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Assessment of tomato ripeness using chlorophyll fluorescence[1]

Avaliação da maturação do tomate usando fluorescência da clorofila

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HIGHLIGHTS:

New mathematical models were developed for measuring tomato maturity.
The suggested models and method can potentially be used in tomato sorting.
Evaluating tomato maturity based on their induction of chlorophyll fluorescence.

ABSTRACT: The ripeness of tomatoes has a direct impact on their quality. This study aimed to develop mathematical models to determine and monitor tomato ripeness based on chlorophyll fluorescence parameters. Three varieties of tomatoes (Alkazar, Lezginka, and Rosanchik) with five ripening stages (green, breaker, pink, light red, and red) were examined using chlorophyll fluorescence analysis. Chlorophyll fluorescence variables (Variable - F_v, maximum - F_m, initial - F_0, and F_v/F_m ratio) were assessed at five stages of maturation. Five mathematical models were proposed for each tomato variety examined to determine the relationship between chlorophyll fluorescence parameters and ripening stages. The experimental results revealed that tomato maturity could be determined using chlorophyll fluorescence. It was found that as the tomato fruits ripened, the chlorophyll fluorescence parameters, such as F_v, F_v/F_m, F_m, and F_0, gradually decreased. The proposed models allowed estimation of the ripening stage of all three tomato varieties. The highest R^2 (0.99) was obtained using chlorophyll fluorescence parameters together.

Key words: model, chlorophyll fluorescence parameters, *Lycopersicon esculentum*

RESUMO: A maturação do tomate tem impacto direto na sua qualidade. Este trabalho teve como objetivo desenvolver modelos matemáticos para determinação da maturação do tomate e monitorar o nível de maturação do tomate com base nos parâmetros de fluorescência da clorofila. Três variedades de tomates com cinco estagios de maturação (verde, rompedor, rosa, vermelho claro e vermelho): 'Alkazar', 'Lezginka' e 'Rosanchik' foram examinadas usando análise de fluorescência da clorofila. Variável (Fv), razão (Fv/Fm), máximo (Fm) e mínimo (Fo) de fluorescência da clorofila foram avaliados em cinco estágios de maturação. Para cada variedade de tomate examinada, foram propostos cinco modelos matemáticos para determinar a relação entre os parâmetros de fluorescência da clorofila e os estágios de maturação. Os resultados experimentais revelaram que a maturidade do tomate poderia ser determinada usando fluorescência da clorofila. Verificou-se que à medida que os frutos do tomate amadureceram, os parâmetros de fluorescência da clorofila como Fv, Fv/Fm, Fm e Fo diminuíram gradativamente. Os modelos propostos permitiram estimar o estágio de maturação das três variedades de tomate. As maiores R^2 (0.99) foram obtidas com a utilização dos parâmetros de fluorescência da clorofila em conjunto.

Palavras-chave: modelo, parâmetros de fluorescência da clorofila, *Lycopersicon esculentum*

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Introduction

The maturation of tomato (*Lycopersicon esculentum*) fruit affects the postharvest physiology, shelf life, and storability (Sharma et al., 2020). Fruit sorting at harvesting is also significantly reliant on human labor (Wang et al., 2022; Guann et al., 2022). Manual sorting and classifying methods have become increasingly expensive and unable to meet market demands in recent years, with rapid urbanization and agricultural labor becoming increasingly scarce (Bai et al., 2022; Rong et al., 2022; Yang et al., 2023).

The visual recognition approach sorts the physical properties of tomatoes based on both size and color, but it cannot detect the interior material composition of fruits (Jin et al., 2021). As tomato fruit's red color and change in redness are connected with lycopene content, visual inspection is important for determining maturation status (Sikorska-Zimny et al., 2019). Chloroplast structural and metabolic changes during fruit ripening cause significant variations in chlorophyll fluorescence (Kitao et al., 2000). This shows clearly that the chlorophyll fluorescence yield and fruit ripening stages are connected.

