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Shelf life of gilo fruits treated with ethylene

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ABSTRACT: Gilo fruits are appreciated for their bitter taste and bright green color. However, they become yellowish a few days after harvest, losing commercial value. It is known that the ethylene is responsible for the degradation of chlorophyll and induction of other responses in fruits sensitive to the hormone. The aim of this study was to determine the degree of sensitivity and responses of gilo fruits treated with ethylene. Fruits were treated with 0.1, 1.0, 10, 100 and 1000 µL L^{-1} ethephon plus a control treatment with water. The fruits were evaluated for the fresh weight loss, color component a*, and chlorophyll, total soluble sugars, reducing sugars, non-reducing sugars, and starch contents. The fruits treated with ethephon at concentrations greater than 0.1 µL L^{-1} had a shelf life of two days, because they showed alterations in their color resulting from an increase in component a* (red color) values. The application of ethephon reduces the chlorophyll content but does not influence the loss of mass and the carbohydrate content in the fruits.

Key words: carbohydrates; chlorophyll; color; ethephon; *Solanum gilo*

Prazo de validade dos frutos de gilo tratados com etileno

RESUMO: Frutos de Jiló são apreciados pelo sabor amargo e cor verde intensa, porém, em poucos dias após a colheita tornam-se amarelados perdendo valor comercial. Sabe-se que o hormônio etileno é responsável pela degradação de clorofila e indução de outras respostas em frutos sensíveis á presença do hormônio. O trabalho teve por objetivo determinar o grau de sensibilidade e respostas de frutos de jiló tratadas com etileno. Frutos foram tratados com 0,1, 1, 10, 100 e 1000 µL L^{-1} de ethephon e controle tratados com água. Os frutos foram avaliados quanto à perda de massa de matéria fresca, o componente da cor a*, o teor de clorofila, teor de açúcares solúveis totais, açúcares redutores, açúcares não redutores e amido. Frutos de jiló tratados com concentrações acima de 0,1 µL L^{-1} de ethephon tiveram vida de prateira de 2 dias, pois apresentam alteração da cor pelo aumento dos valores do componente a* (cor avermelhada). A aplicação de ethephon reduz o teor de clorofila, porém, não influencia na perda de massa e nos teores de carboidratos dos frutos de jiló.

Palavras-chave: carboidratos; clorofila; cor; ethephon; *Solanum gilo*

Introduction

In climacteric fruits, the application of ethylene or analogous agents stimulates the synthesis of ethylene by the fruit itself, the so-called autocatalytic ethylene. In non-climacteric fruits, this behavior is inexistent. The response of the fruits ethylene treatment and his analogous depends on the sensitivity of the tissues, the concentration used, the composition of the atmosphere, the exposure time, the temperature, the sensitivity of the species (Silva et al., 2012), the stage of development and the respiratory pattern (McAtee et al., 2013).

The application of ethylene triggers a signal transduction cascade, initiating by the activation of ethylene receptors to stimulate the expression of specific genes and transcriptional regulators that promote maturation in climacteric fruits (Liu et al., 2015). In non-climacteric fruits, ripening is believed to be independent of ethylene. However, it acts on coloration, berries growth, sugar uptake, acidity increase and anthocyanin accumulation (Liu & Chervin, 2017), emphasizing that ethylene is essential for the maturation of climacteric and non-climacteric fruits (Liu & Chervin, 2017).

Nevertheless, regardless of whether or not the fruit is climacteric, there are various degrees of sensitivity to ethylene, which is provided by hormone receptors. These receptors are present in all tissues, and, thus, all organs of the plant are able to respond to ethylene (Taiz & Zeiger, 2017).

In non-climacteric fruits, the response to ethylene is highly dependent on the concentration and period of exposure to the hormone. A common treatment during post-harvest that depends on the sensitivity of the fruit to ethylene, including the degreening of citric fruits. This treatment consists of the application of ethylene on the fruits with a ripe pulp, but whose skin is still green. The highest reductions in the green color of the skin are observed in fruits remaining exposed to ethylene for a longer period (Sdiri et al., 2012).

Gilo is appreciated for its bitter taste; however, the consumer only cooks mature green fruit with a bright green skin color. Therefore, its harvest should start before the onset of alterations in skin color, when it still has a strong green color. A problem faced by producers, wholesalers, and retailers, however, is the early yellowing of the harvested fruits. Yellowing reduces the product quality, since consumers reject fruits displaying alterations in the characteristic green color of the cultivar.

