Prediction of Days After Anthesis of Developing Tomato (*Solanum lycopersicum*) Fruit from Blossom-End Changes in Color

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ABSTRACT

The development of mathematical models to predict the physiological status of a developing fruit can be a useful tool to reduce the variation in fruit response to postharvest treatments. The objective of this study was to develop a mathematical model to predict Days After Anthesis (DAA) in tomato fruit from changes in the blossom end color. Around 160 tomato flowers from 40 plants of a tomato cultivar 'Rutgers' and the nearly isogenic line tomato ripening mutant *rin* were tagged after artificial pollination and allowed to fruit set. The quantification of color was done from 5 to 85 DAA every 5 days in the tomato fruit blossom-end of both 'Rutgers' and mutant *rin* using a a Minolta chroma meter CR-300 set in the L*, a* and b* color space. The predictive model was developed using the stepwise procedure in forward selection with DAA as the response variable and all the possible combinations between the next group of regressor: L*, a* and b*, square L*, a* and b*, chroma and Hue angle calculated from measured a* and b* values. It was used the F statistic, mean square error, coefficient of determination, Mallows coefficient and distribution of residuals around zero as indicators of model prediction's efficiency. Correlation coefficients between the different variables measured and the DAA were calculated. The reliability of the statistical analysis was tested by using the nearly isogenic line of 'Rutgers': The non-ripening mutant *rin* as a comparative control. It was concluded that the statistical procedure used is robust and sensitive enough to identify data not suitable for developing a good predictive model. Also, it is possible to predict the days after anthesis of a developing tomato fruit from changes in the blossom-end color with almost 80% of accuracy.

Keywords: Tomato Fruit, 'Rutgers', Mutant *rin*, Days After Anthesis, Mathematical Model

1. INTRODUCTION

Studies of the postharvest life of tomato fruit frequently require to control the physiological status of the fruit. In order to do that, usually not senescent flowers are pollinated by hand and tagged to know the day after anthesis of the fruit. This procedure usually takes a long time and effort. This is particularly true for experiments in which a new protocol for postharvest treatment and storage of tomato fruit is being tested. In this context, the development of a tool to know the physiological status of the fruit without the need of tagging flowers can help to save a lot of time. Also, it will be advantageous if this tool entails a non-destructive technique.

To our knowledge, there is only one experiment in which it was attempted to analyze the changes in color during development with the goal to predict the stage of

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tomato fruit development by laser-induced fluorescence using a wavelength of 266. In this study a correlation of 0.78 and -0.513 was found for the carotenoids/flavonoids and carotenoid/chlorophyll ratios, indicating that this methodology can be used to predict the developmental stage of tomato (Lai *et al*., 2007). Furthemore, there is a study in which the optical properties of fresh cut tomato at different stages of development was evaluated (Lana *et al*., 2006). Also, color determination in tomato epicarp at room temperature as well as under refrigeration was evaluated using computer vision system, in which the digitalization of images was performed based on the L*, a* and b* coordinates (Pereira *et al*., 2012).

Besides, fluorescence imaging had been tested for assessing maturity in apple (Greer, 2005; Noh *et al*., 2007), grape berries (Kolb *et al*., 2006), papaya fruit (Bron *et al*., 2004) and mango fruit (Jacobi *et al*., 1998).

By other side, models to predict other characteristics had been developed in tomato, like prediction of the changes in fruit cell number during development (Bertin *et al*., 2003), prediction of tomato fruit transpiration (Leonardi *et al*., 1999), a biophysical model to predict fruit growth (Fishman and Genard, 1998), dry matter accumulation (Bussieres, 1995), water import rate (Bussieres, 1994).

It is possible through simulation to evaluate the quality of a final products in order to identify critical points during postharvest handling and to adjust or improve the decision making related to harvest dates and product commercialization. Adequate models should be mechanistic enough to give a representative description of physiological processes and explain variations in some quality traits (Tijskens *et al*., 2010; Vazquez-Cruz *et al*., 2010).

However, to our knowledge, no papers had been published reporting a model to predict the stage of tomato development based on changes in skin color.

