# **Original Research Article**

# POST HARVEST OF ACID LIME FRUITS 'TAHITI' GRAFTED ON GENOTYPE TSKC x (LCR x TR) – 017, UNDER SALINE STRESS

**Objective:** The objective of this study was to verify the post-harvest quality of fruits of the acid lime fruits 'Tahiti' of the genotype TSKC x (LCR x TR) - 017, under saline stress.

**Experimental design:** The experimental design was completely randomized from the factorial arrangement (2x5), where factor 1 corresponded to irrigation water salinity levels (0.3 and 3.0 dSm<sup>-1</sup>) and factor 2, storage time (0.5, 10, 15 and 20 days).

**Location and duration of the study:** the experiment was conducted at the Center of Science and Technology Agrifood of the Federal University of Campina Grande, Pombal Campus, Paraíba, from February 2016 to February 2017.

**Methodology:** the effect of the treatments was analyzed from the following evaluations: loss of fresh weight (%),soluble solids(%),titratable acidity (%),soluble solids and titratable acidity,hydrogen potential, concentration of H + ions ( $\mu$ M), electrical conductivity (dSm $^{-1}$ ),ascorbic acid (mg 100 mL $^{-1}$ ), total soluble sugars (mg 100 mL $^{-1}$ ),phenolic compounds(mg 100 mL $^{-1}$ ) and flavonoids (mg 100 mL $^{-1}$ ).

**Results:** it was observed the interaction between the saline concentration and the time of storage, on the physical-chemical and chemical characteristics in the acid file 'Tahiti'.

**Conclusion:** the salinity of 3.0 dSm<sup>-1</sup> showed the highest values for soluble solids, SS/AT ratios, total soluble sugars, phenolic compounds, and flavonoids during storage, indicating that the quality of these fruits was better when compared to fruits of the acidic 'Tahiti' file with 0.3 dSm<sup>-1</sup> of salinity.

Keywords: Citrus sp.; rootstock; physicochemical; salinity.

# 1. INTRODUCTION

In the Brazilian Northeast is the second largest citrus crop, where citriculture has great socioeconomic importance, being related to the generation of employment and income. Nevertheless, production and productivity are below the potential of the culture, since it shows an average yield of only 11.8 t ha<sup>-1</sup> [1]. This can be attributed to the use of less productive canopy/rootstock combinations and to the natural water deficit that occurs in the hottest months of the year, which points to the need to use irrigation techniques to increase productivity.

However, another limiting factor is the high presence of salts in the water bodies of the Northeast Region, which according to Oliveira et al. [2] varies according to rainfall and soil origin. Considering that, citrus plants are considered moderately sensitive to salinity, their growth, development, and productivity may be influenced by salinity [3, 4]. The compartmentalization of the saline ions inside the vacuoles and the maintenance of a favorable K<sup>+</sup>/Na<sup>+</sup> balance in the cytosol determines the capacity to resist saline stress [5].

The harvest is the main element of the final quality of the fruits. For the fruit to arrive with high quality to the consumer market it is necessary that the harvest is made at the ideal moment. Because the citrus fruits are non-climacteric and the sugar and acid contents did not change after harvest, the harvest should be made at the ideal maturation point for consumption, increasing the chances of obtaining better quality fruits [6].

The fruit storage is an option to prolong the period of commercialization and regularization in the supply, the method of refrigeration of 'Tahiti' for domestic consumption is little or never used. However, for the external market, the fruit requires at least 30 days of post-harvest life, totaled by maritime transport and commercialization, refrigerated storage is essential [7].

Considering that salinity influences the growth, development, production, and productivity of citrus and that the species have a socioeconomic role for the Northeast Region, the objective was to verify

the post-harvest quality during the storage of fruits of the irrigated Tahiti acid file with two levels of water salinity.