Studies have been conducted to control fruit quality in recent years using non-destructive techniques, such as infrared thermography, microwave imaging, and spectral analysis (Avotins et al., 2020). The chlorophyll fluorescence method is used for non-destructive analysis to classify tomato maturity (Kasampalis et al., 2020; Abdelhamid et al., 2021). This technique considers the internal biochemical changes that occur throughout fruit and vegetable maturation and can provide an improved estimate of ripening (Abdelhamid et al., 2020). This study aims to develop mathematical models to determine and monitor tomato ripeness based on chlorophyll fluorescence parameters.

Material and Methods

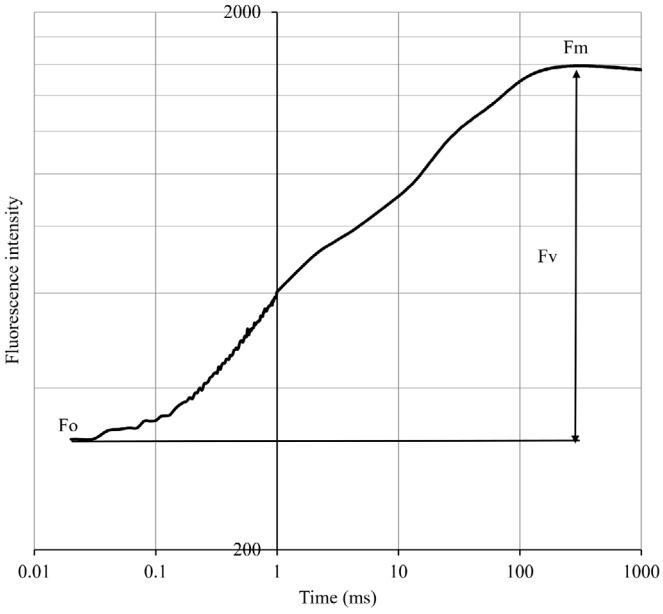
In the study, three botanical varieties of tomato (*Lycopersicon esculentum*) - Alkazar, Lezginka, and Rosanchik - were selected with five varying stages of ripening (green, breaker, pink, light red, and red) as shown in Figure 1. All varieties of tomatoes used were cultivated with the same management concerning irrigation, fertilization, pest control, and diseases during the 2020-2021 crop season. Tomatoes were harvested from greenhouses. For each of the five maturation stages, 25 similarly sized tomatoes were used to observe variations in chlorophyll fluorescence parameters. All fruits were transferred in open carton boxes within half an hour to the laboratory for measurements.

First, a colorimeter (Minolta Chromameter 400, Japan) was used to measure tomato skin color at ripening stages at two opposed locations on the fruit's equator. Color changes were measured in the color spaces L*, a*, and b*. The hue angle [H^o = 180+ $\tan^{-1}$ (b*/a*)] and chroma values [C^* = $(a^{*2}+ b^{*2})^{1/2}$] were computed from the values of a* and b*. The tomatoes were graded into different stages of ripeness using a colorimeter as a reference guide in the sorting process. More details are provided in Abdelhamid et al. (2021). After that, as shown in Figure 2, the initial (F_0) and maximum (F_m) values of chlorophyll fluorescence were measured; then, the variable fluorescence ($F_v = F_m - F_0$) and F_v/F_m ratio were estimated based on the JIP test for all three tomato varieties using the fluorimeter described in Kreslavski et al. (2014). The level of fluorescence is expressed by the F_0 parameter when photosystem II can transmit nearly all the electrons stimulated by the light. The maximum fluorescence intensity is referred to as F_m, while photosystem II can only transmit a small amount of the electrons stimulated by the light. The effectiveness of the energy transfer process and chloroplast activation are measured by the F_v/F_m ratio (Janeeshma et al., 2022).

Chlorophyll fluorescence parameters were measured in laboratory light according to Kasampalis et al. (2020). Readings were obtained at two opposing points on the equator of each tomato, and then the average was calculated. The data was recorded by a computer system. Based on the results of all measurements, the mean values and standard deviations for the F_m, F_0, F_v, and F_v/F_m were determined at each maturity stage.

