

Degreening of 'Murcott' tangor with ethephon treatments

M.L.L. Jomori, I. Sestari, F.A.M. Terra, D.G. Chiou, R.A. Kluge
Department of Biological Science, University of Sao Paulo (ESALQ/USP), Piracicaba-
SP, Brazil

Keywords: postharvest, *Citrus reticulata* Blanco x *Citrus sinensis* Osbeck, ethylene, chlorophyll content, chlorophyllase activity.

Abstract

Brazilian citrus production is located in tropical regions. Due to its climate conditions fruit can reach full internal maturity while the peel is still fully or partially green. This phenomenon makes the fruit unacceptable to consumer. 'Murcott' tangor (*Citrus reticulata* Blanco x *Citrus sinensis* Osbeck) shows problems of green peel near the junction of the pedicel, which thus affects fruit quality. The objective of the present study is to investigate the effect of ethephon dip postharvest in degreening fruits of 'Murcott' tangor. Different concentrations of ethephon were used: 0, 500, 1000 and 2000 mg L⁻¹. After treatments by dipping in different solutions, fruit were stored at 15 °C or 25 °C at 90% RH. Fruit were analyzed for color changes on the equatorial region of the peel and near the pedicel, soluble solids content (SSC), titrable acidity (TA), SSC/TA ratio; juice content in % , ascorbic acid content, ethylene production, respiration rate, total chlorophyll content and the activity of the chlorophyllase. It was observed that at both temperatures 15 °C and 25 °C, there was an increase in color index, getting close to 2.0 that represents yellow color. Treated and untreated fruit with ethephon stored at 25 °C had a greater increase in the index values of color than fruit stored at 15 °C. It was observed that there was a reduction in the total chlorophyll content due to the increase of color index and activity of chlorophyllase. No differences were observed in the levels of SSC, TA and their ration. It was also observed that the production of ethylene and respiration rate were proportional to the concentration of applied ethephon and inversely decreases with lowering storage temperature from 25 to 15 °C. Therefore it was concluded that ethephon treatments may have a potential commercial use for degreening fruit of 'Murcott' tangor.

INTRODUCTION

The citrus has a great importance in brazilian industry as the country is among the major orange producer and the major exporter of concentrated juice. However the exportation of fresh orange represents only 0,5% of total production. This small percentage is due to low external quality of the fruits when compared to others from traditional exporter countries, like Spain and Morocco.

The development of color in citrus peel is greatly influenced by the temperature changes at fruit maturity (Ortolanni, 1991). The full expression of carotenoids and degradation of chlorophyll happens when maturation stage occurs in a period of hot days and cold nights. In Brazil, where citrus crops are concentrated at tropical climate areas, fruits can reach full internal maturity while the peel remains totally or partially green, making them unacceptable for fresh marketing. In subtropical and temperate climates, fruits present its characteristic color due to the exposure to low temperatures during ripening (Casas & Mallent, 1988).

Due to the fact that the large part of Brazilian citrus areas are located in areas of tropical climate conditions, it is necessary to develop means of obtaining colored fruit artificially postharvest. Degreening technique of citrus is the exposure of the fruits to ethylene or the application of ethylene generators such as ethephon treatment. Once executed in adequate conditions, these treatments accelerate the reduction of peel green color and promote the appearance of typical cultivars colors (Mazzuz, 1996). The objective of the present work was to study the effect of ethephon application on degreening of 'Murcott' tangor, in different concentrations and different temperatures.

MATERIALS AND METHODS

The experiment was carried out at Postharvest Physiology and Biochemistry Laboratory, located at Biological Sciences Department of Escola Superior de Agricultura "Luiz de Queiroz", University of Sao Paulo, USP/ESALQ, in Piracicaba, SP, Brazil. 'Murcott' tangors were harvested in a commercial orchard in the city of Engenheiro Coelho, SP, Brazil. Fruit were treated with different concentrations of ethephon (chloroethylphosphonic acid) ranging from 0, 500, 1000 and 2000 mg L⁻¹. The commercial product used is Ethrel® applications were made by dipping method, for 3 minutes at 20°C. After treatments, the fruit were stored at 15°C and 25°C, and at 90% RH for a period of 20 and 12 days, respectively.

