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ETHYLENE APPLICATION AFTER COLD STORAGE IMPROVES SKIN COLOR OF 'VALENCIA' ORANGES[1]

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ABSTRACT- Degreening is a postharvest technique that consists of applying ethylene to oranges fresh fruits to improve skin color. In Brazil, this technique is still not widely applied due to a lack of specific research, resulting in poor fruit coloration, which limits the oranges' exportation. The objective of this study was to investigate the de-greening effect of ethylene application after cold storage in 'Valencia' oranges. The ethylene treatments (0; 5 and 10 μL L^{-1} for 96 h at 22°C) were applied after storage for 30 days at 5°C. After ethylene application, the fruit were stored for three days at 22°C to simulate a shelf life. The skin color rate enhanced after ethylene treatment due to increased chlorophyllase activity. The content of carotenoids did not change, nor did the soluble solids, titratable acidity, ratio, percentage of juice and ascorbic acid content. The levels of acetaldehyde and ethanol in the juice were also assessed, and unpleasant odors or flavors did not occur despite a small increase in the acetaldehyde content. Therefore, the ethylene application after cold storage is efficient to improve the skin color of 'Valencia' orange without changing the internal quality.
Index terms: *Citrus sinensis*; chlorophyllase; color index; acetaldehyde.

APLICAÇÃO DE ETILENO APÓS O ARMAZENAMENTO REFRIGERADO MELHORA A COLORAÇÃO DE LARANJA 'VALÊNCIA'

RESUMO- O desverdecimento é uma técnica pós-colheita que consiste na aplicação de etileno em frutos de laranjeira para melhorar sua coloração externa. No Brasil, essa técnica ainda não é extensivamente aplicada, devido à falta de pesquisas específicas, resultando em má coloração de frutas, a qual limita a exportação. O objetivo deste trabalho é analisar os efeitos da aplicação do etileno após o armazenamento refrigerado de laranja 'Valência', como forma de melhorar a coloração externa da fruta. O etileno (0; 5 e 10 μL L^{-1} durante 96 h a 22°C) foi aplicado após 30 dias de armazenamento da fruta a 5°C. Após a aplicação do etileno, as frutas foram armazenadas a 22°C durante três dias, para simular um período de vida útil. O índice de coloração de casca aumentou após o tratamento com etileno, devido ao aumento na atividade da clorofilase. O conteúdo de carotenoides não sofreu alteração em função dos tratamentos. Também não houve alteração significativa nos sólidos solúveis, acidez titulável, *ratio*, percentagem de suco, teor de ácido ascórbico e de etanol. Os teores de acetaldeído tiveram pequena elevação após os tratamentos com etileno, mas não causaram odor desagradável. A aplicação do etileno após o armazenamento refrigerado de laranja 'Valência' é eficiente para a melhoria na cor da casca, sem alteração significativa na qualidade interna da fruta.
Termos para indexação: *Citrus sinensis*, clorofilase, índice de cor, acetaldeído.

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INTRODUCTION

Low temperatures and wide temperature range during the maturation period stimulate the development of skin color in citrus fruits. Thus, regions with warm days and cool nights favor good skin pigmentation because they allow for the carotenoids synthesis and chlorophyll degradation (CASAS; MALLENT, 1988; GRIERSON et al., 1986; MAZZUZ, 1996). However, the temperatures in most Brazilian citrus growing regions are not sufficient to promote the optimal color development, so oranges present only a pale yellow color and the oranges and tangerines have not developed the orange color by the time they are harvested; moreover, these fruits often show yellow and green patches on their skins.

