were used in the fluorimeter to record the fast chlorophyll fluorescence induction curves over a time range of 0.01 ms to 1 s. This allowed for the computational recording of the transitory fluorescence. The blue light (455 nm; 5,000 μmol quanta m^{-2} s^{-1}; 1 s duration) saturating pulse from the light-emitting diode was used to measure the maximum fluorescence (Fm) for the dark-adapted condition. For further information, refer to (Kreslavski et al., 2014). The signal was captured every 10 μs during 1 ms and every 1 ms beginning from 1 ms up to 1 s during data collecting. After the signal was smoothed, it was transferred from the silicon photodiode to the computer for further processing. The fluorescence induction curve illustrates variations in chlorophyll emitted by fluorescence in a photosynthetic item. It can be separated into two phases, each lasting about a second. This fast phase involves photosynthetic light-phase processes. The slow phase, on the other hand, may last for minutes.

Model Fitting

For data analysis, the time was the independent variable, and chlorophyll fluorescence was the dependent variable based on the 'Kautsky curve'. As a result, the suggested generalized model may be written as follows:

$$\text{FI} \sim f\,\text{Time} \qquad (1)$$

The data values were fitted with multiple alternative models from several types of fit (polynomial, exponential, linear, power, and logarithmic) to identify the most appropriate relation of FI over time and the degree of confidence of the fit statistics of these models were obtained using 'Mathematica v.12'. The most accurate model within the fitted models was a third-order polynomial correlation between the intensity of fluorescence over time, as shown below:

$$FI = a_3\, t^3 - a_2\, t^2 + a_1\, t + c, \text{ at } 0 \le t < 250\ ms. \qquad (2)$$

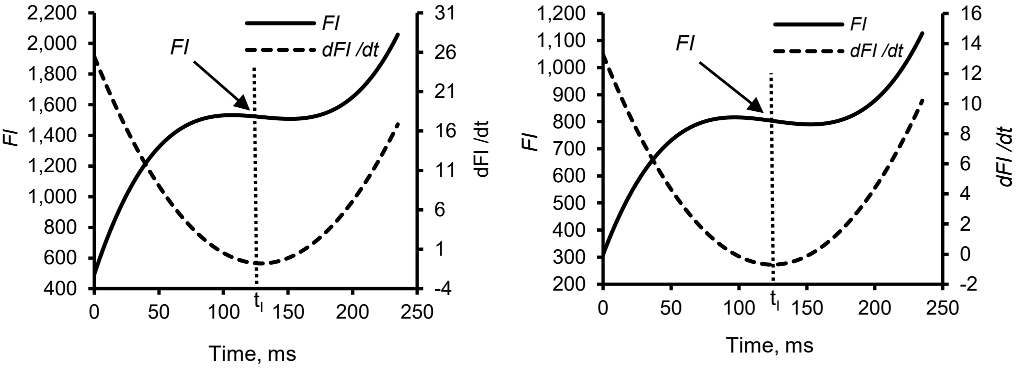
where, FI: chlorophyll fluorescence intensity, rel. units and t: time, ms.

The initial inductive curve was used to derive the first derivative. The FI was then approximated as the fluorescence level at the first bend point on the fluorescence induction curve (at time t_I on the first derivative curve). Calculating the second derivatives of the fluorescence induction curve $d^2FI\ /dt^2$ yielded the time at the first bending point that defines the fluorescence induction curve (t_I). Then, solve $d^2FI\ /dt^2 =$ zero to determine the precise period at which the fluorescence induction curve first inverts. At the time, the FI was calculated for each level of maturity of the tomato cultivars under study.

RESULTS AND DISCUSSION

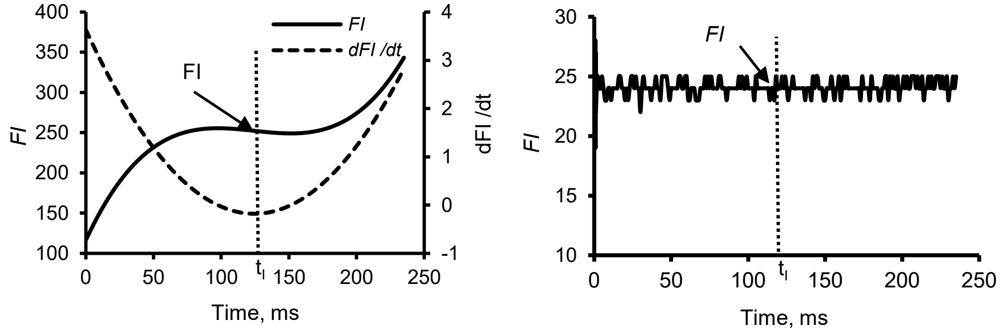
Changes in chlorophyll fluorescence during ripening of tomato fruits