### 2. MATERIAL AND METHODS

# 2.1 Obtaining Plant Material and Experimental Procedure

The fruits of acid lime 'Tahiti' were grown in an experimental area, located in the Science and Technology Center Agrifood - CCTA, the Federal University of Campina Grande - UFCG, the municipality of Pombal, Paraíba, Brazil, the geographic coordinates 6º47'20 "Of latitude S and 37º48'01" of longitude W, at an altitude of 194 m.

The genotype used as rootstock was (TSKC  $\times$  (LCR  $\times$  TR) - 017) from the Citrus Genotypes (PMG-Citrus) breeding program of Embrapa Mandioca and Fruticultura, in Cruz das Almas, Bahia, Brazil. The canopy variety was the acid lime 'Tahiti'. During the cultivation, lysimeters with a capacity of 150 L were used (Fig. 1A), the spacing used was 2 m  $\times$  2 m between rows and between columns. The water supply to the plants was performed according to each level of salinity, from a localized irrigation system, with 18 mm hoses and self-compensating drippers (flow rate of 8 L/h per dripper), with 4 drippers per plant.

Two levels of salinity (electrical conductivity) of the waters (0.3 and 3.0 dSm<sup>-1</sup>) were used, the level of 3.0 dSm<sup>-1</sup> was prepared with the addition of sodium chloride (NaCl), whereas the level of 0.3 dSm<sup>-1</sup> corresponded to salinity level of the local water supply system itself.

The fruits for each treatment were collected manually at the beginning of the morning when they presented physiological maturation, with smooth bark, bright green (Fig. 1B), they were transported in plastic bags to the Laboratory of Chemistry, Biochemistry and Food Analysis of the CCTA to carry out the analyzes. In the laboratory, the fruits were selected aiming the removal of fruits with injuries, in order to obtain samples with quality and visually classified in relation to the color of the fruit peels to obtain standardized samples. After sorting the fruits were distributed in trays of polystyrene, with 3 fruits in each tray (Fig. 1C), placed under a bench and maintained at room temperature ( $28 \pm 2$   $^{\circ}$ C) for 20 days (Fig. 1D). In order to carry out the analyzes, the fruits were manually cut in the middle in the transverse direction with stainless steel knives (Fig. 1E), with the aid of a juicer the cell juice was collected (Fig. 1F and 1G) and physical-chemical and chemical analyzes were performed (Fig. 1H).



Fig.1. Flowchart of harvesting and processing the fruit of acid lime 'Tahiti'.

#### 2.2 Experimental Design

The experiment was carried out in a completely randomized experimental design, in a 2x5 factorial scheme, where the first factor corresponded to the two irrigation water salinity levels (0.3 and 3.0

dSm<sup>-1</sup>) and the second factor to the storage time (0.5, 10, 15 and 20 days), with 3 replicates composed of 3 fruits each.

# 2.3 Physical Chemistry and Chemical Analysis

In order to carry out the physicochemical and chemical analyzes, the cell juice was extracted as described in the flowchart for harvesting and processing the fruits of the Tahiti acid file (Fig. 1) and evaluated by the following characteristics:

- Loss of fresh mass (%): it was quantified gravimetrically, using semianalytic scale(Bel brand) with an accuracy of 0.01 g. The obtained masses were transformed into a percentage of fresh mass loss, taking as 100% the initial mass of the first day of analysis.
- Soluble Solids (%): were determined in digital refractometer (brand Digital refractometer) with automatic temperature compensation. The analysis was performed in duplicate for each repetition, the refractometer was calibrated with distilled water.
- Titratable acidity (%): 1 mL of the cell juice obtained from the Tahiti acid file was used, homogenized in Erlenmeyer flask containing 50 mL of distilled water and 2 drops of phenolphthalein, then titrated with the solution of Sodium Hydroxide 0.1 M [8].
- Solid Solubility Ratio and Titratable Acidity: it was estimated by means of the ratio of soluble solids values to the values of the titratable acidity.
- hydrogen ionic potential or pH: was determined by direct reading in the cell juice obtained from the acid lime 'Tahiti' in digital bench pot (Digimed mark).
- hydrogen ionic potential or pH: was determined by direct reading in the cell juice obtained from the acid lime 'Tahiti' in digital bench potentiometer (Digimed mark).
- Electrical conductivity (dSm<sup>-1</sup>): was measured directly on the sample using a digital conductivity meter (MS Tecnopon brand).
- Ascorbic acid (mg 100 mL<sup>1</sup>): ascorbic acid values were obtained through the titration method using 1 ml of the sample plus 49 mL of 0.5% oxalic acid and titrated with Tillmans solution until reaching pink staining according to the method described by Instituto Adolfo Lutz [8].
- Total Soluble Sugars (mg 100 mL<sup>1</sup>): for the determination of the total soluble sugars, the Yemm method was used; Willis [9], where it used 0.5 ml of the sample and diluted in 100 ml of distilled water by measuring to a volumetric flask, then used a 0.150 ml aliquot of the extract, 1,850 ml of water and 2 ml of anthrone followed by stirring, the tubes were heated at 100 °C for 5 minutes. After this procedure, the samples were read in a spectrophotometer (brand Spectrum) at 620 nm.
- Phenolic Compounds (mg 100 mL<sup>-1</sup>): were quantified following the Waterhouse method [10]. The extracts were prepared by diluting 1 ml of acid lime in 50 mL of distilled water where they remained undisturbed for 30 minutes and were subsequently filtered. The extracts were transferred to glass tubes, followed by the addition of water and Folin-Ciocalteau. The tubes were shaken and after 3 minutes, sodium carbonate was added to 20%. The tubes rested for a period of 30 minutes in a water bath (Hemoquímica brand) at 40 °C. Readings were taken on a spectrophotometer (Spectrum brand) at absorbance 765 nm.
- Flavonoids (mg 100 mL<sup>1</sup>) were determined according to the method described by Francis [11], where 1.5 mL of the Tahiti acidic cell juice was used, which was macerated in a mortar with 10 mL of ethanol HCl (1.5 N) in a dark environment and left to stand for 24 hours in the refrigerator (Consul brand). At the end of the rest the samples were filtered, then aliquots were taken in a cuvette and the spectrophotometer (Spectrum brand) readings were taken at 374 nm absorbance.

#### 2.4 Statistical Analysis

The data were submitted to analysis of variance when detected significant effect for the test F has applied the test of Tukey at the level of 5% of probability. The results were analyzed using the AgroEstat® statistical package [12].

### 3. RESULTS AND DISCUSSION

There was a significant difference between the levels of salinity for fresh fruit weight of acid lime 'Tahiti' with increasing loss of fresh weight over time of storage (Table 1).

Table 1. Fresh Mass Loss in Tahiti acid fruits irrigated with salinities of 0.3 dSm<sup>-1</sup> (S<sub>1</sub>) and 3.0 dSm<sup>-1</sup> (S<sub>2</sub>) over time of storage.

<u> </u>					
Source of variation	(Salinity – S)	(Storage – St)	S X St	<b>Mean</b>	CV %
Fresh mass loss	12355226.00 <sup>**</sup>	80307523.00 <sup>**</sup>	1918832.65 <sup>**</sup>	17.12	0.01
** = significant	at the 0.01 proba	ability level; CV =	coefficient of	variation.	

The greatest loss of fresh mass was observed in fruits irrigated with 3.0 dSm<sup>-1</sup> salinity with a value of 32.32% on the last day of storage, whereas for fruits of irrigated plants with lower salinity 0.3 dSm<sup>-1</sup> was 28.17% (Table 2). According to Chitarra and Chitarra [13] this behavior happens due to the perspiration of the fruits throughout the days, considering that, biochemical transformations occur that generate change in the coloration of the fruit. This performance during storage may also be related to external factors such as the methodology chosen for the harvesting of fruits and other variables that

Table 2. Fresh Mass Loss (%) in Tahiti acid fruits irrigated with salinities of 0.3 dSm<sup>-1</sup> (S<sub>1</sub>) and

may result in mechanical injuries, in addition, the level of salinity in the crop [14].