The measurement of the normal phenotypical changes during tomato fruit ripening can be used to predict the developmental stage of the fruit in a non-destructive way. The ripening phenomena in many fruits is accompanied by changes in skin and pulp color (Monselise, 1986). In the case of tomato fruit, a noticeable color change in the blossom-end of tomato fruit indicates the active initiation of the ripening phenomena (Gomez *et al*., 1998).

Color is a rather subjective quality of fruits and therefore it is not easy to quantify. Indeed, in the past the color of a particular fruit was scored based on comparison with a color chart. In 1976, it was published the last version of the Munsell book of color which was

the result of many improvements from its first creation in 1915. This book of color allowed assigning a number to 1500 color standards (Munsell Color. McBeth Division of Collmorgen Corporation. Baltimore, Maryland, U.S.A). The color in the Munsell book was classified based in their Hue (Munsell Hue), lightness (Munsell Value) and Saturation (Munsell Chroma). However, due to the particular change in color for every fruit, there was an special chart for every fruit. These charts were so useful that even today; many of them are still in use. In this way, there are color charts developed for persimmons (Yamazaki *et al*., 1981), potatos (Coleman, 2004), grapefruit (Ono *et al*., 2000), sweet cherry (Kappel *et al*., 1996), oil palm (Abdullah *et al*., 2002), among others.

Today, there are several methods to express colors numerically (which is called color space): XYZ, Yxy, L*a* b*, L*C*h* and Hunter Lab. Out of this, XYZ (devised in 1931) and L*a*b* (devised in 1976) are the most widely used. Between these last two, L*a*b* color space was created to provide more uniform color differences in relation to visual differences. The color space of these methods is based in the same three different attributes of color in which the Munsell book of color is based: Hue, saturation and brightness. He is related to wavelength and the terms red and blue are primarily describing Hue. However, there are many different mixtures of wavelength that can produce the same perceived Hue like pink and red. From here the importance of the other two attributes. Saturation is related with the effect produced by a mixture of colors. In this way, a pink color is less saturated than a red color even though they have the same Hue. A fully saturated color is one with no mixture of white color and it is considered more vivid. It is important to be cautious when quantifying saturation, since some spectral colors are perceived more saturated than others. For example, monochromatic red and violet are perceived more saturated than monochromatic yellow. Brightness of a color depends upon the illuminance and its reflectivity. It is related with how bright they are. It had been found that equal surfaces with differing spectral characteristics but which emit the same number of lumens will be perceived to be equally bright. From the mentioned above, evaluating subjectively a color is often biased by the perception of the human eye. Now, let us see how this three attributes of color are related with the color space L*, a* and b*. The variable L* is related with the brightness of the color and moves from 0 (black) to 100 (white). Both a* and b* variables are related with Hue and saturation. Negative values of a* indicates green

color, whereas positive ones indicates a red color. Also, negative values of b* indicates blue color and positive ones indicates a yellow color. The scale of both variables a* and b* goes from 0 to 60 or -60 depending upon the color. A fully saturated color shows a value of 60 or -60, whereas a dull or achromatic color shows a value close to zero. If we place a* in the x-axis and b* in the y-axis, we can have a Cartesian coordinate axis. In this cartesian axis, 0 degrees corresponds to a red Hue when a* value is 60, 90 degrees corresponds to a yellow Hue when b* value is 60, 180 degrees corresponds to a green Hue when a* value is -60 and 270 degrees correspond to a blue Hue when b* value is -60. Mixing yellow and red results in yellow-red Hue, mixing yellow and green results in yellow-green Hue; mixing green and blue results in a green-blue Hue and so on. In this way, a continuum of these Hues results in what is call a color circle or chromaticity diagram. The intermediates values of Hue can be exactly calculated using the values of a* and b*. Up to now we have been considering a circle. However, to define a color precisely, it is also needed the brightness of the color which is given by L* variable. Following the same logic as we did with variables a* and b*, L* variable will be place on the z-axis and the circle becomes a sphere. Therefore, the precise definition of a color needs the three variables: saturation and Hue given by the variables a* and b* and brightness, given by the variable L*. In this way, it is possible to precisely quantify all the attributes that can define a color using the color space L*, a* and b* (also referred to as CIELAB). Based on the methods to express color numerically, algorithms had been developed for harvested tomato fruit to classify the fruits based on color, color homogeneity, defects, shape and stem analysis (Laykin *et al.*, 2002).