Figs 2–4 show the chlorophyll fluorescence intensity FI and their first derivatives ($dFI\ /\ dt$) dependencies versus time for the 'Alkazar', 'Lezginka', and 'Rosanchik' cultivars. Figs 2–4 demonstrate that the *FI* increases with time until a specific point (*I*), at which point the curve trend begins to change direction for a reasonably short period and then begins to rise again. The first differential curve of the original chlorophyll fluorescence curve, on the other hand, begins with its maximum value and then begins to drop with time to its lowest value at the point corresponding to point (*I*) on the original fluorescence induction curve. Following that, it begins to rise for a relatively brief amount of time until it reaches its higher values. Then it appeared to fall again.



a) Green stage

b) Turning stage

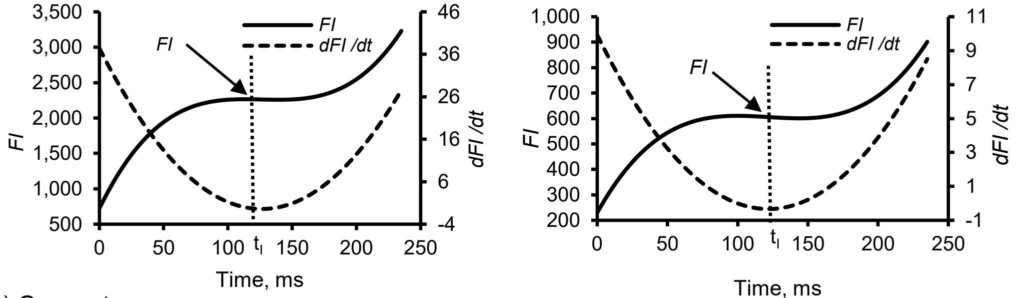


c) Pink stage

d) Red stage

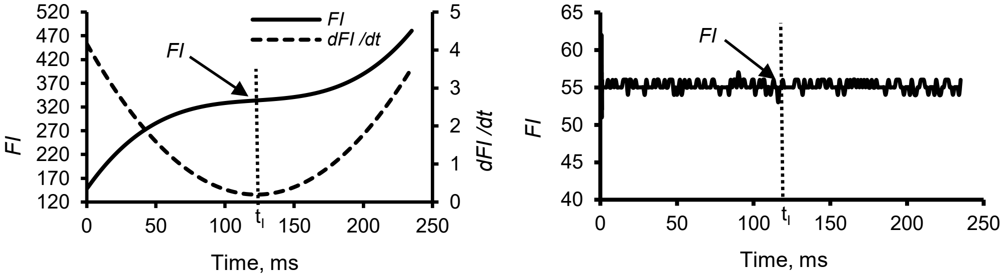
Figure 2. Curves of the fluorescence dependencies of chlorophyll (*FI*) and their first derivatives (*dFI* / *dt*) over time for the ‘Alkazar’ variety for different degrees of ripeness.

A more recent approach is to find the FI parameter using the first derivative. According to the figures, the *FI* value is equal to the signal level at the short-term deceleration point of the fluorescence induction curve's first inflection point. This is the first minimum of time tI on the first derivative curve of the original fluorescence induction curve. Figures reveal that at various stages of maturation, the first inflection point in the fluorescence induction curve occurs with an average duration of 129 ± 4 ms. The dependence is stationary for red tomatoes (Figs 2, d; 3, d, and 4, d) and varies insignificantly (in magnitude) with time.