The degreening technique postharvest is a solution that improves the external quality of fruit by allowing for the variety's typical color development and is already used in others countries for many times (JIMENEZ-CUESTA et al., 1983; KADER; ARPAIA, 1992; CUQUERELLA-CAYUELA, 2004). This technique consists of exposure to ethylene for a determined period of time and controlled temperature. Although the natural citrus ripening process is not following by an increase in respiration and autocatalytic ethylene production, exposure to ethylene can stimulate processes associated with ripening, accelerating the degradation of chlorophyll and increasing the synthesis of carotenoids (PURVIS; BARMORE, 1981; RODRIGO; ZACARIAS, 2007; STEWART; WHEATON, 1972). Several researches on degreening citrus fruits using ethylene or ethylene releasers were previously carried out, but the ideal condition to achieve the desirable effects are not been fully established for the conditions of Brazil**.** This is due, in part, to the different varieties of citrus existing and to the conditions in which they are grown. The application conditions (e.g., time and doses) also influence significantly the efficiency of degreening. In a study conducted by Mendonça et al. (2003) with 'Siciliano' lemon was demonstrated that the application of ethylene 6 μL L^{-1} for 4 h or 6 days was effective to improve the color of the fruit, without affecting other quality parameters. Jacomino et al. (2003) found that doses above 10 μL L^{-1} may increase the incidence of decay of 'Siciliano' lemon. Jomori et al. (2014) had verified that ethylene 5.0 μL L^{-1} for 96 h was enough to trigger the degreening of 'Murcott' tangor and improved the final color of fruit this cultivar. Santos et al. (2012) found that 'Satsuma Okitsu' tangerine showed best rind degreening using ethephon (an ethylene releaser) at concentrations between 250 and 500 mg L^{-1}. Nascimento and Medina (1994) found higher carotenoids synthesis in 'Pera' orange with the application of ethephon 2,000 mg L^{-1}. For 'Baianinha' orange the best concentrations were 2,000 and 4,000 mg L^{-1}. The increase of carotenoids synthesis leads to improved fruit peel color.

Thus, the conditions of degreening with ethylene should be established for each cultivar to improve the external color of the fruit and at the same time to avoid increase decay and establishment of off-odors resulting from the production of acetaldehyde and ethanol. Considering the effects of low temperature to reduce the metabolic processes and maintenance of fruit quality, degreening after cooling may allow the marketing of fruits of better visual quality in off-season periods and enables exportation. Although Valencia orange is most used for juice industry, it has potential as fresh fruit considering its sensory characteristics. Thus, this study aims was to investigate the degreening effect of the application of ethylene after cold storage of 'Valencia' oranges.

MATERIAL AND METHODS

The fruits of 'Valencia' sweet orange [*Citrus sinensius* (L.) Osbeck] were obtained in a commercial orchard localized in Mogi Mirim, Sao Paulo, Brazil. The climate of region is cwa (tropical altitude with dry winter and hot summer). The 'Valencia' orange were grafted on Rangpur Lime (*Citrus limonia* Osbeck) and plants had 8 years old. Fruit were harvested at a skin color index of – 0,75 (± 0,06) (JIMENEZ-CUESTA et al., 1983) , characterized by yellow color and some green spots in the peel. They were then transported to the laboratory, where the damaged fruit were removed to yield a uniform batch. Fruit were placed in plastic boxes high density polyethylene with 52 liters capacity and then stored at 5 ± 0,5°C and 90 ± 5% RH for 30 days and subsequently degreened with ethylene.

The ethylene (gas-Ethyl 5®, White Martins, Americana, São Paulo, Brazil) containing 5% ethylene and 95% nitrogen was applied in airtight chambers with a capacity of 187 L in static application. Two concentrations of ethylene, 5.0 and 10.0 μL L^{-1} evaluated, being applied for a period of 96 h at 22°C. Fruit maintained at the same conditions but not treated with ethylene was considered the control. The ethylene concentration in the chamber was monitored every two hours with samples analyzed in a gas chromatograph (Trace GC Ultra, Thermo Electro Corp., San Jose, California, USA) equipped with two flame ionization detectors. The ethylene concentrations and time of application were defined based on pre-tests.

The oranges were evaluated in four periods: before cold storage, after cold storage, after ethylene application, and three days after ethylene application (simulated market at 22 ± 1,0ºC and 70% RH). These conditions are referred to as the initial, storage, degreening and marketing periods, respectively.

The experimental design was completely randomized, factorial 3 x 4 (treatments x trial periods), with four replicates of 10 fruits each.

