Preharvest Prevention of Regreening in Valencia Oranges [*Citrus sinensis* (L.) Osbeck]

Wan-Jean Hsu,* Charles DeBenedict, Steve D. Lee, Stephen M. Poling, and Henry Yokoyama

Preharvest treatment with bioregulators [(*N*,*N*-diethylamino)ethoxy]benzophenone (1), *N*,*N*-diethyloctylamine (2), and (*N*,*N*-diethylamino)ethyl *p*-bromobenzoate (3) reduced chlorophyll biosynthesis in the flavedo of Valencia oranges [*Citrus sinensis* (L.) Osbeck]. Bioregulator treatment not only reduced regreening but also increased total xanthophyll content and gave the fruit better coloration.

The reappearance of chlorophyll in mature oranges [*Citrus* sinensis (L.) Osbeck] is known as regreening (Thomson et al., 1967). Regreening of mature Valencia oranges is a problem in southern California and Flordia. Two possible approaches to the problem have been considered. One is to degreen the regreened fruit, that is, to destroy the chlorophyll present in the flavedo; the other is to prevent the fruit from undergoing the regreening process, that is, to inhibit chlorophyll biosynthesis. Regreened fruit does not respond well to the regular ethylene degreening treatment, although it may be degreened by prolonged exposure to ethylene in a warm and humid atmosphere. This procedure, however, hastens rind senescence and thus increases storage problems (Biale, 1961). 2,4-Dichloro-1-cyanoethanesulfonanilide (R33417) was found to be effective in postharvest degreening of regreened Valencia oranges, but only when intense light was present. Preharvest treatment with this compound caused leaf abscission (Coggins and Hall, 1975). In view of the impact of regreening on the marketability of fresh fruit, alternative methods need to be developed to overcome the problem. Carotenogenic bioregulator 2-[(4-chlorophenyl)thio]triethylamine (CPTA) was found to inhibit chlorophyll biosynthesis in greening detached pumpkin cotyledons (Simpson et al., 1974). This finding provided promising leads to preventing the regreening process itself. Therefore, 11 carotenogenic bioregulators that appeared to significantly interfere with chlorophyll formation were selected for field tests on Valencia oranges. We now report the results obtained with three of the most promising: *N*,*N*-[(diethylamino)ethoxy]benzophenone (1), *N*,*N*-diethyloctylamine (2), and *N*,*N*-(diethylamino)ethyl *p*-bromobenzoate (3).

MATERIALS AND METHODS

Preharvest Treatment. 1 and 3 were used as their HCl salt forms, and 2 was used as free amine form. Ortho X-77 was used as wetting agent in a concentration of 0.025% in all the bioregulator solutions. Fruits on the trees were spray washed with 0.025% Ortho X-77 solution before the treatment. When the fruit surface was dry, 20–30 fruits on a branch were sprayed with bioregulator and kept wet for 2 min. An equal number of fruit on adjacent branches were left untreated and used as controls.

Chemicals. [(*N*,*N*-Diethylamino)ethoxy]benzophenone (1) was synthesized from (*N*,*N*-diethylamino)ethyl chloride and *p*-hydroxybenzophenone (Schuetz and Baldwin, 1958); *N*,*N*-diethyloctylamine (2) from diethylamine and octanyl bromide (Poling et al., 1975); and (*N*,*N*-diethylamino)ethyl *p*-bromobenzoate (3) from *N*,*N*-diethylethanolamine and *p*-bromobenzoyl chloride (Poling et al., 1976).

Fruit and Vegetable Chemistry Laboratory, U.S. Department of Agriculture—Agricultural Research Service, Pasadena, California 91106.

Table I. Effect of 500 ppm [(*N*,*N*-Diethylamino)ethoxy]benzophenone (1) on Pigments of Valencia Orange Flavedo[a]

pigment	control	treated
carotenes		
phytoene	9.8	66.5
phytofluene	7.5	10.3
ζ-carotene	2.1	3.1
neurosporene		
lycopene		
α-carotene	9.5	7.2
β-carotene	9.4	9.2
γ-carotene		
β-zeacarotene		0.9
total xanthophyll	848.1	1353.8
total chlorophyll	53.6	7.5

[a] Numbers are expressed as micrograms per gram dry weight of flavedo.

Sampling Method. At the end of each experiment, all the flavedo tissues of 20–30 fruits in each treatment were collected, ground through a meat grinder, and thoroughly mixed. Random samples of the flavedo tissue were then taken for pigment determination.

Extraction of Lipid and Preparation of Unsaponifiable Matter. Flavedo tissues of Valencia oranges were extracted, saponified, and separated into carotene and xanthophyll fractions by standard procedures (Davies, 1965).

