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EFFECT OF POSTHARVEST APPLICATION OF SUCROSE AND ASCORBIC ACID ON ETHYLENE-INDUCED COLORATION OF NAVEL ORANGES PEEL DURING DEGREENING

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ABSTRACT

The present work was carried out during two successive seasons (2004 and 2005) to study the effect of postharvest application of sucrose (250 ppm) or ascorbic acid (1000 ppm) on Navel oranges peel coloration, chlorophylls and carotene contents during the commercial degreening process and its effect on Navel orange fruit quality. Advancing the maturity stage and prolonging exogenous ethylene period significantly decreased chlorophyll *a* (Chl *a*), Chlorophyll b (Chl *b*), total chlorophyll (T Chl) contents and significantly increased carotene contents. Sucrose treatment significantly decreased Chl *a*, Chl *b* and increases carotene contents in the advanced maturity stage after two days exogenous ethylene period. Sucrose treatment showed the lowest color index values (the highest coloring). Ascorbic acid treatment showed the highest carotene content. The stepwise regression indicated that color index might depend on Chl *a* and carotene contents.

Keywords: citrus fruit; degreening; ethylene; sucrose; ascorbic acid; chlorophyll; carotene; fruit quality.

INTRODUCTION

The external appearance of the citrus fruits used by the consumer as an indication of citrus fruit quality, which does not have the correct color may not be accepted. Degreening is thus becoming an important commercial aspect in the preparation of citrus fruits for the consumer. Some early season varieties of citrus fruits reach minimum maturity standards before the skin have fully colored. Ethylene enhances the appearance of many fruits by stimulating their ripening. Degreening of citrus fruits with ethylene gas is a common commercial practice (Brown and Miller, 1999). Degreening will stimulate yellow and orange carotenoid production; accelerate chlorophyll degradation and unmasking other colors in flavedo. Postharvest ethylene treatment reproduces and accelerates the biochemical and gene expression changes naturally occurring during ripening. Ethylene application strongly enhances chlorophyll degradation and increase total carotenoid content in the flavedo of the fruit and accelerated fruit coloration (Maria & Lorenzo, 2007). Ethylene also promotes respiration, senescence, and increases susceptibility to decay (Brown & Lee, 1993 and Plaza *et al.*, 2004). During ripening, citrus fruit peel undergoes "color break" a process characterized by the conversion of chloroplast to chromoplast. The process involves the progressive loss of chlorophylls and the gain of carotenoids, changing peel color from green to orange. The rate of the color brake is correlated positively with sucrose content. In vivo sucrose supplementation promoted sucrose accumulation

and advanced color break. The chloroplast to chromoplast conversion in citrus fruit epicarps is simulated by sucrose accumulation (Shu, 1999). The sugar regulation appears to operate via ethylene (Iglesias *et al*, 2001). Changes in the activities of antioxidant enzymes and the content of nonenzymes during ripening indicated that the antioxidant system plays a fundamental role in the ripening of orange fruits (Huang *et al.*, 2007). Ascorbic acid content was positively correlated with antioxidant activity, while a negative linear correlation between the b* color value (color from yellow to red) and the total antioxidant activity in the peel (Drogoudi *et al.*, 2007).

The object of this study was to gather information that could assist the producer to operate and manage his degreening facilities in such way that he can produce a product of high quality. This study investigates the effect of postharvest application of sucrose on the balance of chlorophylls degradation and carotene synthesis during commercial degreening by ethylene. Because ethylene-induced changes may dependent on the maturity stage, the effect of ethylene on the chlorophyll and carotene content was studied in the fruits peel in the two maturity stages (two color degrees). The study is supplemented by an effect of the antioxidant action of ascorbic acid on the ethylene-induced changes.