3.0 dSm (	3.0 dSm <sup>-1</sup> (S <sub>2</sub> ) over time of storage.				
Storage (days)	Salinity – S₁	Salinity – S <sub>2</sub>			
<mark>0</mark>	0.00 eA	0.00 eA			
<mark>5</mark>	8.05 dB	10.79 dA			
<mark>10</mark>	18.89 cB	19.28 cA			
<mark>15</mark>	24.62 bB	29.05 bA			
<mark>20</mark>	28.17 aB	32.32 aA			

Averages followed by the same lowercase letter in the column and averages followed by the same capital letter in the row do not differ according to Tukey's test, p <0.05.

There was a significant difference between salinity levels for soluble solids, where fruits of irrigated plants with 3.0 dSm<sup>-1</sup> conductivity presented higher values on all storage days with values between 11.67% and 10.17% (Fig. 2A). In the fruits of the plants irrigated with salinity 0.3 dSm<sup>-1</sup> obtained values close to the saline level of 3.0 dSm<sup>-1</sup>. There are some reasons to correlate the soluble solids content with the amount of water applied by the irrigation system, since when there are conditions of less amount of water available in the soil the sugar contents tend to increase in the fruits, aiming at the lower water absorption by the plant and consequently a lower dilution of these solids when subjected to water scarcity [15,16]. The soluble solids values found in this study were between 9.0 and 12.0%, it is observed that these values were higher than the results reported by Miranda; Junior [17], who studied the fruits of acidic lime from the western Colorado, Rondônia, Brazil, found 8.46%. Already in the work of Blum; Ayub [18], when evaluating the "Tahiti" lemon fruits treated with 1-methylcyclopropene the contents were 7.9 to 8.4%, respectively.

According to Fig. 2B, the titratable acidity showed a significant difference in the salinity level, where the fruits of the irrigated plants with a salinity of 0.3 dSm<sup>-1</sup> obtained higher results at the end of storage days, varying from 5.69 to 7.46%. It was observed by the linear regression equation that for salinity 3.0 dSm<sup>-1</sup> the values were between 5.45% and 5.57%. The results are close to those found by Sales [19] who, when studying 'Tahiti' file irrigated with waters of different salinities, obtained an average of 5.54% for 0.3 dSm<sup>-1</sup> of salinity.

For the SS/AT ratio, the results showed a significant difference between salinity and storage (Fig. 2C). The linear regression equation was the one that showed better performance of the two salinity levels, these values were low on all storage days. Although the results were close, salinity 3.0 dSm<sup>-1</sup> was the one with the best values ranging from 2.15 to 1.83. The results for SS/AT reported by Miranda; Júnior [17] in 'Tahiti' lemon fruits were 1.56 lower results than those found in this study.

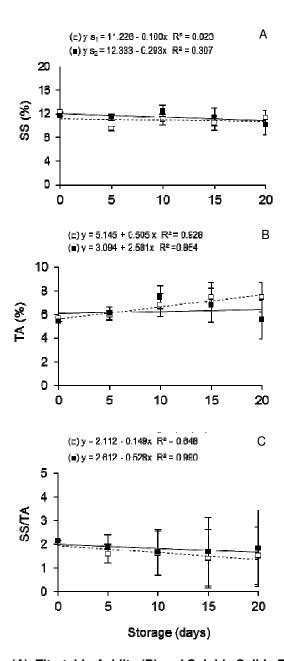


Fig. 2. Soluble Solids (A), Titratable Acidity (B) and Soluble Solids Ratio and Titratable Acidity (C) in Tahiti acid fruits irrigated with salinities of 0.3 dSm<sup>-1</sup> (S<sub>1</sub>) and 3.0 dSm<sup>-1</sup> (S<sub>2</sub>).

