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Use of Nootkatone as a Senescence Indicator for Rouge La Toma Cv. Grapefruit (*Citrus paradisi* Macf.)

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The objective of this research was to study the usefulness of nootkatone as a senescence indicator for Rouge La Toma cv. grapefruit (*Citrus paradisi* Macf.), simulating different treatments that included the normal postharvest handling of citrus fruits: temperature conditioning, cold storage, shipment periods to overseas markets such as Japan and the U.S., marketing conditions, and storage at nonchilling temperature (control treatments). The highest nootkatone levels, determined by GLC−MS analyses, were detected in fruits subjected to control treatments. No significant differences were observed in nootkatone levels between treatments either with or without temperature conditioning prior to the start of the cold storage. Levels of nootkatone increased throughout time for all assayed treatments. The linear regressions of nootkatone levels showed correlation coefficients of 0.80 and 0.83 with storage time (29 and 42 days, respectively). Therefore, nootkatone appears to be a good indicator of senescence for Rouge La Toma grapefruit.

KEYWORDS: Nootkatone; senescence indicator; grapefruit; postharvest treatment

INTRODUCTION

Argentina is one of the most important citrus producers in the southern hemisphere (*1*) and Rouge La Toma is a natural mutation of grapefruit (*Citrus paradisi* Macf.) cultivars selected in Salta, a northwestern region of Argentina. International trade often requires fruit to be stored for long periods at low temperature. This condition could be detrimental to the quality of subtropical fruits such as citrus, because they are known to be susceptible to chilling injury (CI) development during cold storage (*2*). This sensitivity to low temperatures has serious economic implications, because cold storage also provides an important quarantine treatment which is required by many countries to export citrus to fruit-fly-free zones (*3*). One approach to avoid CI under quarantine treatment is to apply postharvest heat treatments to induce cold tolerance and to reduce the development of CI symptoms (*4*). However, the conditions employed in these postharvest treatments can induce various physiological and biochemical alterations such as changes in the volatile components that contribute to fruit flavor. Among these volatile components, the sesquiterpene nootkatone (**Figure 1**) is a major flavor impact compound that contributes to the characteristic flavor and aroma of the grapefruit (*5*). It has been suggested that levels of nootkatone increase with storage time and storage temperature of grapefruit (*6*) and pummelo (*7*). On the other hand, some authors have reported that processes such as harvesting (*8*), degreening, or use of growth regulators (*9*, *10*) can accelerate maturation/senescence processes, and these lead to increases in the levels of nootkatone.

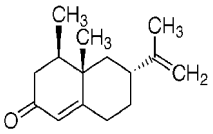

Figure 1. Structural formula of nootkatone.

In this regard, the use of volatile compounds to evaluate citrus quality deterioration during storage, marketing, and senescence is an important objective for the citrus industry. These compounds could help determine the best conditions of postharvest treatments or shipment to market that ensure optimum quality. Therefore, the objective of the present investigation was to study the usefulness of nootkatone as senescence indicator using different postharvest handling regimes for Rouge La Toma cv. grapefruit (*Citrus paradisi* Macf.).

MATERIALS AND METHODS

Plant Material and Postharvest Treatments. The variety of grapefruit (*Citrus paradisi* Macf.) used for this study was Rouge La Toma, a natural mutation selected in the Argentinian northern province

