

Degreening of Citrus Fruit

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ABSTRACT

The practice of postharvest degreening of green but otherwise mature and edible citrus fruit has developed in order to promote external color development, i.e., destruction of the green chlorophyll pigments and accumulation of orange/yellow carotenoid pigments. The degreening process is complicated, since it depends on various endogenous and exogenous factors, such as fruit maturity at harvest and sensitivity of the fruit to ethylene, and is influenced by ethylene concentrations and the duration of the degreening process, the temperature and relative humidity used, efficacy of air circulation and ventilation. Although the commercial beneficial effect of ethylene on color development is well known, packers and exporters must be aware of the detrimental effects of ethylene, and pay special attention to its effects of enhancing decay development and stimulating senescence, which result in the appearance of various peel disorders. Overall, for efficient degreening, it is recommended to harvest the fruit at the onset of natural color development or later, and to use the lowest ethylene concentration and shortest exposure time possible. In addition, it is proposed to degreen the fruit under moderate temperatures of 20-25°C and high humidity of 95% RH, and to be sure to have appropriate air circulation and ventilation. Further details and recommendations regarding minimizing decay development and appearance of peel disorders are discussed.

Keywords: carotenoids, chlorophyll, citrus, degreening, ethylene, postharvest Abbreviations: RH, relative humidity

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INTRODUCTION

In citrus, the internal, edible portion of the fruit (pulp) usually reaches maturity while the external peel is still green, therefore, degreening practices were developed to accelerate fruit color change and render the fruit more acceptable for marketing. Application of degreening treatments is especially important for early varieties, in order to extend the marketing season, and for fruit grown in warm tropical climates where natural color development is late and weak.

The practice of citrus fruit degreening initially developed about 100 years ago. At that time, citrus fruit used to be cured at high temperatures, after harvest, in order to enhance healing of mechanical injuries, and when kerosene stoves were used for this process it was noticed that the green color of the fruit tended to disappear (Sievers and True 1912). Later, Denny reported that the factor responsible for the degreening of the fruit was the hormone ethylene (Denny 1924). Since the recognition of ethylene as the colorpromoting agent, industrially produced ethylene eventually became the preferred choice for degreening, and basic procedures were designed for ethylene degreening in special rooms that featured innovations in room designs, ethylene delivery systems, ventilation, and air replacements.

Nevertheless, besides its beneficial effect in enhancing fruit color change, exposure to ethylene carries certain disadvantages, including acceleration of senescence, button abscission, stem-end rot decay, and development of physiological disorders. Therefore, degreening must be performed under appropriate conditions, using the lowest possible levels of ethylene and the shortest possible exposure times (Cohen 1978a).

The primary effects of ethylene in promoting fruit color change during the degreening process are, on one hand, destruction of the green chlorophyll pigments (Barmore 1975; Purvis and Barmore 1981), and, on the other hand, induction of the biosynthetic pathway and accumulation of the yellow/orange carotenoid pigments (Stewart and Whea-

Ethylene concentration (ppm)



Fig. 1 Visual appearance of 'Satsuma' mandarins after degreening with various concentrations of ethylene. Pictures were taken after 5 days of degreening in the presence of ethylene at 0 (left), 0.1 (middle), and 1 ppm (right) at 20°C.

ton 1972; Young and Jahn 1972; Eilati *et al.* 1975; Rodrigo and Zacarias 2007). The breakdown of chlorophyll by ethylene is mediated by activation of chlorophyllase gene expression and enzyme activity (Barmore 1975; Shimokawa *et al.* 1978; Trebitch *et al.* 1993; Jacob-Wilk *et al.* 1999), whereas the accumulation of carotenoids is caused by the induction of carotenoid biosynthesis genes that results in enhanced accumulation of carotenoid pigments (Stewart and Wheaton 1972; Rodrigo and Zacarias 2007). The conversion of green chloroplasts into colored chromoplasts may also be reversible; a process called regreening (Goldschmidt 1988).