The following parameters were analyzed and included peel color changes (using a colorimeter MINOLTA CHROMA METER model CR-300), determining L, a and b values, and calculating the color index (1000 x a / L x b) (Jimenez-Cuesta *et al.*, 1981); soluble solids content (SSC) by a refractometer ATAGO model PR-101; titratable acidity (TA) was measured mixing 10 ml of the juice with 90 ml H₂O and titrated with 0.1 N NaOH solution. The acidity was expressed as percentage of citric acid; ratio of SSC/TA; ascorbic acid content; juice content in %; chlorophyll content following the method described by Arnon (1949) and modified by Manfroï *et al.* (1996); chlorophyllase activity, as described by Stangarlin *et al.* (2000); respiration rate and ethylene production were analyzed with a gas chromatograph type Thermofinigan, model Trace 2000 GC equipped with flame ionization detector (FID) with column Porapak N, of 2m of length. The injector, column, and detector temperatures were 100, 100 and 250 °C, respectively, and with H₂ carrier gas flow of 0.40mLs⁻¹. The production rate of CO₂ was expressed in mg kg⁻¹ s⁻¹ and the production rate of C₂H₄ was expressed in µg kg⁻¹ s⁻¹.

A completely randomized design was used in this study, in which 10 fruits constituted a single replicate, and each treatment was repeated 4 times. The data were subjected to analysis of variance and the least significance differences were calculated using SANEST software. Differences between any two treatments greater than the sum of two standard deviations were always significant ($P > 0.05$).

RESULTS AND DISCUSSION

During the storage at 15°C and 25°C it was observed that color index (CI) of the peel and especially near the pedicel changes following the concentrations of applied ethephon and the temperature of storage (Fig 1).

The color measured near the pedicel, that initially was -5,42 raised to 1,60 after 20 days of storage at 15°C, on average. However, these changes notice in CI among treatments did not correspond to a visual difference in color, these changes were visually imperceptible (Fig. 1). Wheaton & Stewart (1973) reported that the response to

ethylene decreases in lower temperatures proportionally to the reduction of fruits respiration rates (Fig. 3).

On figure 2, it is possible to observe that decreases in chlorophyll content are associated with the activity of chlorophyllase enzyme. The explanation to this correlation relies on the fact that exogenous ethylene accelerates chlorophyll degradation at the thylakoids membranes through a complex mechanism that includes the action of chlorophyllase and oxidase enzymes (Shemer et al., 2008). Trebitsh et al. (1993) reported that the application of exogenous ethylene promotes peel degreening in citrus by the syntheses *de novo* of chlorophyllase enzyme.

For fruit storage at 25°C the observed behavior was similar from those fruits storage at 15°C. An increase in color index happened, depending on storage period, and on differences among ethephon concentrations (Fig. 1). In this higher temperature, in addition of degreening have been faster, it was visually possible to observe that fruit treated with ethephon had an improvement in its color. According to Korban (1998), ethylene in the form of ethephon is capable of stimulate chlorophyll degradation by chlorophyllase activity, as show on figures 1 and 2, respectively.

Best result was verified in the concentration of 500 mg L⁻¹. Oh et al. (1979), working with tangerines 'Satsuma', obtained similar results at ethephon concentrations of 500 and 1000 mg L⁻¹. Jahn (1973) observed that degreening induced by ethephon application depends on concentration, cultivar and handling temperature of fruit after treatment.

Ethylene production was analyzed until the last day of cold storage at 15°C and 25°C, as well as fruits respiration rate (Fig. 3).

It is clearly visible that fruits exposed to higher ethephon concentrations had a greater ethylene production, in relation to fruits not treated and fruits subjected to low ethephon concentrations. High ethylene production may also be related with the high respiration rate. This high respiration rate increases ATP production, necessary in the process of methionine transformation in SAM (S-adenosylmethionine), one of the precursor molecules of ethylene, therefore, increasing the vegetal hormone concentration (Abeles et al., 1997).

It is observe that, at the temperature of 25°C, the fruits presented higher ethylene production in comparison to fruits stored at 15°C. According to Abeles et al. (1997), the best temperature for ethylene production is situated between 28 and 30°C, at lower temperatures below this range, the enzyme activity is reduced.

In cold storage at 15 e 25°C, not treated fruits presented a respiratory rate stability while the fruits subjected to higher ethephon dosages had a respiratory rate increase.

It was verified small variations in soluble solids content, tritable acidity, ratio, juice percentage and ascorbic acid content in fruits from different ethephon concentrations and storage temperatures. This happens due to the fact that the orange is a non climacteric fruit, what justify the results, considering that the respiratory pattern of these kind of fruits implies small alterations on most fruits physic-chemistry characteristics (Brady, 1987). Similar results were also reported by Jacomino et al. (2003), which worked with 'Siciliano' lemons.