From the above mentioned, today, it is possible to quantify a color. Therefore, the color changes in the blossom end of tomato can be known precisely. In turn, quantification of a variable allows the use of mathematical analysis.

With the objective of developing a non-destructive mathematical tool to predict the days after anthesis of tomato fruits, we recorded the color changes every five days in the blossom-end of tomato cv.'Rutgers' growing in a greenhouse. With this data, we developed a linear mathematical model able to predict in almost 80% the developmental stage of the fruit in days after anthesis from changes in color. Furthermore, with the objective of showing the precision of the procedure used to develop the mathematical tool, we also did the same procedure

with the *rin* tomato, which is a ripening mutant and does not show normal changes in color.

2. MATERIALS AND METHODS

2.1. Tomato Plants

In a greenhouse with automatic control of temperature, light and irrigation, 40 plants of the *rin* tomato mutant and 40 plants of nearly isogenic line of tomato 'Rutgers' were planted in 10-inch-pots filled with a peat-based mix, made up of the next ingredients: peat moss 70-80%, perlite 10-15% and vermiculite 10-15%. The plants were irrigated with a nutritive solution described elsewhere (Jensen and Malter, 1995), once or twice a day depending upon the water or nutrient demands. Pests and diseases were managed following agricultural experimental local station guidelines (INDIF, 1984). Flowers were tagged after artificial pollination and allowed to fruit set.

2.2. Color Analysis of Fruit Blossom-End

The quantification of color was done every five days after anthesis (DAA) starting at day five in both the 'Rutgers' and the *rin* mutant in the tomato fruit blossom-end with a Minolta chroma meter CR-300 (Minolta Corporation. 101 Williams Drive, Ramsey, New Jersey 07446, U.S.A.) set in the L*, a* and b* color space. A linear multiple regression model was developed with Days After Anthesis (DAA) as the response variable and all the possible combinations between the next group of regressor: L*, a* and b* variables measured in the tomato fruit blossom-end, square L*, a* and b* variables, chroma and Hue angle calculated from measured a* and b* values. The correlation coefficients between the different variables measured and the DAA for both the *rin* mutant and tomato 'Rutgers', were also calculated.

2.3. Statistical Analysis

The linear mathematical model was created using the stepwise procedure in forward selection with the level of significance for entry into the model of 0.15 and for staying in the model of 0.10. The F statistics, mean square error, coefficient of determination ($r2$), Mallows Coefficient (Cp) and evenly distribution of the residuals above and below zero, were used as a criteria to select the best model. Correlation between DAA and color variables was calculated based in the Pearson test. Besides, a statistic test was run to discriminate between

slope equal or different with zero of the line describing the relationship between the two variables.

All the statistical analysis was done using the statistical analysis software version 8.2 (Statistical Analysis System. SAS Institute, Inc. Cary, N.C., U.S.A.).

3. RESULTS

It is shown in **Fig. 1**, the changes in color of the blossom end measured using the color space L*, a* and b* during different DAA of the *rin* mutant and 'Rutgers'. In panel A it is included the tomato mutant *rin* results and in panel B the tomato cultivar 'Rutgers' results. The tomato mutant did not show large changes in any of the three components of the color space throughout the tomato development. In the case of tomato 'Rutgers', large changes in the three components of the color space before 50 DAA were not observed and the behaviour was rather similar when compared with the mutant. However, from 50 to 85 DAA, the component L* showed a large decrease of about 30 units.