Separation and Identification of Pigments. The carotene fraction was chromatographed on a MgO–Hyflo Supercel (1:1, w/w) column, and the various fractions were eluted with light petroleum ether (30–60 °C) containing increasing amounts of acetone. The pigments were identified by their UV and visible spectra and adsorption characteristics relative to known compound (Hsu et al., 1972). The xanthophyll fraction was treated as total xanthophyll; no further separation was carried out.

Quantitative Determination. Carotenoids were quantitated according to published method (Davies, 1965). An extinction coefficient of $E^{1\%}_{1cm} = 2500$ was arbitrarily used for calculating the total xanthophyll content. The total chlorophyll content was calculated by use of a published equation (Comar and Zscheile, 1942).

RESULTS AND DISCUSSION

The field tests were carried out at the Citrus Research Center and Agricultural Experiment Station, Riverside, CA, at the end of May before regreening took place. Concentrations of 200, 500, and 1000 ppm were tested for each bioregulator. At the end of each test, fruits were harvested and visually examined for degree of regreening. In the test with 1, fruits were harvested 1 month after treatment. At harvest, half of the flavedo surface of the

Table II. Effect of 1000 ppm *N,N*-Diethyloctylamine (2) and (*N,N*-Diethylamino)ethyl *p*-Bromobenzoate (3) on Pigments of Valencia Orange Flavedo[a]

pigment	control	2 treated	3 treated
carotenes			
phytoene	7.6	13.2	15.5
phytofluene	5.4	8.8	11.9
ζ-carotene		1.4	1.4
neurosporene			
lycopene			
α-carotene	4.4	7.2	7.7
β-carotene	14.8	8.9	12.0
γ-carotene			
β-zeacarotene		1.2	1.3
total xanthophyll	485.9	968.4	1277.0
total chlorophyll	79.1	28.3	27.7

[a] Numbers are expressed as micrograms per gram dry weight of flavedo.

control fruits and of the fruits treated with 200 ppm 1 was green. Fruits treated with 500 ppm 1 were slightly green around the stem end and had a better coloration than the control fruits. Fruits treated with 1000 ppm 1 appeared less green than the control fruits; however, there were red spots on the flavedo. Visually, the best concentration of 1 for reducing the regreening process was 500 ppm. The control fruits and fruits treated with 500 ppm 1 differed only slightly in levels of all the flavedo carotenes except phytoene and phytofluene (Table I). On the other hand, the treated and untreated fruits differed substantially in total xanthophyll and total chlorophyll contents of the flavedo. Fruits treated with 500 ppm 1 contained only one-seventh as much total chlorophyll and more than 1.5 times as much total xanthophylls as control fruits.

Fruits in the tests with **2** and **3** were harvested 2 months after treatment. At harvest, at least 75% of the control fruit flavedo was deep green; treated fruits were less green and only half of the surface was green. The orange coloration on the control fruit was much paler than that on the control fruit picked 1 month earlier in the test with 1. During the 1-month interval between harvests of the control fruits in tests with 1 and **2** or **3**, the regreening process progressed. It was accompanied by an increase in total chlorophyll content (53.6–79.1 μg/g dry weight of flavedo) and a decrease in total xanthophyll content (848–486 μg/g dry weight of flavedo) (Tables I and II). The best concentration for both **2** and **3** was 1000 ppm. Table II showed little difference in flavedo carotene content between no treatment and treatment with 1000 ppm **2** or **3**, and it showed that xanthophyll content was much higher and chlorophyll content much lower in the treated than in the untreated fruits.

Field tests with bioregulator 1 were conducted again the following year. Fruits were treated with 200, 500, and 1000 ppm 1 at the beginning of June and retreated 5 weeks later. Fruit samples were taken at different intervals. During the 12-week observation period, no change in total carotene content was observed; however, total xanthophyll content decreased and total chlorophyll content doubled in the control fruit flavedo (Table III). In treated flavedo, 1 progressively enhanced the orange coloration for 8 weeks. During that time, total xanthophyll content increased, whereas total chlorophyll content remained low. Between 5 and 8 weeks after first treatment (3 weeks after second treatment), control fruit aquired additional 4.7 μg of chlorophyll while treated ones only increased 2.7 μg of chlorophyll/g dry weight of flavedo tissue. There was a 44% reduction in chlorophyll accumulation. However, if the fruit was allowed to stay on the tree for 4 weeks further

Table III. Effect of 500 ppm [(*N,N*-Diethylamino)ethoxy]benzophenone (1) on Pigments of Valencia Orange Flavedo

no. weeks after treatment: first (second)	total carotene		total xanthophyll		total chlorophyll	
	control	treated	control	treated	control	treated
5	13.9	20.9	1174	1390	14.6	7.6
8 (3)	14.2	18.7	1014	1437	19.3	10.3
12 (7)	13.8	25.3	980	1240	28.2	25.9

[a] Numbers are expressed as micrograms per gram dry weight of flavedo.