MATERIALS AND METHODS

Navel orange fruits (Citrus Sinensis) were selected from citrus packinghouse during two successive seasons 2004 and 2005, respectively. The fruits were sorted into two color categories by visually selecting fruit according to color categories. The first color degree (first maturity stage) was light green (degree= 6) and the second color degree (second maturity stage) was yellowish green (degree= 5). The fruits were treated with the standard packinghouse treatments including a drench with 750 ppm imazalil + 2000 ppm thiobendazol (TBZ) at ambient temperature (22 ^{0}C). The fruits were completely air dried over night. The fruits of two color degrees, free of visual defect, were selected, dipped for 10 min in water (control) or 250 ppm sucrose or 1000 ppm ascorbic acid. The treated fruits were completely air dried and stored in degreening room, where treated with 5 ppm ethylene at 27±1 ^{0}C and 90-95 % RH. Fresh air was entered the room at the rate of one air change per sex hour, based on the volume of the empty room. Half of the treated fruits were stored out after 2 days and the second half was stored out after 4 days. Each treatment was represented by three replications with 10 fruits each. The stored out fruits were represented for the visual assessment and chemical analysis. The peel color index for the stored out fruits was visually evaluated according to color chart. These color categories are based on a series of photographs depicting various stages of rind color development for Navel orange fruit, and given a score 8, 7, 6, 5, 4, 3, 2, and 1 which corresponds to a rating of dark green, green, light green, yellowish green, light yellow, orange yellow and orange, respectively. The outer colored (flavedo) portion of the rind was removed with a sharp knife, ensuring that the white (albedo) portion of the rind was not included in the sample. Rind

samples were collected from five fruits and combined to form one rind sample per treatment for each replicate. Chlorophyll a, chlorophyll b, total chlorophyll and carotene contents were determined (mg/g fresh weight) (mg/g FW) in flavedo samples according to Sadasivam & Manickam (1992). The juice sample of five fruits per treatment of each replicate were analyses to determine the following: soluble solids content (SSC) (%), titratable acidity (% of citric acid) and ascorbic acid content (mg/100 ml juice) by the official methods (A.O.A.C, 1990). Analysis of variance (ANOVA) was performed by the Irristatc statistical program according to three factors, randomized complete block design (Byrkit, 1987). Data calculated as percentage was transformed to arcsine. Means comparisons were calculated, where applicable; by Duncan multiple rang test (DMRT, *P* 0.05).

RESULTS AND DISCUSSION

Data in Table (1) show that, chlorophyll a (Chl *a*) content was significantly decreased by prolonging exogenous ethylene period, in both the maturity stages. Aiso, sucrose treatment significantly decreased Chl *a* content (0.10 & 0.10 mg/g FW), whereas ascorbic acid treatment maintained Chl *a* content (0.12 & 0.13 mg/g FW) compared to the control treatment (0.13 & 0.14 mg/g FW) in the two seasons, respectively. Sucrose treatment had a pronounced effect in decreasing Chl *a* content, in the second maturity stage (M_2) (0.10 & 0.12 mg /g FW) and the first maturity stage fruits (M_1) (0.13 & 0.14 mg /g FW), after two days of exogenous ethylene exposure in the two seasons, respectively. While, after four days of exogenous ethylene exposure, Chl *a* content was not significantly affected by both sucrose and ascorbic acid treatments in the two seasons. Noaki *et al.*, (1997) indicated that Chl *a* content was degraded enzymatically by chlorophyllase, Mg-dechelatase and decarbomethoxylase.

Chlorophyll b content (Chl *b*), as shown in Table (1), was significantly decreased by advancing the maturity stage and prolonging exogenous ethylene period in the two seasons. Sucrose treatment significantly decreased Chl *b* content in the second maturity fruits after two days exogenous ethylene period (0.16 0 & 0.14 mg/g FW) as compared with the control treatment (0.20 & 0.22 mg/g FW) in the two seasons, respectively. Ascorbic acid treatment maintained Chl *b* content during two days exogenous ethylene period in the two maturity stages, whereas after four days of exogenous ethylene period there was no significant difference among sucrose, ascorbic acid and control treatments in the two maturity stages.