The a* component of the color space showed the largest changes during the tomato fruit development, in such a way that from 50 to 85 DAA, an increase of approximately 60 units was observed. Out of the three components of the space color b* was the one showing the smallest changes throughout the development of the tomato fruit.
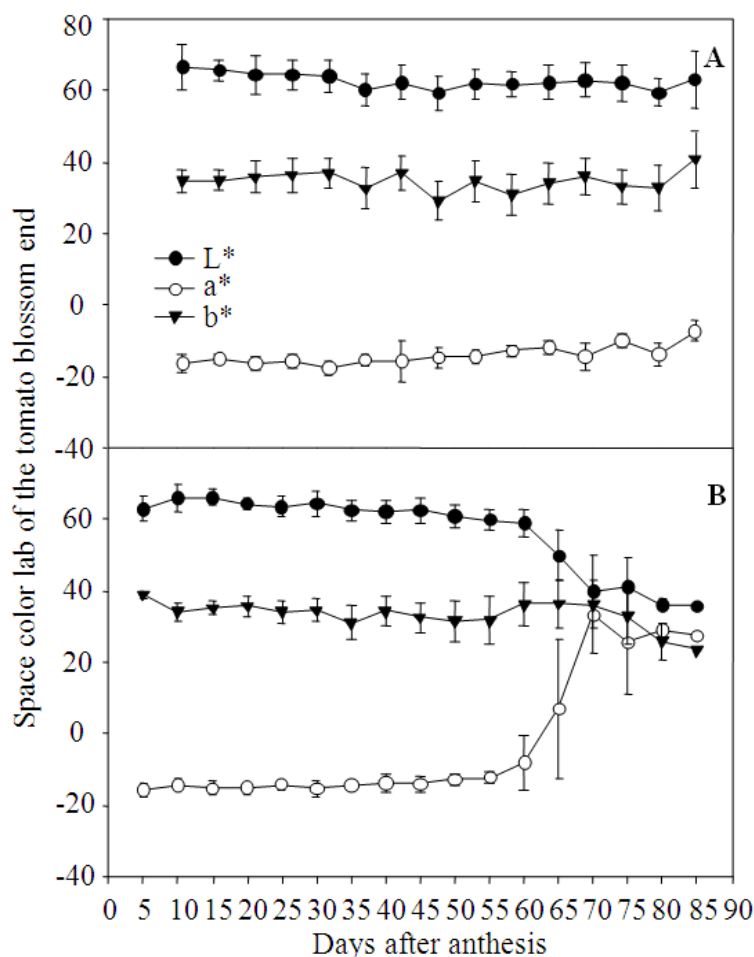

Fig. 1. Changes in the L*, a* and b* color space components measured in the tomato blossom end during different days after anthesis: The L*, a* and b* color space components were measured every five days after anthesis of tomato fruit blossom end using a Minolta chroma meter CR-300. In panel A it is shown the tomato mutant *rin* results and in panel B the tomato cultivar 'Rutgers'
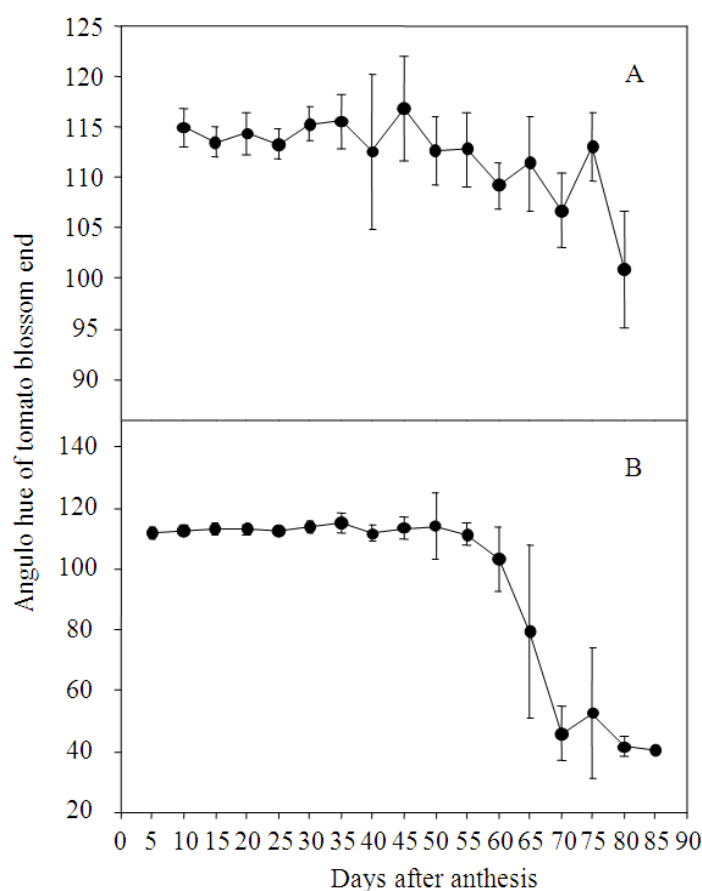
Fig. 2. Changes in the Hue angle of the tomato blossom end during different days after anthesis: Hue angle was calculated from the a* and b* color space components measured in the tomato fruit blossom end every five days after anthesis using a Minolta chroma meter CR-300. In panel A it is shown the tomato mutant *rin* results and in panel B the tomato cultivar 'Rutgers'