(7 weeks after second treatment) without treatment, the chlorophyll content in treated fruit increased to a level almost equal to that of the control fruit. The results of 5 weeks after first treatment, 3 weeks after second treatment, and 7 weeks after second treatment indicated that the duration of action of the bioregulator 1 is about 1 month. Thus, monthly treatment during the summer months may be necessary to reduce regreening. These results showed that [(*N,N*-diethylamino)ethoxy]benzophenone (1) applied before harvest was consistently effective in reducing regreening. During the entire experimental periods with 1–3, the leaves and new crop of small green fruits surrounding the treated fruits appeared to be healthy. No leaves or young fruit abscised. Results of the field tests indicated that these three bioregulators may be useful as preharvest sprays to prevent regreening in Valencia oranges.

Bioregulators 1–3 were shown to profoundly affect carotenogenesis in Marsh white grapefruit (Hsu et al., 1975; poling et al., 1975, 1976). The effects of these bioregulators were found to be similar to that of 2-[(4-chlorophenyl)-thio]triethylamine. It was proposed that these bioregulators act as derepressors of a gene regulating the synthesis of a specific enzyme(s) in the primary carotenoid pathway and also they act as inhibitors of the cyclase(s) and lead to the accumulation of the acyclic carotene lycopene (Hsu et al., 1972). At lower concentrations, the stimulatory effect on carotenogenesis was more evident than the inhibitory effect on the cyclase(s). In our field tests, the concentrations of bioregulators were much lower (200–1000 ppm) than those previously used (5000, 30 000, and 37 000 ppm) in the studies on carotenogenesis in citrus (Hsu et al., 1975; Poling et al., 1975, 1976). When applied at 500 and 1000 ppm, 1–3 did not induce lycopene accumulation but did increase the levels of phytoene, phytofluene, and total xanthophylls (Tables I and II). These results agree with those observed previously (Hsu et al., 1972, 1974). Ultrastructural evidence indicates that regreening of Valencia orange fruit epicarp involves the reversion of chromoplast to chloroplast (Thomson et al., 1967). The plastid interconversion was found to be regulated by the concentrations of sugar and nitrogen in the epicarp of Valencia orange (Huff, 1983, 1984). High sugar concentrations favored chromoplasts over chloroplasts especially when the nitrogen status of the epicarp was low. Nitrogen, in a variety of forms, promoted the chloroplasts over chromoplasts. Whether bioregulators 1–3 affected the regreening process by influencing the relative sugar and nitrogen state of the epicarp or direct involving in the chlorophyll biosynthetic pathway needs further study.

ACKNOWLEDGMENT

This work was supported in part by the California Citrus Advisory Board and the Florida Citrus Commission.

LITERATURE CITED

Biale, J. B. Postharvest physiology and chemistry. In *The Orange: Its Biochemistry and Physiology*; Sinclair, N. B., Ed.; University

of California Division of Agricultural Science: Berkley, CA, 1961.
Coggins, C. W.; Hall, A. E. Effect of light, temperature, and 2′,4′-dichloro-1-cyanoethanesulfonanilide on regreening of regreened Valencia oranges. *J. Am. Soc. Hortic. Sci.* **1975**, *100*, 484–487.
Comar, C. L.; Zscheile, F. P. Analysis of plant extracts for chlorophylls a and b by a photoelectric spectrophotometric method. *Plant Physiol.* **1942**, *17*, 198–209.
Davies, B. H. Analysis of carotenoid pigments. In *Chemistry and Biochemistry of Plant Pigments*; Goodwin, T. W., Ed.; Academic Press: London, New York, 1965.
Hsu, W.-J.; Yokoyama, H.; Coggins, C. W. Carotenoid biosynthesis in *Blakeslea trispora*. *Phytochemistry* **1972**, *11*, 2985–2990.
Hsu, W.-J.; Poling, S. M.; Yokoyama, H. Effect of amines on the carotenogenesis in *Blakeslea trispora*. *Phytochemistry* **1974**, *13*, 415–419.
Hsu, W.-J.; Poling, S. M.; DeBenedict, C.; Rudash, C.; Yokoyama, H. Chemical inducers of carotenogenesis. *J. Agric. Food Chem.* **1975**, *23*, 831–834.
Huff, A. Nutritional control of regreening and degreening in citrus peel segments. *Plant Physiol.* **1983**, *73*, 243–249.
Huff, A. Sugar regulation of plastid interconversions in epicarp of citrus. *Plant Physiol.* **1984**, *76*, 307–316.
Poling, S. M.; Hsu, W.-J.; Yokoyama, H. *Structure*-activity relationships of chemical inducers of carotenoid biosynthesis. *Phytochemistry* **1975**, *11*, 1933–1938.
Poling, S. M.; Hsu, W.-J.; Yokoyama, H. Synthetic regulators of carotenoid biosynthesis in *Citrus paradisi*. *Phytochemistry* **1976**, *15*, 1685–1687.
Schuetz, R. D.; Baldwin, R. A. The synthesis and properties of some substituted phenyl-w-(N,N-dialkylamine)-alkyl sulfides. *J. Am. Chem. Soc.* **1958**, *80*, 162–164.
Simpson, D. J.; Chichester, C. O.; Lee, T. H. Chemical regulation of plastid development. I. Inhibition of chlorophyll biosynthesis in detached pumpkin cotyledons by CPTA. A pigment and ultrastructural study. *Aust. Plant Physiol.* **1974**, *1*, 119–133.
Thomson, W. W.; Lewis, L. N.; Coggins, C. W. The reversion of chromoplasts to chloroplasts in Valencia Orange. *Cytologia* **1967**, *32*, 117–124.