To find a better-fit model between the parameters of chlorophyll fluorescence with the maturity stages and the correlation between them, a transformed regression analysis was performed with several possible models, including linear, polynomial, exponential, and logarithmic. Five mathematical models were proposed for each tomato variety examined to determine the relationship between chlorophyll fluorescence parameters (F_m, F_0, F_v, and F_v/F_m) and ripening stages. The first four models express the relationship between each parameter of the chlorophyll fluorescence parameters and the maturity of fruits separately. In contrast, the fifth model relied on a

Figure 1. Tomato at different maturity stages



F_0- Initial fluorescence; Fm- Maximum fluorescence; Fv- Variable fluorescence

Figure 2. Typical Kautsky fluorescence induction curve

multiple linear regression of the chlorophyll fluorescence parameters by the Statistical Package for the Social Sciences - SPSS program. Models were evaluated, and the most accurate ones were chosen to assess the degree of maturity.

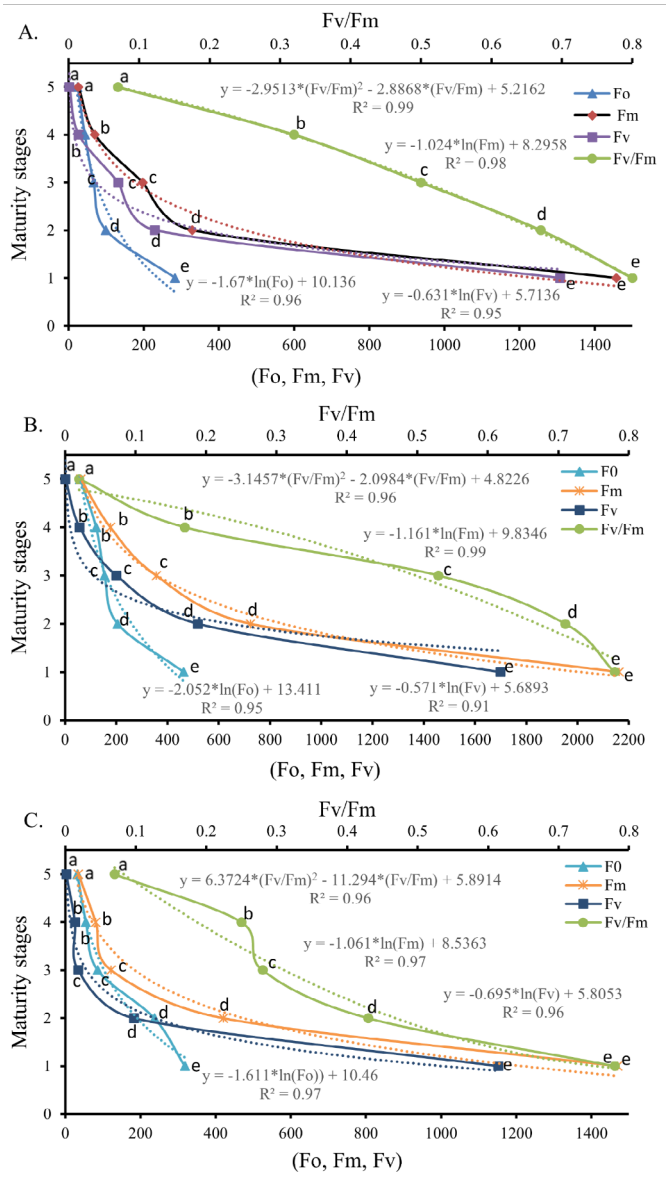
The degree of fit of the proposed models was evaluated using the coefficient of determination (R^2). The coefficient of determination illustrates the percentage of variance among the obtained data explained by the model. R^2 has a value between 0 and 1. Higher values indicate lesser error variance, and standards greater than 0.5 are sufficient (Chicco et al., 2021). Data were analyzed using SPSS, v. 20, USA, and the Tukey test ($p < 0.05$) was used to compare means. The models between the ripening of fruit and chlorophyll fluorescence parameters F_v, F_m, F_v/F_m, and F_0 were evaluated.