The analyzes carried out were:

a) skin color index (SCI): was calculated using the following formula: SCI = (1000 x *a*) / (L* x *b*) according to the methodology of Jimenez-Cuesta et al. (198*3*). The values of L*, *a* and *b* were obtained using a colorimeter (CR-400 Chroma meter, Konica Minolta Optics, Inc., Japan); four readings were performed in the equatorial region of each fruit**.** According Cuquerella-Cayuela (2004), in citrus, the SCI ranges from -26 (intense green) to +29 (intense orange)**.** Values slightly above zero means peel more yellow and values just below to zero means less intense is yellow color peel and some greenish spots. Value above + 1.0 means that the fruit progresses in the intensity of the yellow color.

b) **c**hlorophyllase activity, chlorophyll and total carotenoids content: The chlorophyllase activity (chlorophyllidohydrolase, EC 3.1.1.14) was determined using the method described by Amir-Shapira et al. (1987), as adapted by Yamauchi et al. (1991). The results are expressed in UE per mg protein (UE mg^{-1}). The protein content was determined according to Bradford (1976) using a BSA solution (Bovine Serum Albumin) for the standard curve. The chlorophyll and total carotenoids contents of flavedo were determined using the methodology of Lichtenthaler (1987), and the results are expressed as mg per gram fresh weight (mg g^{-1}).

c) acetaldehyde and ethanol: the levels of acetaldehyde and ethanol were determined by juice head-space analysis according to the methodology of Cohen et al. (1990) using a gas chromatograph (Trace GC Ultra, Thermo Electro Corp., San Jose, California, USA) equipped with two flame ionization detectors. The results were expressed as μL per liter of juice (μL L^{-1}).

d) soluble solids (SS), titratable acidity (TA) and *ratio*: were determined according to AOAC (2010), and the results are expressed in °Brix and % of citric acid, respectively. The *ratio* was calculated using the contents of SS and AT.

e) ascorbic acid (AA) content: was determined according to Carvalho et al. (1990), with the results expressed in mg of ascorbic acid per 100 mL of juice.

f) juice percentage: was calculated by weighing the entire mass of the fruit (MF) in each replicate and weighing its extracted mass of juice (MJ). The resultant data were then applied to the following formula: % juice = (MJ / MF) x 100.

The results obtained were subjected to analysis of variance (F test), and when significant, the means were compared by Tukey test at $P \leq 0.05$ by SANEST statistical program (ZONTA; MACHADO, 1995).

RESULTS AND DISCUSSION

The initial SCI values of approximately -0.5 increased up to +0.5 in all treatments during cold storage (Figure 1). According to Sposito et al. (2006), 'Valencia' oranges are suitable for export when they reach a SCI of + 2.0. Immediately after the ethylene application, both ethylene dosages presented a SCI increase with values between +2.0 and +3.0, which were higher and therefore more yellow than the control, which yielded values of +1.0. Control fruit showed increase in SCI during cold storage, and then the values stayed practically unchanged up to the end of shelf life (marketing). The skin of these fruits still had green spots and pale-yellow color. Thus, the ethylene treatments effectively produce fruit with a desirable appearance for exportation. Other authors also found improvement in the external color of citrus fruits with the application of ethylene or ethylene releasers (NASCIMENTO; MEDINA, 1994; JACOMINO et al., 2003; MENDONÇA et al., 2003; SANTOS et al., 2012; JOMORI et al., 2014).

The development of the skin color in oranges treated with ethylene is due to the reduction in the chlorophyll content (Figure 2A), especially given the increase in the activity of chlorophyllase. Our results show that the activity of chlorophyllase began when the oranges were withdrawn from cold storage and dramatically increased after the application of ethylene, differing from control fruits until the end of the simulated marketing period (Figure 2B). As the chlorophyllase activity increased, the chlorophyll content decreased in oranges treated with ethylene at the end of the simulated marketing period, while the control fruit presented higher values of chlorophyll until the end of observation (Figure 2A e 2B).

The relationship between exogenous ethylene and chlorophyllase occur because ethylene increases the gene expression of chlorophyllase, which provokes the formation of degradation products, which are not green. Moreover, ethylene allows oxygen to access the chlorophyll oxidation reactions (JOHNKARUPPIAH; BURNS, 2010; SHIMOKAWA et al.,

1978; TREBITSH et al., 1993).