CONCLUSION

Ethephon application is efficient for postharvest degreening of 'Murcott' tangor in a concentration of 500 mg L⁻¹ at 25°C. Treatment with ethephon has great potential for commercial use.

ACKNOWLEDGEMENTS

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). Grant n°. 2006/05154-2.

Literature Cited

- ABELES, F.B.; MORGAN, P.W.; SALTVELT, M.E. 1997. Ethylene in plant biology. 2 ed. San Diego: Academic Press.
- ARNON, D.I. 1949. Cooper enzymes in isoled chloroplasts polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*. 24: 1-15.
- BRADY, C.J. 1987. Fruit ripening. *Annual Review of Plant Physiology*. 38:155-178.
- CASAS, A.; MALLET, D. 1988. El color de los frutos cítricos. I. Generalidades. II. Factores que influyen en el color. Influencia de la especie, de la variedad y de la temperatura *Revista de Agroquímica y Tecnología de Alimentos*. 28: 185-202.
- JACOMINO, A.P.; MENDONÇA, K.; KLUGE, R.A. 2003. Armazenamento refrigerado de limões 'Siciliano' tratados com etileno. *Revista Brasileira de Fruticultura*. 25: 45-48.
- JAHN, O. L. 1973. Degreening citrus fruit with postharvest applications of (2-chloroethyl) phosphonic acid (ethephon). *Journal of the American Society for Horticultural Science*. 98: 230-233.
- KORBAN, S.S. 1998. Influence of growth regulators on fruit plant growth and development. In: CONGRESSO BRASILEIRO DE FRUTICULTURA, Poços de Caldas. Conferências... Poços de Caldas: Sociedade Brasileira de Fruticultura, 1998. p. 56-81.
- MANFROI, V.; LUCCHESI, O.A.; CARVALHO, R.I.N.; FIOVARANÇO, J.C.; BENDER, R.J. 1996. Efeito do 2,4-D na frigoconservação de limão Tahiti (*Citrus latifolia* Tanaka). *B. CEPPA*. 14: 77-88.
- MAZZUZ, C.F. 1996. Calidad de frutos cítricos: manual para su gestion desde la recoleccion hasta la expedicion. Barcelona: Ediciones de Horticultura, S.L.
- OH, S.F.; KIM, Y.Y.; HONG, S.B.; CHUNG, S.K. 1979. Effect of postharvest application of ethephon, ethylene and methionine on colour and quality of Satsuma. *Journal of the Korean Society for Horticultural Science*. 20: 142-147.
- ORTOLANI, A.A.; PEDRO JUNIOR, M.J.; ALFONSI, R.R. 1991. Agroclimatologia e o cultivo dos citros. In: RODRIGUEZ, O.; VIÉGAS, F.; POMPEU JUNIOR, J.; AMARO, A.A. (Eds.) *Citricultura brasileira*. Campinas: Fundação Cargill, 1991. 1: 153-195.
- SHAMAILA, M.; POWRIE, W.D.; SKURA, B.J. 1992. Sensory evaluation of strawberry fruit stored under modified atmosphere packaging (MAP) by quantitative descriptive analysis. *Journal of Food Science*. 57: 1168-1172.
- SHEMER, T. A.; HARPAZ-SAAD, S.; BELAUSOV, E.; LOVAT, N.; KROKHIN, O.; SPICER, V.; STANDING, K.G.; GOLDSCHMIDT, E.E.; EYAL, Y. 2008. Citrus chlorophyllase dynamics at ethylene-induced fruit color-break: a study of chlorophyllase expression, posttranslational processing kinetics, and in situ intracellular localization. *Plant Physiology*. 148: 108-118.
- STANGARLIN JR AND PASCHOLATI SF. 2000. Atividades de ribulose 1,5 bifosfato carboxilase-oxigenase (rubisco), clorofilase, b-1,3 glucanase e quitinase e conteúdo de clorofila em cultivares de feijoeiro (*Phaseolus vulgaris*) infectados com *Uromyces appendiculatus*. *Summa Phytopathol*. 26: 34-42.

TREBITSH, T.; GOLDSCHMIDT, E.; RIOV, J. 1993. Ethylene induces *de novo* synthesis of chlorophyllase, a chlorophyll degrading enzyme, in *Citrus* fruit peel. *Plant Biology*. 90: 9441-9445.