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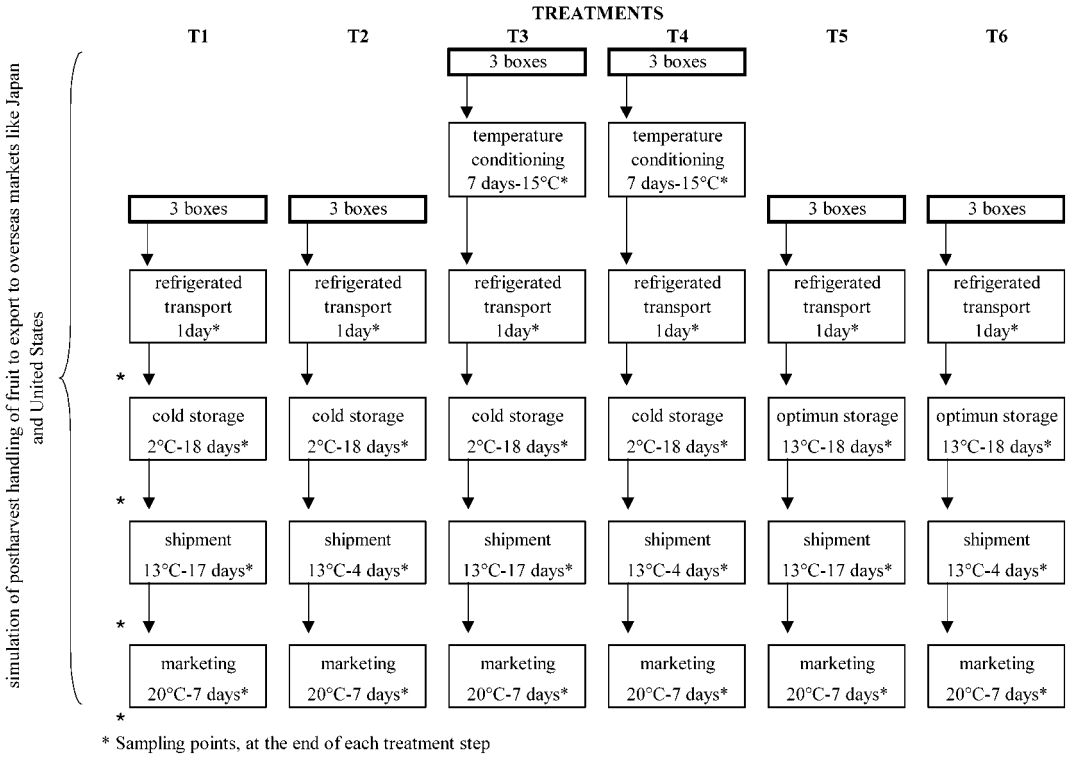

Figure 2. Treatments simulating temperature conditioning, cold storage, shipment periods to overseas markets such as the U.S. and Japan, marketing conditions, and storage at nonchilling temperature (control treatments).

of Salta, harvested in June 1999. Grapefruits subjected to temperature conditioning were harvested 7 days earlier than fruits for other treatments. Fruits were degreened with 3.5 ppm ethylene and 1.5 ppm CO_2 at a temperature of 26 °C and 90% relative humidity. Then, all fruits were washed, disinfected with sodium orthophenylphenate (SOPP), rinsed, dried, and coated with wax containing 5 000 ppm thiabendazole (TBZ). After that, all fruits were packed and the boxes were randomly distributed among different treatments before being transported from the packinghouse to our laboratory, located about 1200 km south (a 36-h trip), in refrigerated transport at 7−8 °C.

Six different treatments, simulating different steps used in postharvest handling of grapefruit, were applied to determine their effects on nootkatone levels: normal postharvest treatments (temperature conditioning and cold storage), simulated shipment periods to overseas markets such as Japan and the U.S., marketing conditions, and storage at nonchilling temperature (control treatments) (**Figure 2**).

Three commercial boxes of grapefruit, containing 40 fruits each, were used for each treatment. Fruits were sampled to determine nootkatone levels immediately after transportation, cold storage, simulated shipment periods to overseas markets, and marketing conditions.