The content of carotenoids did not significantly vary in the different evaluation periods and between treatments (Figure 3). We believe that the carotenoids of the skin had already been synthesized during fruit ripening but were being "hidden" by the chlorophylls. Studies show that carotenoids may be synthesized in citrus after harvest and withdrawal from cold storage (CARMONA et al., 2012; ZHOU et al., 2010). However, a balance may have occurred between synthesis and degradation because these compounds are highly susceptible to oxidation, which precludes the observation of variations between treatments and during the evaluation period. Although there were no significant differences in the carotenoid content between the treatments we found that untreated fruit had pale yellow color with greenish spots on the skin, while fruits treated with ethylene showed no green spots and were more brightness.

Changes in the internal fruit quality are a major concern for producers. Therefore, we evaluated the main characteristics of the fruit, such as the SS, TA and percentage of juice and AA, as well as the concentrations of acetaldehyde and ethanol, which are closely linked to the development of undesirable flavor in the fruit.

We observed that the parameters of internal fruit quality did not differ between the days of analysis or between treatments (Table 1). The SS content was approximately 10.5 °Brix, the TA was approximately 0.63% citric acid, the *ratio* was 16.7, the percentage of juice was 51% and the AA content was approximately 19 mg 100 mL^{-1}. These amounts are consistent with those observed in previous studies, which were not affected by ethylene application (PLAZA et al., 2004; ZHOU et al., 2010). As verified by Mayuoni et al. (2011), we have demonstrated that the effect of exogenous ethylene was restricted to the skin and that the internal quality of the oranges was not affected.

We found an increase in acetaldehyde content in all treatments after removal from cold storage (Figure 4A). However, this increase was small, as it did not provoke unpleasant odors and flavors, as verified by informal sensory evaluations. Thus, this increase may be considered typical of the physiology of the fruit. . Also, the fact that the control fruit and those treated with ethylene having remained closed for 96 h may have stimulated the production of acetaldehyde. The flavor of citrus fruit is linked to an increased accumulation of volatile compounds, including ethanol and acetaldehyde. Thus, their presence in certain quantities may actually be an important component of fruit quality (COHEN et al., 1990; KE; KADER, 1990).

The ethanol content did not differ between treatments or over time (Figure 4B). Similarly, undesirable changes in the levels of ethanol were not observed in a study of 'Oronule' mandarins when the fruit were degreened with ethylene (CARVALHO et al., 2006). Furthermore, according to Ke and Kader (1990), strange odors and flavors occur when the ethanol concentration reaches values above 1000 $\mu L\ L^{-1}$, and this value exceeds those observed in our study.

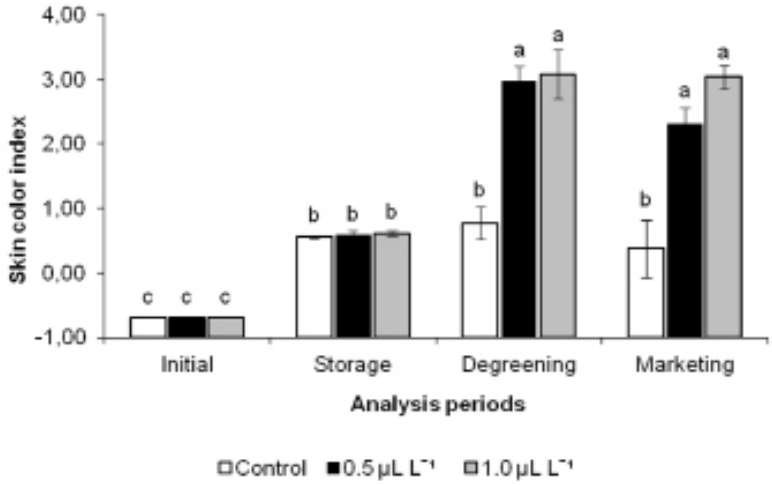

FIGURE 1- Skin color index of 'Valência' oranges after to ethylene application at different concentrations during 96 h at 22°C. (Storage: 30 days at 5°C; Marketing: simulated marketing at 22°C during 3 days). Means followed by the same letter do not differ by Tukey's test ($p \leq 0.05$). Vertical bars represent the standard error ($n = 4$).