Overall, the present review summarizes current knowledge regarding the various factors affecting the degreening process in citrus, including the effects of fruit maturity stage, ethylene concentration and duration, temperature, relative humidity (RH), ventilation, and waxing. In addition, the detrimental effects of ethylene, i.e., enhancement of decay, and stimulation of senescence, including the enhancement of peel disorders and defects. For further information on the degreening process, readers are referred to previous reviews by Grierson and Newhall (1960), Eaks (1977), Ritenour (1999), and Wardowsky *et al.* (2006).

FACTORS AFFECTING THE DEGREENING PROCESS

Fruit maturity

Although there is not much published information on this issue, it is known to be much more difficult, if at all possible, to degreen grossly dark-green, immature fruit than fully mature fruit harvested at the onset or beginning of natural degreening. For example, Rodrigo and Zacarias (2007) reported that 'Navelate' orange fruit harvested completely green in late October achieved a pale yellow-orange color after 7 days of degreening under 10 ppm ethylene, whereas fruit harvested in mid-November, at the onset of natural degreening, developed, under the same conditions, a full characteristic orange color. Similarly, we found that 'Rishon' mandarin fruit, an Israeli early variety, harvested at the beginning of October at the dark-green stage, developed a pale yellow-orange color only after 5 days of degreening under 1.5 ppm ethylene, whereas fruit were harvested 2 weeks later, at the onset of natural degreening, reached the same external color after a much shorter period of just 3 days under the same conditions (unpublished data). Therefore, it is definitely recommended, for commercial practice, to degreen only mature fruit that reached the minimum acceptable internal quality standards required for harvest. For example, in Israel, it is allowed to harvest and degreen early 'Satsuma' mandarins only after the soluble solids content of the juice exceeds 9% and acidity levels are below 1.3%. Setting such strict criteria for harvesting provides two major advantages: it ensures that the fruit is mature enough

for the degreening process to succeed, and it forces the growers to harvest only high-quality, tasty fruit.

Ethylene concentration and exposure time

Ethylene is a naturally occurring plant hormone that has numerous effects on fruit-quality parameters, including induction of changes in pigmentation, and of ripening, abscission and senescence (Saltviet 1999). In commercial practice, after harvest, ethylene is often applied at high concentrations of 100 to 150 ppm in tightly closed rooms or chambers, in order to promote uniform ripening of various commodities, such as banana, tomato, and avocado. In the case of citrus, ethylene is applied in degreening rooms at much lower concentrations of just 1-5 ppm, to promote chlorophyll destruction and color development, therefore, the citrus degreening process requires precise control and monitoring of ethylene levels.

It was found that ethylene has a threshold level of between 5 and 10 ppm, depending on the variety tested, above which it does not accelerate degreening but rather enhances losses caused by decay and senescence (Wardowski et al. 2006). For example, Cohen (1978a) reported that exposure of 'Shamouti' oranges to various concentrations - 10, 20, 30 or 40 ppm – of ethylene did not cause any apparent differences in fruit color development. Furthermore, exposure of the fruit to a very high concentration of 100 ppm led to faster color development during the first 24 h of the degreening process, but that was followed by very slow color development. In fact, Cohen (1978a) noted that the duration of the degreening process was actually much more important for rind color development than the ethylene concentration used, and that it was not possible to shorten this duration by applying higher concentrations of ethylene.

In practice, the recommended concentration of ethylene for degreening of citrus fruit in most places around the world is about 5 ppm (Ritenour 1999; Wardowsky et al. 2006). Nevertheless, in order to eliminate the detrimental effects of ethylene, characterized by stimulation of decay, rind senescence, button abscission and appearance of peel blemishes, more and more packers are currently degreening the fruit under extremely low ethylene concentrations of 1 to 2 ppm. However, especially in these cases, it is important to monitor ethylene levels consistently, since too low levels will result in slow and inefficient degreening. Fig. 1 shows the visual appearance of 'Satsuma' mandarins after 5 days of exposure to various concentrations of ethylene at 20°C it can be seen that without ethylene the fruit remained green; exposure to 0.1 ppm ethylene resulted in partial degreening and chlorophyll destruction; whereas exposure to 1 ppm ethylene resulted in complete yellowing. It should be noted that while ethylene promotes degreening of most citrus fruit, in some particular varieties, such as 'Oroblanco' (a grapefruit × pummelo hybrid), ethylene exposures of up to 100 ppm for 3 days had only very slight effects on fruit Time zero