Nootkatone Extraction and Analysis. Each nootkatone determination was performed using the grapefruit peels (flavedo plus albedo) from six fruits of one box. At each sampling time, three replicates were analyzed for each treatment. Isolation of the nootkatone was performed as described by Del Río et al. (*8*). Briefly, the methodology consists of chopping, into 0.5-cm pieces, the same quantity of flavedo plus albedo from each fruit. Then the six fruit flavedo+albedo pieces were mixed, and 4 g of fresh weight (FW) of this pool was homogenized 3× with *n*-pentane (1 g of FW/4 mL), adding 500 μL of internal standard, lauric acid methyl ester, of 400 μg/mL. The homogenates were decanted, and the organic layer was dried with anhydrous Na_2SO_4 and then concentrated to 0.5 mL under nitrogen at room temperature before being analyzed. The extracts were analyzed by Shimadzu series 14B gas−liquid chromatography (GLC), equipped with a flame ionization detector (FID) and a glass capillary column (J&W Scientific, Folsom, CA) coated with Carbowax 20 M (30 m × 0.53 mm i.d., 1.0 μm film thickness). The flow rate of carrier gas N_2 was 5 mL/min. The injection volume was 2 μL, and the split ratio was 10/1. The injector and detector temperatures were 270 °C and 280 °C, respectively. The following column temperature-programming sequence was used: an initial temperature of 75 °C was maintained for 5 min before being increased to 200 °C at 40 °C/min, then raised at 10 °C/min to 240 °C and held for 15 min. Each peak area on the gas chromatogram was calculated automatically with Class-VP Software. For capillary gas−liquid chromatography−mass spectrometry (GLC−MS) a Hewlett-Packard 5989A mass spectrometer was used with a column similar to that used above. Peak identification was confirmed by comparing the retention times and mass spectra with those of an authentic sample. Quantitative determinations were based on the known amount of added standard.

Reagents. Nootkatone and lauric acid methyl ester were purchased from Sigma-Aldrich of Argentina S. A.

Statistical Analysis. The experimental procedure consisted of a completely randomized design, with three replicates per treatment, with each box being a replicate.

Data for nootkatone level were analyzed using a fixed effects model by means of the general linear model (PROC GLM) procedure of SAS v. 6.12 (*11*) after a Box−Cox transformation of the data (eq 1) (*12*)

$$y^{(\lambda)}\,(\text{transformed variate}) = [(y^{\lambda} - 1)/(\lambda \times \hat{y}^{(\lambda-1)})] \qquad (1)$$

where $\hat{y}$ (geometrical mean of observations) = $\exp[(1/n)\,\Sigma \ln y]$, and $\lambda = 0.5$; for nootkatone levels (mg/100 g FW).

Linear contrasts were used to determine the effects of the different treatments and processes. The correlation coefficients between nootkatone levels and storage time was determined by linear regression using the PROC REG procedure of SAS.

RESULTS AND DISCUSSION

The analysis of variance of transformed nootkatone data showed highly significant ($P < 0.0001$) effects of treatment, of time, and of interaction (**Table 1**).

Table 2 shows the statistical tests of the contrasts tests for the transformed data of nootkatone among the different treatments at the end of the marketing conditions. These results

Table 1. Analysis of Variance of Transformed Nootkatone Data in Grapefruits Rouge La Toma Conditioned to Six Different Treatments

source	degrees of freedom	mean square[a]
treatments	5	8.65***
time	3	173.10***
treatments × time interaction	15	14.88***

[a] Significance at *** $P \leq 0.0001$.

Table 2. Linear Contrasts Tests between Different Treatments of Transformed Nootkatone Data at the End of the Marketing Conditions

treatment	estimate[a]
T1 vs T5	−1.13***
T3 vs T5	−0.90***
T1 vs T3	−0.23
T2 vs T6	−1.72***
T4 vs T6	−1.97***
T2 vs T4	0.25

[a] Significance at *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$.

Table 3. Linear Contrasts Tests between Different Times of Transformed Nootkatone Data

treatment	storage days	estimate[a]
T1	1–18	−0.82***
	19–35	−0.79**
	36–42	−2.06***
	1–42	−3.67***
T2	1–18	−1.37***
	19–22	−1.00***
	23–29	−0.95***
	1–29	−3.31***
T3	1–18	0.32
	19–35	−1.13***
	36–42	−2.13***
	1–42	−2.95***
T4	1–18	0.14
	19–22	−0.49*
	23–29	−0.94***
	1–29	−1.29***
T5	1–18	−5.22***
	19–35	−1.35***
	36–42	−0.54*
	1–42	−7.11***
T6	1–18	−4.58***
	19–22	−1.66***
	23–29	−0.77***
	1–29	−7.02***

[a] Significance at *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$.