Very little is known about the postharvest behavior of gilo fruits. Thus, the aim of this study was to determine the degree of sensitivity of harvested gilo fruits to the exposure to ethylene applied at the mature green stage.

Material and Methods

Gilo fruits of cultivar 'Tinguá' were planted in an experimental field in November 2012. Plants were arranged in two rows approximately 10 m long spaced 0.50 m apart, with 0.80 m space between plants, totaling 24 plants. The fruits were harvested at the commercial point, characterized by the green color and a length of approximately 7 cm. After the harvest, the fruits were selected for absence of physiological defects and mechanical and/or phytopathogenic damage.

To evaluate the sensitivity of the fruits to ethylene, 30 fruits were immerged for 20 min in 18 L buckets containing the commercial product Ethrel® diluted in water at the final ethephon concentrations of 0.1, 1.0, 10, 100 and 1000 µL L^{-1} and control treated with water. Next, the fruits were stored in open trays until complete reduction of the postharvest quality (unsuitable for sale) at 24 ºC and 59 % of relative air humidity. The fruits were analyzed daily for the fresh weight loss, color, chlorophyll content of the skin, and carbohydrates (total soluble sugars, reducing sugars, non-reducing sugars, and starch).

For fresh weight loss, we determined the fresh mass of five pre-selected fruits by gravimetry, and this variable was estimated relative to the initial mass of the fruits and expressed in % of fresh weight loss.

The fruit color was determined in two opposite points in the mid part of the skin using a colorimeter (Color Reader CR-10, Minolta). In the evaluation, we determined the component a* values, which indicate the alteration from green color (negative values) to red (positive values)

For the chlorophyll analysis, four disks were taken off the fruit skin with 3 mm thickness and 11 mm diameter. These were weighed and placed in test tubes containing 10 mL N, N-dimethylformamide, under refrigeration at 4 ºC in the dark. Ten days later, the material was filtered through filter paper and the filtrate's absorbance was read in a spectrophotometer at the wavelengths of 647 and 664.5 nm (Inskeep & Bloom, 1985). The chlorophyll concentration was expressed in µg g^{-1} fresh matter.

For the extraction of the carbohydrates, approximately 5 g of the fruit pulp were ground in warm 80 % ethanol, followed by centrifugation for a subsequent collection of the supernatant. This procedure was repeated three times, and, at the end, the alcoholic extract volume was completed to 50 mL. This alcoholic extract was used for the analyses of total soluble and reducing sugars. The precipitate resulting from the concentrations was dried in an oven at 65 ºC for 24 h and kept in the oven for a subsequent quantification of starch (McCready et al., 1950).

To quantify the total soluble sugars (TSS), 250 µL of the alcoholic extract, 250 µL 5 % phenol, and 125 mL concentrated sulfuric acid were pipetted in a test tube with a cork. Subsequently, the tubes were shaken and kept in a water bath at 30 ºC for 20 min for a later reading in the spectrophotometer. The wavelength used in the readings was 490 nm, and results were compared with the standard 1 % sucrose curve and expressed in % of total soluble sugars (Dubois et al., 1956).

The reducing sugars (RS) were quantified by the method of Somogy-Nelson (Nelson, 1944). A test tube with cork received 200 µL of the alcoholic extract and 200 µL of Nelson

4 reagent. After the tubes were shaken, boiled for 15 min, and chilled in an ice bath, 200 µL of Nelson reagent 5 plus 600 µL deionized water were added. The material was read in a spectrophotometer after the tubes were agitated. The wavelength used for the readings was 540 nm, and the results were compared with the 1 % standard glucose curve and expressed as % of reducing sugars.

The non-reducing sugars (NRS) content was obtained as the difference between the total soluble sugars and reducing sugars contents, and it was expressed in % values.

The method described by McCready et al. (1950) was adopted for the extraction of starch. From the residue obtained in the extraction of total sugars, 2.5 mL of deionized water and 3.5 mL 52 % perchloric acid were added to the dry material in centrifuge tubes. Next, the tubes were centrifuged at 2000 *g* for 15 min, and the supernatant was collected in 25 mL beakers. After this procedure was repeated for 15 min, the volume of the beakers was completed to 25 mL with deionized water. The starch was quantified in the same way as performed for the quantification of total soluble sugars, according to Dubois et al. (1956), and was expressed in % of starch.