Data in Table (1) show that, total chlorophyll (T Chl) was significantly decreased as maturity stage advanced and prolonging the exogenous ethylene period. Sucrose had the superiority effect on decreasing T Chl content, in the two maturity stages, after two days ethylene exogenous ethylene period. Whereas, after four days ethylene exogenous ethylene period, there were no significant differences among sucrose, ascorbic acid and control treatments on T Chl content, through the two maturity stages, in both seasons. The previous studies showed that chlorophyll content was

declined in response to different kinds of sugars, epically sucrose (Shu, 1999). Also, it was concluded that the activities of chlorophyllase, Mg-dechelatase, and peroxidase-linked chlorophyll bleaching could be regulated by external application of ethylene. Chlorophyll content in flavido decreased and chlorophyllase activity increased. A significant chlorophyllase protein was detected in ethylene-treated fruit indicating that ethylene induces de novo synthesis of chlorophyllase (Jacob *et al.*, 1999)

Carotene content (Table, 1) was increased as the maturity stage advanced and elongation of the ethylene exposure period, in the two seasons. Carotene content was significantly higher in both sucrose (4.82 & 5.44 mg/g FW) and ascorbic acid (5.86 & 5.36 mg/g FW) treatments compared to the control treatment (3.82 & 3.88 mg/g FW) in the two seasons, respectively. The present data showed highly negative correlation between carotene content and both Chl *a* ($r = -0.61$ $P = 0.000$) and T Ch ($r = -0.67$ $P = 0.000$) more than Ch *b* content ($r = -0.39$ $P = 0.000$). These results are in accordance with previous data showing that citrus maturation-related genes are also induced by ethylene, which suggests the involvement of the hormone in the molecular regulation of the ripening process (Alonso *et al.*, 1995 and Goldshmidt, 1998). It has been previously suggested that ethylene may be involved in the regulation of carotenoid biosynthetic genes during fruit maturation despite of the non-climacteric behavior of these fruit (Alonso *et al.*, 1995). The pattern of expression of carotenoid biosynthetic genes in response to ethylene in the flavedo of orange is similar to that observed during natural ripening. Maria & Lorenzo (2007) revealed that the transcriptional activation of genes is the early steps of the carotenoid biosynthesis appears to be the common response to ethylene which will increase the production of precursors and the flux into the pathway, independently of the climacteric or non-climacteric behavior of the fruit.

Color index, as shown in Table (1), was significantly decreased (the color was improved) as the maturity stage advanced and prolonging the exogenous ethylene period, in the two seasons. Sucrose treatment showed the lowest color index values (the highest coloring) (3.31 & 3.12) in the two seasons, respectively. Also, the lowest color index was recorded after four days exogenous ethylene period for the second maturity stage in the presence of sucrose treatment (3.00 & 2.08) followed by the control treatment (3.11 & 3.44) and ascorbic acid treatment (3.22 & 3.11). Fruit peel coloration was improved by increasing exogenous ethylene period and was correlated to Chl *a* degradation and carotene synthesis. The extra period required to complete the degreening process in fruit with an initial color closer to commercial color could be a deterrent to the adoption of this procedure by the citrus industry. The present data showed that the color index might depend on Chl *a* content ($r = 0.79$ $P = 0.000$) more than T Ch ($r = 0.77$ $P = 0.000$), Chl *b* content ($r = 0.47$ $P = 0.000$) and carotene content ($r = -0.45$ $P = 0.000$). The stepwise regression indicated that color index might depend on Chl *a* (St Coeff=0.80 $P=0.000$), Chl *b* (St Coeff =0.29 $P=0.000$) and carotene contents (St Coeff =0.01 $P = 0.005$). As was expected, the degreening treatment was more effective as the maturity stage advanced and prolonging exogenous ethylene period. Color changes during degreenig involve both destruction of