WHEATON, T.A.; STEWART, I. 1973. Carotenoids in citrus: their accumulation induced by ethylene. *Journal of Agriculture and Food Chemistry*. 20: 448-449.

Figures

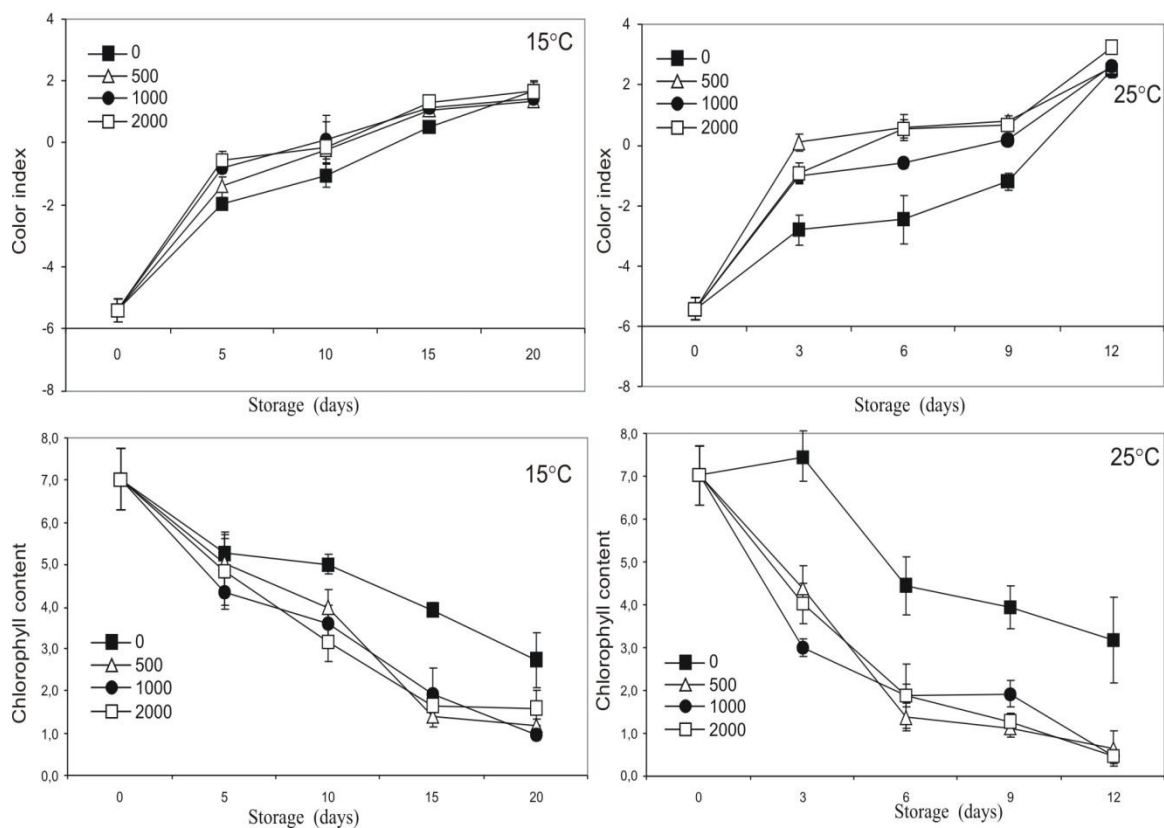


Fig. 1. Effect of ethephon (mg L^{-1}) on color index and chlorophyll content (mg g^{-1}) on regions of the stalk in the tangor 'Murcott' storage at 15°C and 25°C . The vertical bars represent \pm SD ($n=4$).