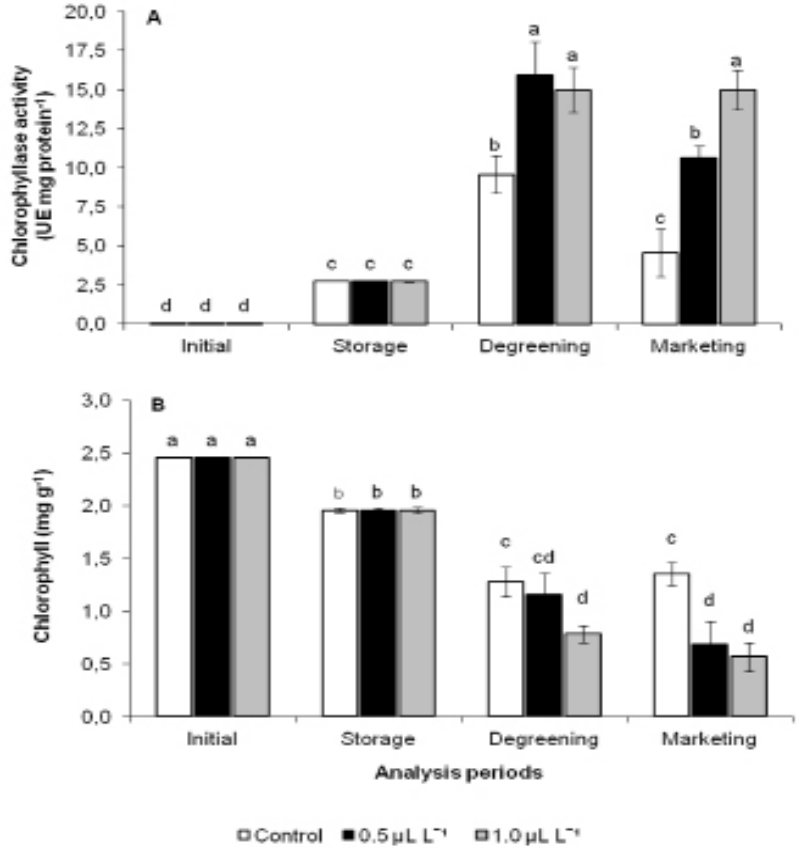

FIGURE 2- Chlorophyllase activity (A) and content of chlorophyll (B) of 'Valência' oranges after to ethylene application at different concentrations for 96 h at 22°C. (Storage: 30 days at 5°C; Marketing: simulated marketing at 22°C during 3 days). UE = Unit of the enzyme activity was defined as a change of 0.01 in absorbance at 663 nm per min. Means followed by the same letter do not differ by Tukey's test ($p \leq 0.05$). Vertical bars represent the standard error ($n = 4$).

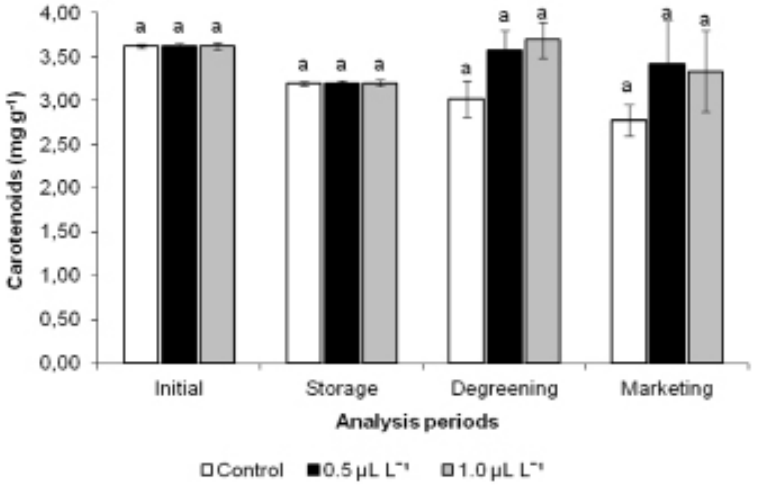

FIGURE 3- Total carotenoids content of 'Valência' oranges after ethylene application at different concentrations for 96 h at 22°C. (Storage: 30 days at 5°C; Marketing: simulated marketing at 22°C during 3 days). Means followed by the same letter do not differ according to Tukey's test ($p \leq 0.05$). Vertical bars represent the standard error ($n = 4$).