a) Green stage

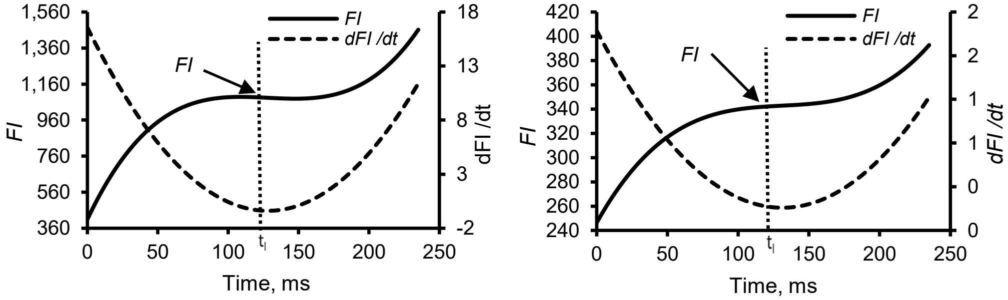
b) Turning stage



c) Pink stage

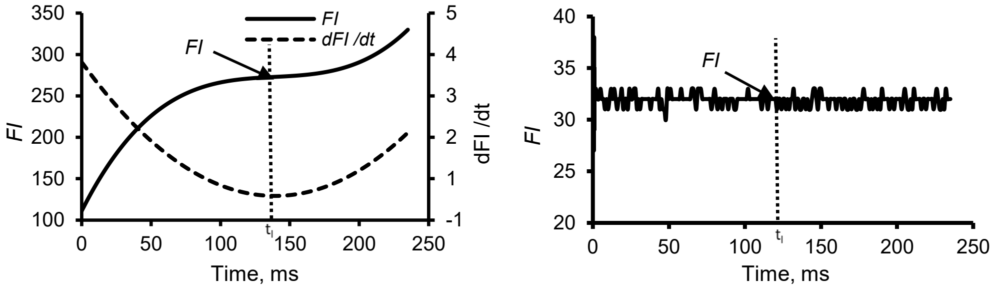
d) Red stage

Figure 3. Curves of the fluorescence dependencies of chlorophyll (*FI*) and their first derivatives (*dFI* / *dt*) over time for the 'Lezginka' variety for different degrees of ripeness.



a) Green stage

b) Turning stage



c) Pink stage

d) Red stage

Figure 4. Curves of the fluorescence dependencies of chlorophyll (*FI*) and their first derivatives (*dFI* / *dt*) over time for the 'Rosanchik' variety for different degrees of ripeness.

Development of mathematical models

Table 1 shows the calculated time t_I at the first inflection point of the chlorophyll fluorescence intensity curve (the shortest period for operational control of the degree of ripeness of tomatoes), which was derived by calculating its second derivative (in this case, $d^2FI/dt^2 = 0$), as well as the determined time t_I at the second curvature point of the chlorophyll fluorescence intensity curve (the minimum time for operational control of the degree of ripeness).

Table 1. Mathematical models of chlorophyll fluorescence curves of ‘Alkazar’, ‘Lezginka’, and ‘Rosanchik’ varieties with different degrees of maturity

Tomato variety	Degree of ripeness	Equations of mathematical models
Alkazar	Green	$FI = 0.000525\,t^3 - 0.2033\,t^2 + 25.45\,t + 494$ $dFI/dt = 0.001574\,t^2 - 0.4066\,t + 25.45$ $d^2FI/dt^2 = 0.003149\,t - 0.4066$ (t_I=129 ms)
	Turning	$FI = 0.000299\,t^3 - 0.1119\,t^2 + 13.26\,t + 309$ $dFI/dt = 0.000897\,t^2 - 0.2237\,t + 13.26$ $d^2FI/dt^2 = 0.001793\,t - 0.2237$ (t_I=124 ms)
	Pink	$FI = 0.000082\,t^3 - 0.0306\,t^2 + 3.64\,t + 116$ $dFI/dt = 0.000246\,t^2 - 0.0613\,t + 3.64$ $d^2FI/dt^2 = 0.000492\,t - 0.0613$ (t_I=124 ms)
	Red	$FI = 24 \pm 5$
Lezginka	Green	$FI = 0.000790\,t^3 - 0.2994\,t^2 + 37.40\,t + 721$ $dFI/dt = 0.002370\,t^2 - 0.5989\,t + 37.40$ $d^2FI/dt^2 = 0.004741\,t - 0.5989$ (t_I=126 ms)
	Turning	$FI = 0.000230\,t^3 - 0.0842\,t^2 + 9.92\,t + 229$ $dFI/dt = 0.000691\,t^2 - 0.1684\,t + 9.92$ $d^2FI/dt^2 = 0.001381\,t - 0.1684$ (t_I=122 ms)
	Pink	$FI = 0.000088\,t^3 - 0.032369\,t^2 + 4.16\,t + 147$ $dFI/dt = 0.000264\,t^2 - 0.0647\,t + 4.16$ $d^2FI/dt^2 = 0.000528\,t - 0.0647$ (t_I=122 ms)
	Red	$FI = 55 \pm 5$
Rosanchik	Green	$FI = 0.000344\,t^3 - 0.1322\,t^2 + 16.56\,t + 409$ $dFI/dt = 0.001032\,t^2 - 0.2645\,t + 16.56$ $d^2FI/dt^2 = 0.002063\,t - 0.2645$ (t_I=128 ms)
	Turning	$FI = 0.000039\,t^3 - 0.0154\,t^2 + 2.09\,t + 246$ $dFI/dt = 0.000118\,t^2 - 0.0309\,t + 2.09$ $d^2FI/dt^2 = 0.000235\,t - 0.0309$ (t_I=131 ms)
	Pink	$FI = 0.000055\,t^3 - 0.0232\,t^2 + 3.32\,t + 111$ $dFI/dt = 0.000167\,t^2 - 0.0464\,t + 3.32$ $d^2FI/dt^2 = 0.000333\,t - 0.0464$ (t_I=139 ms)
	Red	$FI = 33 \pm 5$