revealed that the fruit stored at nonchilling temperature (control treatments T5 and T6), had levels of nootkatone significantly different when compared with those of the other treatments (T1−T2 and T3−T4). No significant differences in the nootkatone levels were observed between treatments either with or without temperature conditioning prior to the start of the cold storage (T1 vs T3 and T2 vs T4) (**Table 2**). This could be explained because the time and temperature used in the temperature conditioning (15 °C for 7 days) were low enough to avoid promoting appreciable changes in the metabolic processes. Moreover, the temperature employed in this postharvest heat treatment is similar to the optimum storage temperature of the control treatments. On the other hand, contrasts for the changes in nootkatone levels at different times showed an increase in levels of nootkatone throughout time for all treatments (**Table 3**). However, examination of nootkatone levels has revealed that the least variation and lowest levels were obtained under cold storage (T1−T2 and T3−T4)

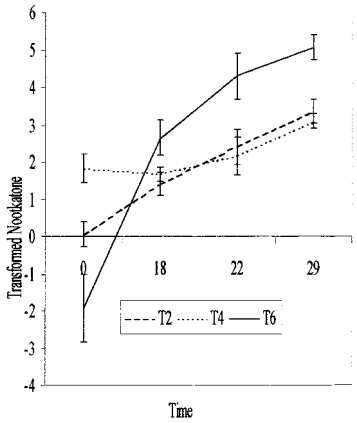

Figure 3. Transformed nootkatone data from different treatments simulating the postharvest handling of grapefruit Rouge La Toma cv. to overseas U.S. market. Vertical bars indicate SE ($n = 3$).

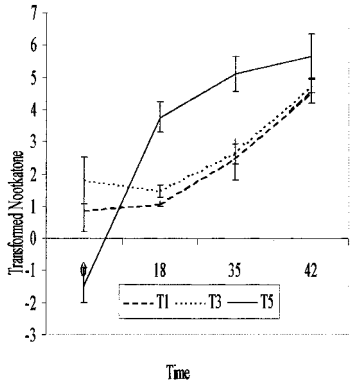

Figure 4. Transformed nootkatone data from different treatments simulating the postharvest handling of grapefruit Rouge La Toma cv. to overseas Japan market. Vertical bars indicate SE ($n = 3$).

(**Figures 3** and **4**), excepting T1 and T2 higher initial values. These higher levels could be explained by variability in the initial amount of nootkatone in the fruit, because at that point there was no difference among the treatments applied.

Under these refrigerated conditions it is possible to retard the biochemical and physiological changes associated with senescence. In contrast, the fruit stored at optimum conditions (T5−T6) showed the highest and most variable levels of nootkatone (**Figures 3** and **4**). These results suggest that the biosynthesis of this compound can be regulated by storage conditions. Moreover, a high correlation coefficient between the transformed nootkatone data and the storage time of grapefruit for the treatments studied was obtained. The estimated correlation at 42 days ($r = 0.83$) was slightly higher than that at 29 days ($r = 0.80$). Storage temperature and storage period at which fruits are held affect both fruit respiratory demand and coating permeability to gases. These combined factors contribute to alter the atmosphere and thus may affect volatile compounds (*13*). It is noteworthy that in the present study, nootkatone levels, showed for the first time for Rouge La Toma grapefruit (which is a pigmented variety extremely appealing for the consumer because of its flavor and general appearance), were higher at higher temperatures and longer storage times. The results in the present research are in agreement with those obtained by Sun and Petracek (*6*), who observed in Marsh cv. white grapefruit that nootkatone levels increased with storage time, but the increment was lower when the fruit was stored in a cold temperature (4.5 °C) than when stored at 21 °C, even though other conditions were used. Likewise, Sawamura et al. (*7*)

suggested that the levels of nootkatone may increase during pummelo storage. Considering that nootkatone levels are much higher in grapefruit than in other citrus species (*14*), and its content increases constantly with storage time, it is proposed to be used as an indicator of senescence. And, therefore, nootkatone could be used to monitor the quality of Rouge La Toma grapefruit (*Citrus paradisi* Macf.).

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