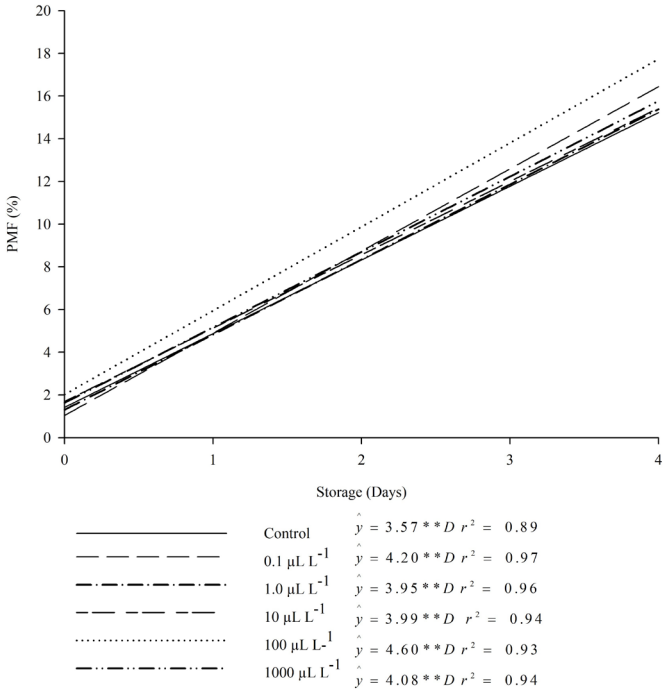
The experiment was implemented in a split-plot arrangement, in which the plots were the different concentrations of ethephon and the storage days were the subplots, in a completely randomized design with four replications. The data were subjected to variance and regression analyses, and the models were chosen according to the coefficient of determination and the biological phenomenon. Tukey's test at the $p > 0.05$ probability level was used for the comparison of the ethephon concentrations.

Results and Discussion

The fresh weight loss of the fruits increased linearly throughout the storage days. Fruit treated or untreated with ethephon showed a mass loss of 6.9 % in the first storage day, and by the end of the storage, 4nd day, the fruits had lost around 15.5 % of their initial fresh mass (Figure 1). The largest mass loss observed, 13 % higher than in control fruits, occurred in those treated with 100 µL L^{-1} ethephon in the 4nd day.

In the fruits of summer squash the PMF increased with time of storage, regardless the application of ethylene (Araújo et al., 2017). This was also observed in 'Uba' mango (Silva et al., 2012).

The alterations in a* values followed a quadratic response for all treatments (Figure 2). After the 2nd day of storage, fruits treated with ethephon concentrations greater than 0.1 µL L^{-1} showed an increase in a* contents, indicating the beginning of alterations in the skin color, which changes from green to red, when completely ripe (Table 1). On the 3rd and 4th days of storage, only the fruits treated with 100 and 1000 µL L^{-1} ethephon were different from control fruits, suggesting that these concentrations accelerated the process of loss of green color of the gilo fruits. The positive



** Significant by t-test p < 0,01 probability.

Figure 1. Mass loss of fresh matter (PMF), in % in gilo fruits control and treated with different concentrations of ethephon during the day storage (D).

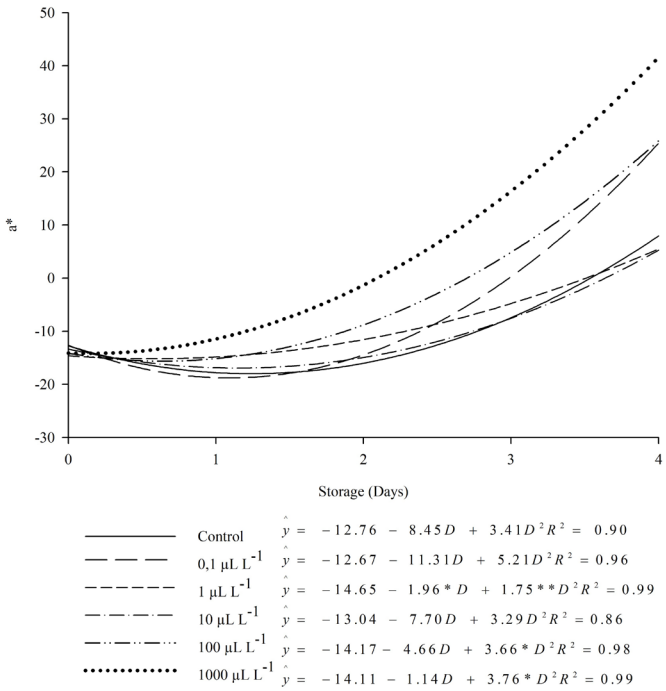


Figure 2. Color changes in the skin of gilo fruits control and treated with ethephon during days of storage (D).

a* values for all treatments show that on the 4th day, the fruits were already reddish or completely red, indicating the end of the shelf life of these fruits. Therefore, considering the skin color, the postharvest life of the fresh fruits was only two days, if exposed to ethephon concentrations above 0.1 µL L^{-1}. Concentrations greater than 100 µL L^{-1} significantly accelerate the alteration of skin color in these fruits.