In **Figure 2** it is depicted the changes of the Hue angle in the blossom end of the tomato mutant and 'Rutgers' throughout the fruit development. In panel A it is shown the tomato mutant *rin* results and in panel B the tomato cultivar 'Rutgers' results. As it can be seen, both tomatoes started at the same value of Hue and showed a completely different pattern of Hue changes. In the case of the tomato mutant, Hue angle essentially did not change until 80 DAA. At this time, it showed a large decline from 115 to 100 proximately. This change in Hue means a change in color from green to yellow. These two colors are pretty close each other in the chromaticity diagram. In the case of the tomato 'Rutgers', it can be seen a large decline from about 115 at the beginning of the ripening phenomena to 40 when the fruit was fully ripe. In terms of color, this change in Hue means a change from green to red in the chromaticity diagram

which are the typical changes in color during ripening of a normal variety of tomato fruit.

In **Table 1**, it is presented the Pearson correlation coefficients between DAA and the color space components L*, a*, b*, calculated Hue and chroma for both tomato mutant and 'Rutgers'. Coefficients for the tomato mutant are in general very low. In fact, the largest coefficient was found between DAA and calculated Hue (-0.315). In contrast, for tomato 'Rutgers', several of the calculated coefficients were closer to one. Indeed, the largest (-0.873) was found between DAA and L* component. Further, between DAA and a* component and calculated Hue, we found 0.756 and –0.756, respectively. These correlation coefficients are most likely due to the large changes that both the L* component and calculated Hue showed during the tomato fruit development, as shown in **Fig. 1 and 2**.

Table 1. Pearson correlation coefficients between days after anthesis of tomato *rin* mutant, tomato cv. 'Rutgers' and the measured color space components L*, a*, b* and calculated Hue and Chroma

Measured color space components and calculated Hue and chroma	Days after anthesis of tomato *rin* mutant	Days after anthesis of tomato cv. 'Rutgers'
L*	-0.246	-0.873
a*	0.415	0.756
b*	-0.128	-0.178
Hue	-0.315	-0.756
Chroma	-0.197	0.368

Although there is not a similar experiment in the literature to compare with, the results just mentioned agrees with a study in which the analysis by fluorescence imaging of epicarp tissue of tomato fruit during ripening was done. In this study, a correlation of 0.78 for the ratio carotenoids/flavonoids was recorded (Lai *et al*., 2007). Also, correlation analysis between the amount of anthocyanin in seven apple strains and L*, Hue and a*/b* components recorded values of 0.79, 0.80 and 0.82, respectively (Iglesias *et al*., 1999). From here, it is clear that the changes in the color of fruits can have a good correlation with the analysis using the components of the space color.

In **Table 2**, it is presented the summary of the stepwise procedure to develop the mathematical equation for the tomato mutant. For this equation, it was found that Hue was the variable able to explain almost 50% of the variation in the blossom end color out of the total variation explained by the equation. Several statistics showed that this is not a good predictive equation. First of all, the correlation coefficient of the model is 0.20, which means that the equation is able to predict only 20% of the DAA variable based in changes in color of the tomato fruit during development. Also, the Mallows coefficient is 67, which is too high for the three regressors of the equation.

Furthermore, the residuals were found not located randomly around zero (data not shown).

The mathematical model developed for the tomato mutant is:

$$DAA = 255.728 - 0.731*L - 1.3*Hue - 0.593*Chroma$$

Where:
DAA   = Days after anthesis
L   = L* component of the space color
Hue   = Hue angle
Chroma = Saturation

Table 2. Summary of the stepwise selection procedure to develop the mathematical model for the tomato *rin* mutant, all the variables left in the model are significant at 0.1 level

Step	Variable Entered	Variables in the Model	Partial correlation coefficient	Model correlation coefficient	Mallows coefficient
1	Hue	1	0.0991	0.0991	187.01
2	L*	2	0.0701	0.1692	114.37
3	Chroma	3	0.0392	0.2084	67.06