In Fig. 3A is the pH results that did not differ statistically between the salinity levels 0.3 dSm<sup>-1</sup> and 3.0 dSm<sup>-1</sup>. Salinity levels and storage did not affect fruit quality when it comes to the pH parameter. According to Benevides et al. [20], low pH values are important, since they guarantee the preservation of the pulp without being subjected to some thermal treatment, thus avoiding loss of nutritional quality of the fruit.

The results obtained for the concentration of  $H^+$  ions (Fig. 3B) were not significant between the levels of salinity and storage. According to Paixão et al. [21] evaluating the acid lime 'Tahiti' grown in Northern Minas Gerais, Brazil, found 2.26 pH value converted to  $H^+$  ion concentrations has the value of 5.50  $\mu$ M. This value reported by the author is close to that found in this study.

There was a significant difference for the results of the electrical conductivity (Fig. 3C), where on the last day of storage the highest value was found in the fruits of the irrigated plants with 0.3 dSm<sup>-1</sup> salinity, which varied between 3.64 and 4.44 dSm<sup>-1</sup>. The results of the 3.0 dSm<sup>-1</sup> salinity decreased on the last day of storage. Sales [19] when studying the quality of 'Tahiti' acidic fruits irrigated with waters of different salinities obtained an electrical conductivity of 8.02 dSm<sup>-1</sup>.

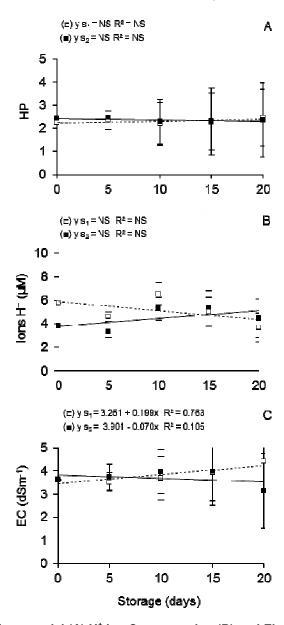


Fig.3. Hydrogenionic potential (A) H<sup>+</sup> ion Concentration (B) and Electrical Conductivity (C) in fruits of acid lime 'Tahiti' irrigated with 0.3 salinity dSm<sup>-1</sup> (S<sub>1</sub>), 3.0 dSm<sup>-1</sup> (S<sub>2</sub>).

It was observed that there was a significant difference between the results of ascorbic acid in the fruits of the acidic 'Tahiti' It was observed that there was a significant difference between the results of ascorbic acid in the fruits of the Tahiti acid file (Fig. 4A), it was observed that the fruits of the plants irrigated with salinity water 0.3 dSm<sup>-1</sup> varied considerably during the storage, it was verified that the value decreased by day 5, this may have occurred due to an analytical error, considering that in the other days the results were high, with best results between 61.20 and 69.84 mg 100 mL<sup>-1</sup> of ascorbic acid.

In the irrigation water salinity level of 3.0 dSm<sup>-1</sup>, there was a decrease on day 15 followed by a considerable increase on day 20. However, the fruits of the plants irrigated with 0.3 dSm<sup>-1</sup> were maintained with the ascorbic acid content higher than the fruits of the plants that were irrigated with 3.0 dSm<sup>-1</sup>, in all the days of analyzes. According to Couto; Canniatti-Brazaca [22], when studying ascorbic acid and antioxidant capacity of citrus varieties, reports that the ascorbic acid content modifies according to the chosen harvest time, crop region and climate, even fruits belonging to the same species. Thus, it was observed that the conductivity of 3.0 dSm<sup>-1</sup> had no effect when it came to the quantification of ascorbic acid in the acid file since it presented a greater degradation of ascorbic acid during storage. The results are shown in Fig. 4A shows that fruits irrigated with higher salinity obtained higher results than fruits such as ponkan tangerine (41.8 mg 100 mL<sup>-1</sup>), clove lemon (32.8 mg 100 mL<sup>-1</sup>) and lemon gale (34.5 mg 100 mL<sup>-1</sup>), reported in the Brazilian Table of Food Composition [23].