Changes in fruit color are due to the induction of activity of the chlorophyllase enzyme, stimulated by the ethylene, which degrades and reduces the concentration of chlorophyll in the fruit skin. The ethylene increases the activity of chlorophyllase and oxidases responsible for the degradation of chlorophyll by the regulation of its gene expression, in the presence or absence of light (Finger et al., 2015).

Gilo fruits treated with ethylene doses greater than 1 µL L^{-1} have lower chlorophyll contents than control fruits (Table 2). These results indicate that the chlorophyll degradation was stimulated by the presence of the ethylene. The chlorophyll content followed a quadratic behavior for the control fruits and the response of the root square for the fruits treated with 1, 100 and 1000 µL L^{-1}. Thus, initially there was an increase with a subsequent decrease in the chlorophyll content. The increase in the chlorophyll content occurs up to days 1.22, 0.62, 0.37 and 0.52 in treatments control, 1.0, 100 and 1000 µL L^{-1}, respectively. This increase may be due to the loss of water by the fruit, which reduced its fresh mass and generated a relative increase in the chlorophyll concentration in the skin. However, subsequently, the chlorophyll degradation process begins, as evidenced by the decrease in the contents.

The application of 0; 5 and 10 µL L^{-1} of ethylene for 96 h at 22 ° C in 'valencia' oranges reduced chlorophyll content (Jomori et al., 2016). In the summer squash the reduced chlorophyll was 67.97 %, 69.63 %, and 73.39 % in response to the three highest doses of ethylene (10, 100 and 1000 µL L^{-1}), respectively, after eight days of storage (Araújo et al., 2017). Both authors attributed chlorophyll reduction to increased chlorophyllase activity. What was also observed by Paul et al. (2012).

In gilo fruits, ethephon concentrations of 1.0 µL L^{-1} were effective in reducing the chlorophyll content, contributing to changes in color determined by the color component a*.

The total soluble, reducing, and non-reducing sugars contents were changed over the storage days only in the control fruits and in those treated with 0.1, 1.0 and 10 µL L^{-1} ethephon (Table 3). During the storage period, control fruits and those treated with 0.1 µL L^{-1} had an increase in the NRS and TSS contents, respectively. In the summer squash, the application of 10 µL L^{-1} got to a greater reduction in sugars than the highest doses, with a reduction of 22 and 35 % for total soluble sugars and reducing sugars, respectively (Araújo et al., 2017).

There were reductions in the RS contents for control fruits and for those treated with 1.0 and 10 µL L^{-1}. Starch

Table 2. Mean values with standard error of the mean and equation of gilo fruits chlorophyll content treated with ethephon, depending on the day storage (D).

Concentrations in ethephon	Chlorophyll content (µg g^{-1} MF)	Adjusted equations	R^2
Control	0.0885 ± 0.009 a	$\hat{Y}=0.0560-0.00136D+0.0058D^2$	0.81
0.1 µL L^{-1}	0.0742 ± 0.006 ab	$\hat{Y}=0.0742$	
1.0 µL L^{-1}	0.0592 ± 0.005 bc	$\hat{Y}=0.0527+0.0789*D^{1/2}-0.0498*D$	0.92
10 µL L^{-1}	0.0502 ± 0.002 cd	$\hat{Y}=0.0592$	
100 µL L^{-1}	0.0376 ± 0.005 d	$\hat{Y}=0.0516+0.0351*D^{1/2}-0.0285*D$	0.98
1000 µL L^{-1}	0.0409 ± 0.002 cd	$\hat{Y}=0.0527+0.0772*D^{1/2}-0.0534*D$	0.93

Same letter in the column do not differ by Tukey test, at p > 0.05 of probability.
* Significant by t test, at p < 0.05 probability.

Table 3. Equations adjusted content of soluble sugars (TS), reducing sugars (RS), non-reducing sugars (ANR) and starch in gilo fruits treated with ethephon, throughout the storage period (D).