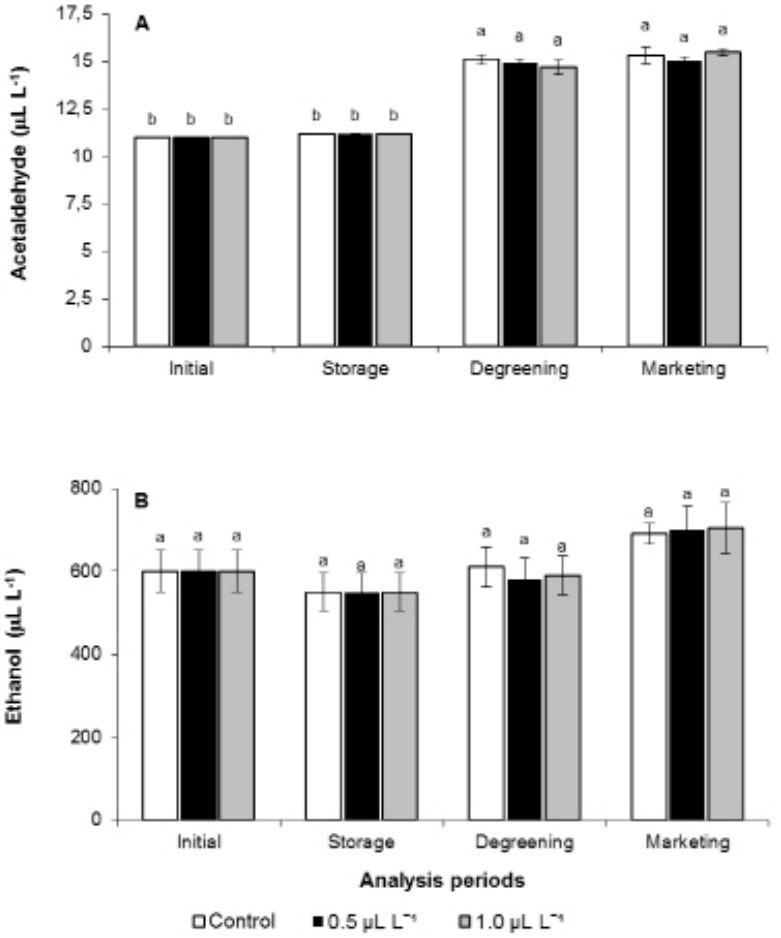

FIGURE 4- Content of acetaldehyde (A) and ethanol (B) of 'Valência' oranges after ethylene application at different concentrations for 96 h at 22°C. (Storage: 30 days at 5°C; Marketing: simulated marketing at 22°C during 3 days). Means followed by the same letter do not differ according to Tukey's test ($p \leq 0.05$). Vertical bars represent the standard error ($n = 4$).

TABLE 1 - Internal quality of 'Valência' oranges submitted to ethylene application at different concentrations during 96 h at 22°C.

		Soluble solids (%)	Titratable acidity (% citric acid)	Ratio (SS/AT)	Percentage of juice (%)	Ascorbic acid (mg 100mL^{-1})
Ethylene concentration	Control	10.52 a	0.63 a	16.66 a	50.87 a	18.95 a
	0.5 μL L^{-1}	10.52 a	0.63 a	16.41 a	51.19 a	19.35 a
	1.5 μL L^{-1}	10.55 a	0.65 a	16.41 a	51.03 a	19.16 a
Analysis periods	Initial	10.23 a	0.65 a	15.78 a	53.65 a	19.58 a
	Storage	10.88 a	0.65 a	16.59 a	49.67 a	18.89 a
	Degreening	10.63 a	0.64 a	16.33 a	51.35 a	18.81 a
	Marketing	10.39 a	0.58 a	17.27 a	49.13 a	19.33 a
	C.V.	2,44%	3,21%	8,82%	2,98%	4,71%

Storage: 30 days at 5°C; Marketing: simulated marketing at 22°C during 3 days. SS = soluble solids; TA = titratable acidity. Means followed by the same letter in line do not differ by Tukey's test ($p \leq 0.05$).

CONCLUSION

The application of ethylene at concentrations of 5 or 10 μL L^{-1} is effective for degreening 'Valencia' oranges by inducing chlorophyll breakdown, and this method does not affect the internal quality parameters.