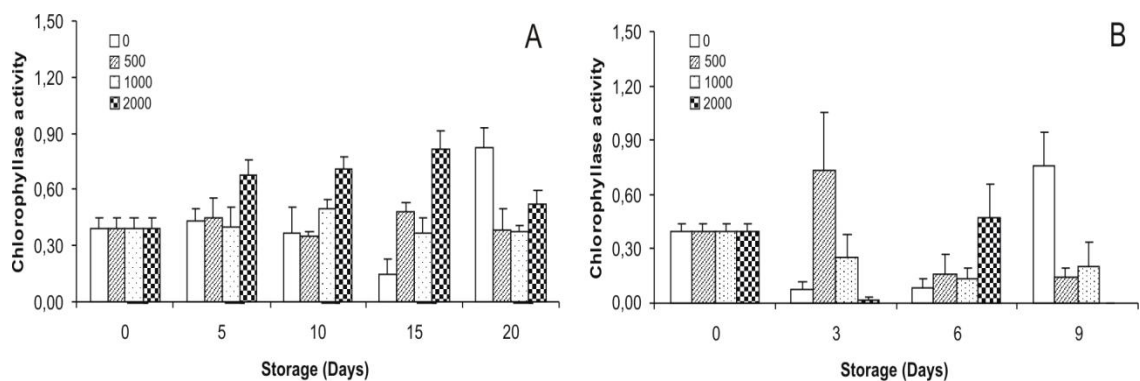


Fig. 2. Effect of ethephon (mg L^{-1}) on chlorophyllase activity on regions of the stalk in the tangor 'Murcott' storage at 15°C (A) and 25°C(B). The vertical bars represent \pm SD ($n=4$).

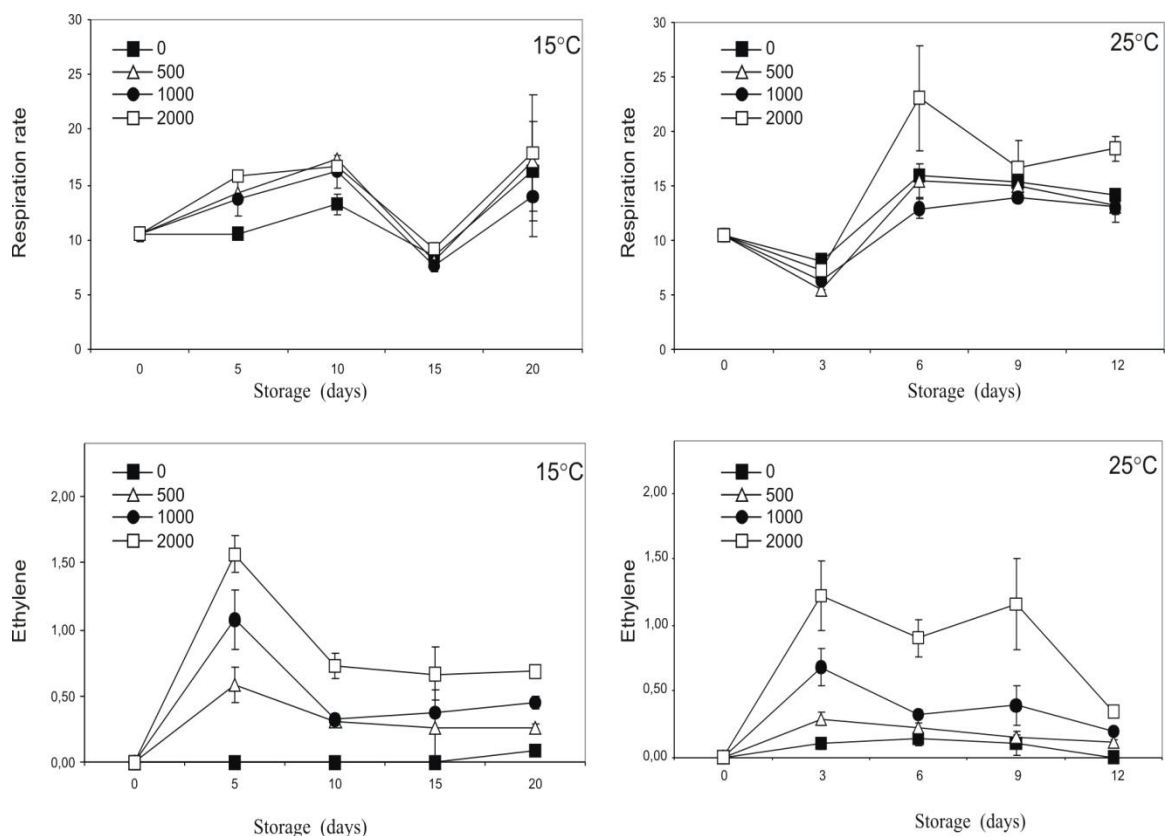


Fig. 3. Effect of ethephon (mg L^{-1}) on respiration rate ($\text{mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$) and ethylene production ($\mu\text{g C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$) in the tangor 'Murcott' storage at 15°C (A and C) and 25°C (B and D). The vertical bars represent \pm SD ($n=4$).