Received for review September 14, 1987. Revised manuscript received May 9, 1988. Accepted July 18, 1988. Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

Reduction of Bitter Components in Grapefruit and Navel Orange Juices with β-Cyclodextrin Polymers or XAD Resins in a Fluidized Bed Process

Charles W. Wilson, III, Charles J. Wagner, Jr., and Philip E. Shaw*

β-Cyclodextrin polymer and XAD-4 and XAD-16 resins were used in a pilot-scale fluidized bed process to reduce bitterness from naringin and limonin in grapefruit juice and limonin in California navel orange juice. For β-cyclodextrin polymers cross-linked with epichlorohydrin, naringin reduction in grapefruit juice ranged from 18 to 61% and limonin reduction ranged from 28 to 67%. Limonin reduction in navel orange juice ranged from 29 to 55%. The polymer was regenerated 30 times with 2% sodium hydroxide at ambient temperature without loss in debittering capacity. Bitterness reduction in juices processed at 5 °C was similar to that found at ambient temperature. Bitterness reduction in grapefruit juice from XAD-16 resin was 55–58% for naringin and 90–97% for limonin; with navel orange juice limonin was reduced 93%. For grapefruit juice debittered with XAD-4 naringin reduction was 32–38%, and for both grapefruit and navel orange juice limonin reduction was 58%. The ability of the XAD resins to withstand repeated regeneration with 2% sodium hydroxide was not determined.

Excessive bitterness in some processed citrus juices adversely affects the flavor and marketability of products made from these juices. The bitter juices are often stored for later blending with less bitter juices. Thus, a larger portion of processed juice products is affected by these bitter juices (Shaw and Buslig, 1986). Bitterness in processed citrus juices occurs mainly in early-season grapefruit juice from Florida (processed from fruit harvested between Aug 1 and Dec 1) and in processed California navel orange juice (Wagner et al., 1988). Reducing bitterness in juices from early-season grapefruit and processed navel oranges could provide the consumer with a more acceptable juice product and the producer with a better quality product for blending.

Bitterness has been related to naringin and limonin in grapefruit juice and to limonin in processed navel orange juice. Adsorptive removal of bitter principles and titratable acid from citrus juices has been reviewed by Johnson and Chandler (1988), and some of the methods reported to remove these bitter components from citrus juices include treatment of the juice with enzymes (Kefford and Chandler, 1970), with immobilized bacteria (Hasegawa et al., 1985), and with insoluble polymers (Johnson and Chandler, 1982, 1985; Puri, 1984; Shaw and Buslig, 1986; Shaw et al., 1984; Shaw and Wilson, 1983, 1985). Recent work reported preliminary pilot scale debittering studies with grapefruit juices using insoluble β-cyclodextrin polymers in a fluidized bed process (Wagner et al., 1988).

In the current study, procedures for commercial applicability to debitter grapefruit and navel orange juices with β-cyclodextrin polymers in a pilot-scale fluidized bed column were thoroughly studied. In addition, a comparison was made between cyclodextrin polymers and neutral XAD resins used under similar fluidized bed conditions.

EXPERIMENTAL SECTION

Juices were prepared by reconstituting commercial concentrated grapefruit juice from Florida and navel orange juice from California to 10.5–11.0° Brix, except for one experimental single-strength grapefruit juice (juice E,

U.S. Citrus and Subtropical Products Laboratory, South Atlantic Area, U.S. Department of Agriculture—Agricultural Research Service, P.O. Box 1909, Winter Haven, Florida 33883-1909.