Table 3. Summary of the stepwise selection procedure to develop the mathematical model for the tomato cv. 'Rutgers' , all the variables left in the model are significant at 0.1 level

Step	Variable Entered	Variables in the Model	Partial correlation coefficient	Model correlation coefficient	Mallows coefficient
1	L*	1	0.7630	0.7630	47.927
2	Chroma	2	0.0104	0.7734	7.949
3	Hue	3	0.0015	0.7749	4.000

In **Table 3**, it is presented the summary of the stepwise procedure to find the best mathematical equation for the tomato 'Rutgers'. Several statistics showed that this is good mathematical model. First of all, the correlation coefficient is 0.77, which is very high. Furthermore, the residuals showed a random pattern of deviations around zero. In this equation, L* variable explains 98.46% of the variation in the blossom end color out of the total variation explained by the equation. However, there was the need to introduce the other two regressors in order to reduce the value of the Mallows statistic from 47.9 to 4. This is very close to the number of regressors (3) of the equation, indicating a robust model.

The mathematical model developed for the tomato 'Rutgers' is:

$$DAA = 175.46 - 1.869*L - 0.054*Hue - 0.386*Chroma$$

Where:
DAA   = Days after anthesis
L   = L* component of the space color
Hue   = Hue angle
Croma = Saturation

4. DISCUSSION

The equations which can describe life phenomena are very useful because they allow to know the behaviour of the phenomena under a certain set of conditions which

can be changed without the need of doing the actual experiment, saving in this way time and money.

In this research, we had developed a mathematical tool to know the tomato fruit developmental stage in DAA from the changes in color of the blossom end. With this tool, it will possible to harvest tomatoes, without flower tagging and still identifying the fruit developmental stage. In this way, the variability due to the differences in the physiological status of the fruit will be lower and the fruit responses to the treatment effects will be more easily observed. Furthermore, since the procedure of quantifying the color is rather easy, it is possible to harvest as many tomatoes as needed and to determine their DAA in a relatively short period of time before the beginning of the experiment. The reliability of the procedure was tested utilizing a tomato mutant which does not show the characteristic changes in color of a normal ripening tomato variety. Furthermore, the mutant *rin* is a nearly isogenic line of the tomato 'Rutgers' which means that both tomatoes have essentially the same genetic background. This piece of information further indicates the sensitivity of the mathematical tool used in this study.

The stepwise procedure found an equation which is able to explain a very small amount of tomato blossom end color variation for the tomato mutant and actually it is not appropriate to use. Besides of the low correlation coefficient of the model, there were other statistics supporting the unreliability of the model like low correlation coefficients between the DAA and the color space components L*, a*, b* and calculated Hue and chroma, very dissimilar value between the Mallows coefficient and the number of the equation's regressors and lack of random dispersion of the residuals around zero. In order to have a good mathematical equation, the correlation coefficients between the regressors and the response variable must be very different to zero. Also, the correlation coefficient of the model must be very different to zero and the Mallows coefficient must be close to the number of regressors in the equation (Mallows, 2000). Another indication of the robustness of a model is the random dispersion of residuals around zero. Therefore, the statistical procedure showed in this paper can easily identify when the mathematical model developed is not accurately describing the phenomena. It is also important to draw attention to the fact that Hue and chroma are not calculated from the component L* avoiding the multicolinearity within the equation. It is interesting to mention that the stepwise procedure to develop the equations for mutant and 'Rutgers' found the same three group of regressors as the best to be included.

It was reported that the analysis of 41 tomato breeding lines showed the highest variability in L*, Hue and chroma (Sacks and Francis, 2001), which agrees with the finding of the statistical analysis done in this work to develop the prediction model. Also, the changes in tomato color show a little variance among different varieties (Gomez *et al*., 1998). Therefore, it is quite possible that the model developed in this study can be used to predict the days after anthesis of any other variety of tomato fruit although with slight changes in the correlation coefficient.

5. CONCLUSION

In this research we have shown that it is possible to develop a mathematical model using well known statistical tools that can help to know the tomato fruit developmental stage in terms of days after anthesis from the changes in color of the blossom end with almost 80% of accuracy. The statistical procedure used is robust and sensitive enough to identify data not suitable for developing a good predictive model.

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