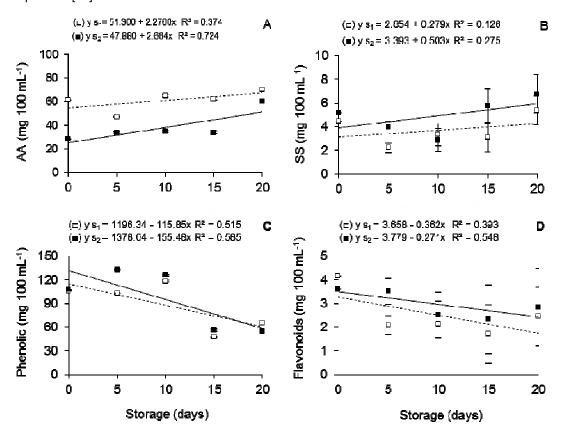


Fig. 4. Ascorbic acid (A), Total Soluble Sugars (B), Phenolic Compounds (C) and Flavonoids (D) in fruits of the acidic lime 'Tahiti' irrigated with a salinity of 0.3 dSm<sup>-1</sup> (S<sub>1</sub>) and 3.0 dSm<sup>-1</sup> (S<sub>2</sub>).

Fig. 4B shows the values of the total soluble sugars that presented significant difference, where the salinity level at 0.3 dSm<sup>-1</sup> presented results between 4.45 and 5.39 mg 100 mL<sup>-1</sup>, it is observed that these values were lower when compared to the level of salinity at 3.0 dSm<sup>-1</sup> which were between 5.16 and 6.76 mg 100 mL<sup>-1</sup>, respectively. At the end of the storage the fruits of the plants that were irrigated with higher conductivity 3.0 dS m<sup>-1</sup> presented higher total soluble sugars content. According to Chitarra; Chitarra [13] with the decrease of organic acids and hydrolysis of polysaccharides to glucose increases the sugar content according to the stage of maturation.

There was a significant difference between the phenolic compounds results (Fig. 4C). The fruits with the highest concentration of phenolic compounds were those irrigated with 3.0 dSm<sup>-1</sup> salinity ranging from 107.52 to 54.51 mg 100 mL<sup>-1</sup>, which delayed the loss of these compounds. The fruits of the irrigated plants with 0.3 dSm<sup>-1</sup> salinity ranged from 105.73 to 65.26 mg 100 mL<sup>-1</sup>. This is an important characteristic to be studied, since phenolic compounds are of fundamental importance for the

metabolism of plants, exerting several functions, them being, protection against UV rays, insects, fungi, bacteria and against the action of enzymes that cause browning, through antioxidant activity and being cited as food preservatives [24].

Among the flavonoid results, there was a significant difference (Fig. 4D). The highest levels of flavonoids were observed in the fruits of the plants irrigated with salinity water 3.0 dSm<sup>-1</sup>, it was observed that on the last day of storage the value was 2.85 mg 100 mL<sup>-1</sup>. The fruits of the plants irrigated with salinity water at 0.3 dSm<sup>-1</sup> presented a smaller amount of this compound with a value of 2.45 mg 100 mL<sup>-1</sup>. Duzzioni et al. [25] when determining antioxidant levels in citrus fruits, found for flavonoids a value of 96.27 mg/ml corresponding to 0.9627 mg 100 mL<sup>-1</sup>. It can be noticed that the value presented by the author was inferior if compared to this study, this behavior can be characterized by the studied cultivar, type of soil or applied treatment.

#### 4. CONCLUSION

During storage, the salinity of 3.0 dSm<sup>-1</sup> was highlighted with the best post-harvest quality results in relation to the fruits of the plants irrigated with the salinity of 0.3 dSm<sup>-1</sup>, mainly for the characteristics of soluble solids, SS/AT ratio, total soluble sugars, phenolic compounds, and flavonoids.

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