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REFERENCES

AMIR-SHAPIRA, D.; GOLDSCHMIDT, E.E.; ALTMAN, A. Chlorophyll catabolism in senescing plant tissues: In vivo breakdown intermediates suggest different degradative pathways for citrus fruit and parsley leaves. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, v.84, n.7, p.1901-1905, 1987.

AOAC - Association of Official Agricultural Chemistry. **The official methods of analysis of AOAC International**. 18th ed. Washington, 2010. 2500 p.

BRADFORD, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, New York, v.72, n.1–2, p.248-254, 1976.

CARMONA, L.; ZACARIAS, L.; RODRIGO, M.J. Stimulation of coloration and carotenoid biosynthesis during postharvest storage of 'Navelina' orange fruit at 12 degrees C. **Postharvest Biology and Technology**, Amsterdam, v.74, p.108-117, 2012.

CARVALHO, C.P.; MONTERDE, A.; MARTÍNEZ JÁVEGA, M.A.; SALVADOR, A. Efecto del tratamiento de desverdización en la calidad de mandarinas 'oronules' con vistas a la exportación a Japón.**Revista Iberoamericana de Tecnología Postcosecha**, Hermosillo,v.7, n.2, p.104-108, 2006.

CARVALHO, C.R.L.; MANTOVANI, D.M.B.; CARVALHO, P.R.N.; MORAES, R.M.M. **Análise química de alimentos**. Campinas: ITAL, 1990. 121 p. (Manual Técnico)

CASAS, A.; MALLENT, D. The color of citrus fruits. I. Generality. II. Factors that influence the color. Influence of the species, variety and temperature. **Revista de Agroquímica y Tecnología de Alimentos**, Valencia, v.28, n.2, p.184-201, 1988.

COHEN, E.; SHALOM, Y.; ROSENBERGER, I. Postharvest ethanol buildup and off-flavour in 'Murcott' tangerine fruits. **Journal of the American Society for Horticultural Science**, Alexandria, v.115, p.775–778, 1990.

CUQUERELLA-CAYUELA, J. Nuevo sistema de medida de color para cítricos. Levante Agrícola: **Revista Internacional de Cítricos**, Barcelona, n.372, p.298-304, 2004.

GRIERSON, W.; COHEN, E.; KITAGAWA, H. Degreening. In: WARDOWSKI, W.F.; NAGY, S. (Ed.). **Fresh citrus fruits**. Wesport: Avi Publishing Company, 1986. chap.10, p.253-274.

JACOMINO, A.P.; MENDONCA, K.; KLUGE, R. A. Armazenamento refrigerado de limoes 'Siciliano' tratados com etileno. **Revista Brasileira de Fruticultura**, Jaboticabal, v.25, n.1, p.45-48, 2003.

JIMENEZ-CUESTA, M.; CUQUERELLA-CAYUELA,J.; MARTINEZ-JAVEGA, J.M. **Teoria y practicca de la desverdización de los cítricos**. Madrid: INIA, 1983. 22p. (Hoja técnica, 46.)

JOHN-KARUPPIAH, K.J.; BURNS, J.K. Degreening behavior in 'Fallglo'and 'Lee× Orlando'is correlated with differential expression of ethylene signaling and biosynthesis genes. **Postharvest Biology and Technology**, Amsterdam, v.58, n.3, p.185-193, 2010.

JOMORI, M.L.L.; SASAKI, F.F.C.; BERNO, N.D.; GIMENES, L.C.; KLUGE, R.A. Desverdecimento e armazenamento refrigerado de tangor 'Murcott' em função de concentração e tempo de exposição ao etileno. **Semina**: Ciencia Agrárias, Londrina, v.35, n.2, p.825-834, 2014.

KADER, A.; ARPAIA, M. Postharvest handling systems: subtropical fruits. In: KADER, A.A. (Ed.). **Postharvest technology of horticultural crops**. 2. Oakland: University of California, 1992. p.233-240.

KE, D.; KADER, A.A. Tolerance of 'Valencia' oranges to controlled atmospheres, as determined by physiological responses and quality attributes. **Journal of the American Society for Horticultural Science**, Alexandria, v.115, n.5, p.779-783, 1990.