Results and Discussion

The results of experimental studies are shown in Figures 3A, B, and C and demonstrate that in the three varieties, there is a general pattern: The values of the chlorophyll fluorescence parameters (F_v, F_m, F_v/F_m, and F_0) decrease ($p < 0.05$) as tomatoes ripen. At the green maturity stage, tomatoes have high F_v, F_m, F_v/F_m, and F_0 values and then reduce as the fruits ripen. In contrast, the lowest values for (F_v, F_m, F_v/F_m, and F_0) were observed in fully ripe tomatoes. Thus, when the tomato ripens further, the chlorophyll fluorescence parameters (F_v, F_m, F_v/F_m, and F_0) decrease. Consequently, maturation stages were well separated using chlorophyll fluorescence parameters. As a rule, when the fruit ripens, the peel's color changes due to the breakdown of chlorophyll, which occurs as the fruit's color transitions from green to yellow or red and causes the loss of green spots (Gould, 1992). Other chlorophyll fluorescence parameters were similarly lowered with ripeness, as reported in apples (Song et al., 1997) and papaya (Bron et al., 2004).

It should be mentioned that cell membrane breakdown throughout maturation is related to the decrease in chlorophyll fluorescence (Mir et al., 1996). The decrease in F_v/F_m, F_0, and F_v additionally sheds light on the loss that occurs to chloroplast function with rapid maturation and aging (Mir et al., 1996). The F_m changes reflect changes in the ability to use light in photosynthesis reactions, reflecting the loss of chloroplast activity with advanced maturation. The decrease in F_m suggests a rise in heat release at the photosystem II antenna and the reaction center. At the same time, thylakoid membrane structural changes are the cause of changes in F_0 (Bron et al., 2004). Therefore, the decrease in chlorophyll fluorescence parameters reflects both the loss of chloroplast efficiency and the degradation of chlorophyll as the fruit ripens.

As the fruits ripen, chlorophyll degradation causes a decrease in the release of chlorophyll fluorescence (Figure 3). The chlorophyll is degraded when the chloroplasts transform into chromoplasts and a pool of carotenoids forms alongside it. This pool eventually spreads to the fruit peel (Bramley, 2002). Additionally, as the fruit ripens, the chloroplasts undergo structural and metabolic changes that affect chlorophyll fluorescence production. The primary changes are the control of chloroplast grana, decreased plastoquinone transport capability, and chlorophyll degradation followed by concurrent



The dashed lines represent the most appropriate equations, n=25. F_0 – Initial fluorescence; Fm – Maximum fluorescence; Fv – Variable fluorescence; Fv/Fm – Ratio; Maturity stages (1 - Green, 2 – Breaker, 3 - Pink, 4 - Light red, and 5 – Red). * Significant at 0.05 probability. Means followed by different letters differ by Tukey test ($p<0.05$)

Figure 3. Relationship between chlorophyll fluorescence parameters and tomato ripening stages for the Alkazar (A), Lezginka (B), and Rosanchik (C) varieties

carotenoid buildup (Kitao et al., 2000). The processes of maturation and aging are known to cause destabilization and loss of membrane function since chlorophyll protein complexes are attached to thylakoid membranes and exhibit chemical and biophysical changes, chlorophyll fluorescence may act as an internal measure of membrane stability, regulation, and fluidity. Indeed, the use of chlorophyll fluorescence as an indirect indicator of the physiological condition of green tissue is supported by the fact that fluorescence properties change when the photosystem II is subjected to any environmental stimuli or influenced by physiological changes (Schreiber & Bilger, 1987).

Tomato fruits contain both forms of chlorophyll, chlorophyll a and b, which stimulates their ability to photosynthesize activity. Additionally, Kasampalis et al. (2020) noticed that with the ripening of the fruit, chlorophyll

a and b significantly decreased, with a rather obvious drop in chlorophyll a, resembling patterns found. As a result, the reduction in chlorophyll concentration is tracked by evaluating the fluorescence of chlorophyll released by the fruit tissues. Thus, a decrease in fluorescence emission may be reflected in the decomposition of chlorophyll as maturation progresses.

Figures 3A, B, and C show the results of the approximation of linear models to describe the relationship between chlorophyll fluorescence parameters during the tomato ripening stages of the three studied varieties. The best models obtained are shown in Table 1. All models showed a relatively strong relationship between the chlorophyll fluorescence parameters and tomato ripening.