The mathematical models have been created based on the findings of experimental studies in which tabular data was obtained and related curves were constructed for the time dependences of the chlorophyll fluorescence intensity (FI) and its first derivatives (dFI/dt) for the tomato variety ‘Alkazar’, ‘Lezginka’ and ‘Rosanchik’ and its four stages of maturity (green, turning, pink, and red).

Effect of chlorophyll on fluorescence induction

Fig. 5 shows that during the green ripening stage, tomatoes have maximum fluorescence intensity values which gradually decrease as the fruits mature. On the contrary, fully ripe tomatoes had the lowest levels of fluorescence intensity. A general trend was observed, during tomato ripening the fluorescence intensity decreases. When fruits ripen, the color of the skin changes due to the decomposition of chlorophyll. This occurs when the color of the fruit turns from green to yellow or red and causes the green spots to disappear (Bramley, 2002). This reduction in fluorescence intensity could be utilized to determine tomato maturity. Many recent investigations showed that excitation-based chlorophyll fluorescence indices applying various wavelengths may estimate grape fruit quality (Agati et al., 2013) apples (Seifert et al., 2014) and tomatoes (Abdelhamid et al., 2020). Chlorophyll fluorescence induction has been used in a number of recent research to classify tomato fruit ripening phases (Hoffmann et al., 2015, Fatchurrahman et al., 2020; Abdelhamid et al., 2021). During the ripening, changes in the fruit's pigment distribution and composition had varying effects on fluorescence. Identifying red or far-red fluorescence by combining several excitation lamps allowed for the observation and definition of distinctive maturation curve patterns. The light absorption spectrum undergoes a major change when the green to red maturation stage is reached (Qin & Lu, 2008). The breakdown of chlorophyll results in a decrease in fluorescence emission. Carotenoids are synthesized in tandem with the breakdown of chlorophyll when chloroplasts transform into chromoplasts, initially in the fruit's core and subsequently in the pericarp (Bramley, 2002). The most reliable indicator of this decline in chlorophyll content is the FI index, which also exhibits high connections with the ripening stage. Thus, fluorescence measurements can be taken as a useful tool for assessing the general state of a fruit and its maturity at various stages. Furthermore, as with apples (Betemps et al., 2012), significant fruit quality attributes might be assessed using this technique. The fluorescence approach could be used to precisely define the optimal harvest time or as a robust tool for fruit categorization in high-speed sorting processes.

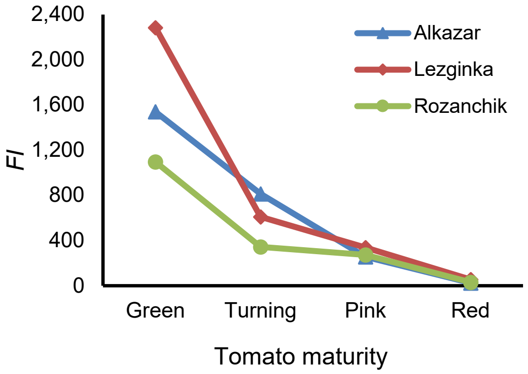


Figure 5. Relationship between *FI* and the degree of maturity for ‘Alkazar’, ‘Lezginka’ and ‘Rozanchik’ varieties.

CONCLUSION

This study investigated the ability to classify the degree of ripeness of tomato fruits using fast fluorescence intensity. The results showed that during the maturity stages, the fluorescence intensity decreases. Mathematical models of chlorophyll fluorescence levels in tomatoes based on ripeness are defined by third-order polynomials. The results indicated that according to the mathematical models obtained, the optimal time to monitor the degree of tomato maturity was $t_I = 129 \pm 4$ ms. The proposed mathematical models can be used in sorting and grading operations for fresh vegetables and fruits. It can also be used as a system that can be integrated into harvesting and post-harvesting machinery for agricultural products.

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