Variable (%)	Concentrations in ethephon	Adjusted equations	R^2
AST	Control	$\hat{Y}=7.05$	
AST	0.1 µL L^{-1}	$\hat{Y}=7.16-2.13*D^{1/2}+1.25*D$	0.96
AST	1.0 µL L^{-1}	$\hat{Y}=6.86$	
AST	10 µL L^{-1}	$\hat{Y}=6.24$	
AST	100 µL L^{-1}	$\hat{Y}=6.46$	
AST	1000 µL L^{-1}	$\hat{Y}=7.81$	
AR	Control	$\hat{Y}=5.7945-0.3756*D$	0.83
AR	0.1 µL L^{-1}	$\hat{Y}=4.70$	
AR	1.0 µL L^{-1}	$\hat{Y}=5.72-0.98D+0.13D^2$	0.91
AR	10 µL L^{-1}	$\hat{Y}=5.5392-0.5562*D$	0.82
AR	100 µL L^{-1}	$\hat{Y}=4.59$	
AR	1000 µL L^{-1}	$\hat{Y}=4.94$	
ANR	Control	$\hat{Y}=1.92-0.83D^{1/2}+0.68*D$	0.95
ANR	0.1 µL L^{-1}	$\hat{Y}=1.88-0.32D+0.20D^2$	0.73
ANR	1.0 µL L^{-1}	$\hat{Y}=2.97$	
ANR	10 µL L^{-1}	$\hat{Y}=1.84$	
ANR	100 µL L^{-1}	$\hat{Y}=2.14$	
ANR	1000 µL L^{-1}	$\hat{Y}=3.10$	
Starch	Control	$\hat{Y}=4.93+1.12D-0.40D^2$	0.79
Starch	0.1 µL L^{-1}	$\hat{Y}=5.30-2.03D^{1/2}-1.57D$	0.90
Starch	1.0 µL L^{-1}	$\hat{Y}=5.30+0.89D-0.38D^2$	0.80
Starch	10 µL L^{-1}	$\hat{Y}=5.03+0.68D-0.31D^2$	0.86
Starch	100 µL L^{-1}	$\hat{Y}=5.32+2.01D^{1/2}-1.70*D$	0.92
Starch	1000 µL L^{-1}	$\hat{Y}=5.32+2.62*D^{1/2}-2.03*D$	0.97

* Significant by t test, at p < 0.05 probability.

Table 1. Mean values of a * and standard error of mean of gilo fruits treated with ethephon over days storage.

Storage days	Control	0.1	1.0	10	100	1000
		µL L^{-1}				
0	-14.76±0.48 a	-14.76±0.48a	-14.76±0.48 a	-14.76±0.48a	-14.76±0.48a	-14.76 ±0.48a
1	-14.01±0.99a	-14.84±0.60a	-14.75±0.73a	-15.11±0.38a	-12.86±0.92a	-9.49±1.89a
2	-15.43± 0.97b	-13.69±1.12ab	-11.28±0.58ab	-12.02±0.62 ab	-12.16±0.42ab	-3.38±1.79ab
3	-12.07±1.51b	-4.71±1.97b	-5.31±1.85b	-13.25±1.90b	6.99±1.07a	17.06±1.70a
4	10.14±1.50c	13.74±1.90c	5.70±0.82c	11.60±0.17c	25.32±1.76b	41.51±1.04a

Equal letters on the line do not differ by Tukey test at p > 0.05 probability.

contents decreased on average twice in control fruits and in those treated with ethephon, throughout the storage days. Due to the respiratory process, there is an interconversion of carbohydrates, with a possible degradation of starch into glucose (RS) and degradation of sucrose (TSS and NRS) into glucose and fructose (RS). The glucose and fructose reducing sugars can be converted to sucrose by the sucrose synthase enzyme (Taiz & Zeiger, 2017). Thus, any factor resulting in increased respiratory rate can cause a reduction in the starch and/or TSS contents, because they are substrates for respiration. For the gilo fruits, there was consumption of starch, glucose, and fructose (RS), and the last ones may be under use by the sucrose synthase enzyme to form sucrose (TSS and NRS).

One of the factors inducing respiration is the ethylene; therefore, higher concentrations of ethylene applied are expected to increase the respiratory rate, resulting in alterations in the concentrations of TSS, RS, NRS, and starch. However, in gilo fruits, ethylene seems not to influence the carbohydrate contents of the fruits.

Conclusions

Ethephon accelerates the loss of chlorophyll in harvested gilo fruits, reducing the green color and shortening the shelf life. Ethephon does not influence the loss of mass or the carbohydrate metabolism of the fruits.

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