During tomato ripening, a decrease in both chlorophyll fluorescence parameters was observed. Accordingly, five models were established for each tomato variety, as shown in Table 1. Regardless of tomato variety, there is a significant correlation between chlorophyll fluorescence parameters with ripeness in the models used. The highest R^2 (0.99) was obtained in each tomato variety with the model that uses chlorophyll fluorescence parameters together (M_5, M_{10}, and M_{15}). Also, it was found that among the chlorophyll fluorescence parameters, the Fm is the best for assessing the degree of maturity, with R^2 of 0.97 in M_2 for the Alkazar, 0.98 in M_7 for Lezginka, and 0.97 in M_{12} for Rosanchik, as shown in Table 1.

Buying and consuming tomatoes before they reach the maturity stage is not desirable for consumers, so it is necessary to monitor and sort the tomatoes according to the degree of maturity since it indicates their quality at the harvest. The ability to anticipate fruit ripeness using contemporary non-destructive techniques, including hyper-spectral imaging (Zhu et al., 2015), electronic nose (Gómez et al., 2006), multi-spectral imaging (Hahn, 2002), VIS-NIR spectroscopy (Lu et al., 2017), has also been proven successfully. Modern methods use some advanced statistical software and techniques, but these methods are expensive and take a long time to process the data. Furthermore, assessing tomato ripening using chlorophyll fluorescence measurements is simple and inexpensive. Although it has been shown that ripening stages for papaya (Bron et al., 2004), kadam (*Neolamarckia cadamba*) (Baran et al., 2023), Avocado (Rubinovich et al., 2023), and seeds (Zhang et al., 2022) may be predicted using chlorophyll fluorescence induction. Our research proved that by employing chlorophyll fluorescence inductive methods, tomato fruits might be precisely classified according to their maturity stage. Our research is the first of its kind to propose mathematical models based on parameters of chlorophyll fluorescence that may be used to categorize tomatoes depending on their state of maturity precisely.

Table 1. Suggested models for assessing the ripeness of tomatoes based on chlorophyll fluorescence parameters

Variety/Models		Models	R^2
Alkazar	M_1	Maturity = 10.136 - 1.67 ln(F_0)	0.95
	M_2	Maturity = 8.296 - 1.24 ln(F_m)	0.97
	M_3	Maturity = 5.714 - 0.631 ln(F_v)	0.94
	M_4	Maturity = -2.951(F_v/F_m)^2 - 2.887 (F_v/F_m) + 5.216	0.97
	M_5	Maturity = -0.002 F_m + 0.003 F_0 - 6.422 F_v/F_m + 5.819	0.99
Lezginka	M_6	Maturity = 13.411 - 2.052 ln(F_0)	0.94
	M_7	Maturity = 9.835 - 1.161 ln(F_m)	0.98
	M_8	Maturity = 5.689 - 0.571 ln(F_v)	0.90
	M_9	Maturity = 3.146 (F_v/F_m)^2 - 2.098 (F_v/F_m) + 4.823	0.95
	M_{10}	Maturity = - 0.001 F_m + 0.001 F_0 - 3.122 F_v/F_m + 4.86	0.99
Rosanchik	M_{11}	Maturity = 10.460 - 1.611 ln(F_0)	0.97
	M_{12}	Maturity = 8.536 - 1.061 ln(F_m)	0.97
	M_{13}	Maturity = 5.805 - 0.695 ln(F_v)	0.95
	M_{14}	Maturity = 6.372 (F_v/F_m)^2 - 11.294 (F_v/F_m) + 5.891	0.95
	M_{15}	Maturity = 0.002 F_m - 0.001 F_0 - 4.902 F_v/F_m + 5.87	0.99

Conclusions

1. The actual ripening process of the tomato fruit at each stage of ripeness is more accurately reflected by the proposed models of chlorophyll fluorescence parameters.

2. The highest R^2 (0.99) is obtained using chlorophyll fluorescence parameters together for the Alkazar, Lezginka, and Rosanchik varieties.

3. The proposed method and models can be used to design, manufacture, and control a robot for harvesting and sorting tomatoes.

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