LICHTENTHALER, H.K. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. **Methods in Enzymology**, New York, v.148, n.22, p.350-382, 1987.

MAYUONI, L.; TIETEL, Z.; PATIL, B.S.; PORAT, R.Does ethylene degreening affect internal quality of citrus fruit? **Postharvest Biology and Technology**, Wageningen, v.62, n.1, p.50-58, 2011.

MAZZUZ, C.F. **Quality of citrus fruits**: manual for management from collection to the expedition. Madrid: Ediciones de Horticultura, 1996. 317p.

MENDONCA, K.; JACOMINO, A. P.; MELHEM, T. X.; KLUGE, R. A. Concentracao de etileno e tempo de exposicao para desverdecimento de limao 'Siciliano'. **Brazilian Journal of Food Technology**, Campinas, v.6, n.2, p.179-183, 2003.

NASCIMENTO, L.F.; MEDINA, V.M. Uso do ethephon no desverdecimento de laranjas 'Pera' e 'Baianinha'. **Revista Brasileira de Fruticultura**, Jaboticabal, v.16, n.3, p.127-140, 1994.

PLAZA, P.; SANBRUNO, A.; USALL, J.; LAMARCA, N.; TORRES, R.; PONS, J.; VIÑAS, I. Integration of curing treatments with degreening to control the main postharvest diseases of clementine mandarins**. Postharvest Biology and Technology**, Wageningen, v.34, n.1, p.29-37, 2004.

PURVIS, A.C.; BARMORE, C.R. Involvement of ethylene in chlorophyll degradation in peel of citrus-fruits. **Plant Physiology**, Rockville, v.68, n.4, p.854-856, 1981.

RODRIGO, M.J.; ZACARIAS, L. Effect of postharvest ethylene treatment on carotenoid accumulation and the expression of carotenoid biosynthetic genes in the flavedo of orange (Citrus sinensis L. Osbeck) fruit. **Postharvest Biology and Technology**, Wageningen, v.43, n.1, p.14-22, 2007.

SANTOS, L.O.; DURIGAN, J.F.; MARTINS, R.N.; DURIGAN, M.F.B.; STUCHI, E.D. Degreening of 'Satsuma Okitsu' Tangerine. **Acta Horticulturae**, The Hague, n.934, p.887-892, 2012.

SHIMOKAWA, K.; SHIMADA, S.; YAEO, K. Ethylene enhanced chlorophyllase activity during degreening of citrus unshiu marc. **Scientia Horticulturae**, Amsterdam, v.8, n.2, p.129-135, 1978.

SPÓSITO, M.B.; JULIANETTI, A.; BARBASSO, D.V.Determination of the minimum color index required for harvest of valencia sweet orange for degreening. **Laranja**, Cordeirópolis, v.27, n.2, p.373-379, 2006.

STEWART, I.; WHEATON, T.A. Carotenoids in citrus: Their accumulation induced by ethylene. **Journal of Agricultural and Food Chemistry**, Columbus, v.20, n.2, p.448-449, 1972.

TREBITSH, T.; GOLDSCHMIDT, E.E.; RIOV, J. Ethylene induces synthesis of chlorophyllase, a chlorophyll degrading enzyme, in citrus fruit peel. **Proceedings of the National Academy of Sciences of the United States of America**,Washington, v.90, n.20, p.9441-9445, 1993.

YAMAUCHI, N.; HASHINAGA, F.; ITOO, S. Chlorophyll degradation with degreening of kabosu (Citrus sphaerocarpa Hort. ex Tanaka) fruits. **Journal of the Japanese Society for Horticultural Science**, Tokyo, v.59, n.4, p.869-875, 1991.

ZHOU, J-Y.; SUN, C-D.; ZHANG, L-L.; DAI, X.; XU, C-J.; CHEN, K-S.Preferential accumulation of orange-colored carotenoids in Ponkan (Citrus reticulata) fruit peel following postharvest application of ethylene or ethephon. **Scientia Horticulturae**, Amsterdam,v.126, n.2, p.229-235, 2010.

ZONTA, E. P.; MACHADO, A. A. **SANEST**: sistema de análise estatística para microcomputadores. Pelotas: UFPel, 1995. 48 p.