chlorophyll, revealing carotenoids already present, and development of carotenoid pigments to produce the orange color. Carotenoid synthesis would be impaired whereas chlorophyll degradation would slow down. Therefore, color changes on early maturity stage fruit (M_1), which contain more chlorophyll, would be primarily due to chlorophyll degradation, unmasking orange carotenoids already present. On late maturity stage (M_2), fruit was more colored and had lower Chl *a* and higher carotene contents. Maria & Lorenzo (2007) revealed that prolonged exogenous ethylene period, fruit at a more advanced stage of ripening accumulated more phytoene, phytofluene and the typical chromoplastic flavedo pigments (9z)-violaxanth and ß-citraurin.

Table (1): Effect of postharvest application of sucrose, ascorbic acid and exogenous ethylene period on peel coloration, chlorophyll a, chlorophyll b, total chlorophyll and carotene contents during degreening process for two maturity stages of Navel Oranges

	Color index (8-1)		Chlorophyll *a* (mg/g FW)		Chlorophyll *b* (mg/g FW)		Total chlorophyll (mg/g FW)		Carotene (mg/g FW)	
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
Before treatment										
Maturity (M)										
M_1	5.33	5.89	0.32	0.29	0.11	0.14	0.43	0.43	3.09	2.73
M_2	4.55	4.67	0.27	0.29	0.12	0.15	0.39	0.44	4.52	3.54
F test	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
After treatment										
Maturity (M)										
M_1	3.65	3.81	0.12	0.15	0.13	0.12	0.25	0.26	4.38	4.83
M_2	3.22	3.01	0.11	0.15	0.10	0.10	0.21	0.23	4.62	4.96
F test	**	**	NS	NS	**	**	**	**	**	NS
Ethylene (P)										
2 days	3.67	3.83	0.15	0.20	0.18	0.16	0.32	0.36	3.37	4.00
4 days	3.20	3.00	0.09	0.09	0.05	0.06	0.14	0.14	5.64	5.78
F test	**	**	**	**	**	**	**	**	**	**
Treatments (T)										
Control	3.42 b	3.32 b	0.13 a	0.17 a	0.12 a	0.10 a	0.25 a	0.26 a	3.82 b	3.88 b
Sucrose	3.17 c	3.25 b	0.10 b	0.14 b	0.11 a	0.11 a	0.21 b	0.25 ab	4.83 b	5.44 a
Ascorbic acid	3.72 a	3.67 a	0.12 a	0.14 b	0.12 a	0.11 a	0.24 a	0.24 a	4.86 a	5.36 a
T x M	NS	NS	NS	**	NS	**	**	NS	NS	**
T x P	NS	NS	NS	NS	NS	**	NS	**	**	**
M x P	**	NS	NS	**	NS	**	NS	NS	**	NS
T x M x P	NS	**	NS	**	NS	*	NS	NS	**	NS

M: maturity stage, M_1 = 6, M_2= 5; P = 250 ppm ethylene; T= treatments: control = 0, sucrose= 250 ppm, ascorbic acid = 1000 ppm. In the same cell of the treatment, means followed by the same letter are not significantly different at the level of 5% according to DMRT.

Data in Table (2) reveal that the fruit quality parameters were improved as the maturity stage advanced. The fruits became juicier (the juice percentage was increased); SSC and ascorbic acid were increased. The titratable acidity and SSC: acid ratio showed different trends in both seasons

depending on the titratable acidity content. Also, prolonging exogenous ethylene period improved the fruits quality. After four days, the fruits became juicier and had higher contents of SSC and titratable acidity compared to two days exogenous ethylene period. Ascorbic acid treatment showed non significant differences in the fruit quality parameters compared to the control treatment.

Table (2): Effect of postharvest application of sucrose, ascorbic acid and exogenous ethylene period on some fruit quality parameters during degreening process for two maturity stages of Navel Oranges

	Juice volume (%)		SSC (%)		Titratable acidity (%)		SSC : acid ratio		Ascorbic acid (mg/100ml juice)	
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
Before treatment										
Maturity (M)										
M_1	47.83	50.03	11.00	11.20	1.14	1.31	9.66	8.55	49.93	51.36
M_2	47.82	47.78	11.20	11.33	1.09	1.21	10.31	9.36	49.04	48.15
F test	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
After treatment										
Maturity (M)										
M_1	39.04	38.49	11.25	11.33	1.14	1.13	6.87	10.03	45.93	45.23
M_2	41.94	41.29	11.51	11.58	1.03	1.10	11.17	10.53	46.49	48.22
F test	*	*	*	NS	**	NS	**	*	NS	**
Ethylene (P)										
2 days	39.11	39.49	11.13	11.29	1.17	1.12	9.51	10.08	45.27	46.93
4 days	41.87	40.28	11.64	11.62	1.03	1.11	11.30	10.47	47.154	46.52
F test	*	NS	**	*	**	NS	**	NS	NS	NS
Treatments (T)										
Control	40.82 a	40.46 a	11.421a	11.36 a	1.16 a	1.10 a	9.84 b	10.33 a	46.49 a	46.44 ab
Sucrose	40.76 a	38.49 a	11.28 a	11.42 a	1.05 a	1.10 a	10.74 a	10.38 a	44.42 a	45.93 b
Ascorbic acid	39.89 a	40.72 a	11.45 a	11.58 a	1.09 a	1.17 a	10.50 a	10.07 a	47.71 a	48.11 a
T x M	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T x P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
M x P	NS	NS	NS	NS	**	NS	*	NS	*	**
T x M x P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

M: maturity stage, M_1 = 6, M_2= 5; P = 250 ppm ethylene; T= treatments: control = 0, sucrose= 250 ppm, ascorbic acid = 1000 ppm. In the same cell of the treatment, means followed by the same letter are not significantly different at the level of 5% according to DMRT.

In conclusion, in the present work the effect of postharvest application of sucrose and ascorbic acid on Navel Oranges peel color, the chlorophyll degradation and carotene synthesis, sucrose treatment improved fruit peel coloration depending, mainly, on Chl *a* degradation and carotene synthesis. Ascorbic acid treatment reduced the fruit peel coloration as a result of maintaining of T Ch content. In general, fruit peel color might, mainly depend on chlorophyll degradation more than carotene synthesis.

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تأثير اضافة السكروز والأسكوربيك أسيد بعد الجمع على التلـــوين النـــاتج عـــن المعاملة بالإثيلين لثمار البرتقال بسرة أثناء عملية التلوين

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اجريت هذه الدراسة خلال موسمين متتاليين (٢٠٠٤ & ٢٠٠٥) وذلك لدراسة تأثير معاملـــة ثمار البرتقال بسرة بكل من محلول السكروز (٢٥٠ جزء فـــي المليـــون) أو محلـــول حـــامض الأسكوربيك (١٠٠٠ جزء في المليون) بعد الجمع وذلك على تلوين قشرة الثمار ومحتواهـــا مـــن الكلوروفيل والكاروتين وكذلك تأثيرهاعلىجودة الثمار وذلك اثناء عملية التلوين التجارية بواسطة غاز الإثيلين (٥ جزء في المليون) . وقد أوضحت النتائج أن هناك انخفاض معنوي في محتـــوى قشرة الثمار من الكلوروفيل (أ) والكلوروفيل (ب) والكلوروفيل الكلي بينمـــا حـــدثت زيـــادة معنوية في المحتوى من الكاروتين وذلك بتقدم مرحلة النضج و بإطالة فترة تعرض ثمار البرتقـــال بسرة للإثيلين. وأن المعاملة بالسكروز اظهرت أعلى تلوين للثمار. كما أظهرت النتائج أن تلـــون القشرة قد يعتمد على تكسير محتواها من الكلوروفيل أكثر من بناء الكاروتين.