5 days degreening at 20°C

5 days degreening at 20°C + 10 days transport at 5°C



Fig. 2 Color development of 'Satsuma' mandarins following degreening and subsequent cold storage. Pictures were taken at time 0 (left), after 5 days of degreening with ethylene at 1.5 ppm, at 20°C (middle), and following subsequent 10 days of transport at 5°C (right).

color change (Porat *et al.* 2001). At least part of this observed ineffectiveness of ethylene may be ascribed to the counteracting effects of high levels of endogenous or exogenously applied gibberellic acid (Goldschmidt *et al.* 1977; Porat *et al.* 2001). For further technical information regarding commercial ethylene sensors and analyzers, and calculations of ethylene amounts and flow rates as related to room size and air exchange rates, readers are referred to the papers of Ritenour (1999) and Miller (2002).

Ethylene carry-over effect

It was noticed in some citrus varieties, that the degreening process continued after the fruit were removed from the ethylene degreening room: a phenomenon termed the "ethylene carry-over effect" or "post-ethylene effect". This continued and uninterrupted color change during 24 h or more in air after removal from ethylene was first reported for 'Valencia' oranges and 'Duncan' grapefruit (Grierson and Newhall 1960), and was later found with 'Navel' oranges also (Eaks 1977). However, only very little or no ethylene carry-over effects were observed in 'Hamlin' oranges (Jahn *et al.* 1973) and lemons (Eaks 1977).

Continuation of degreening after removal from ethylene is commercially important, since it may enable shortening of the degreening process so that the fruit color can continue to develop during shipment. Indeed, we found in Israel that we were able to degreen early-harvested 'Satsuma' mandarins in ethylene for just 3-5 days, to promote chloro-phyll breakdown and yellowing, and that carotenoid accumulation and development of a typical desired orange color were able to develop during subsequent shipment to export markets (Fig. 2). In another study, Petracek and Montalvo (1997) took advantage of this ethylene carry-over effect in order to develop a protocol for degreening of 'Fallglo' tangerines. These particular fruit are hypersensitive to ethylene and suffer from enhanced decay and appearance of peel disorders as a result of overexposure to ethylene. Therefore, they recommended to degreen the fruit only for a short period of just 1-2 days, and afterwards to allow continuation of color development during subsequent storage without ethylene, at 15°C. Use of this "under-degreening" approach enabled promotion of color change without massive enhancement of decay and peel disorders.

Temperature

After ethylene concentration, temperature is the second most important factor that determines the efficacy of the degreening process. However, choosing the optimum degreening temperature is somewhat tricky, since different temperature regimes favor either chlorophyll degradation or carotenoid build-up. In addition, the degreening temperature also markedly influences rates of decay development, weight loss and senescence.

It was found, that high degreening temperatures (up to 30-35°C) accelerated chlorophyll destruction but, at the same time, inhibited carotenoid synthesis, resulting in achievement of pale yellow fruit. On the other hand, degreening at low temperatures of 15-20°C resulted in desired rind color, but the process was too slow (Stewart and Wheaton 1972; Cohen 1978b; Ahrens and Barmore 1986). In light of these findings, Aharoni *et al.* (1973) and Cohen (1978b) recommended degreening of 'Navel' and 'Shamouti' oranges at an optimum temperature of 25°C, which is a compromise that favors both chlorophyll destruction and carotenoid accumulation.

In Florida, it is recommended to degreen citrus fruit at a relatively high temperature of 29°C. The main reason for this is that citrus grown in warm humid areas suffer severely from development of *Diplodia* stem-end rot, which is enhanced by exposure to ethylene, therefore, the prime consideration in Florida is to shorten the degreening period as far as possible, even at the expense of achieving poor carotenoid accumulation (Ritenour 1999; Wardowsky *et al.* 2006). On the other hand, in California, as well as in Mediterranean countries such as Spain and Israel, it is recommended to degreen citrus at moderate/low temperatures of 20-25°C (Eaks 1977; Cohen 1978b). At these temperatures, the degreening process is somewhat slower but results in achievement of a desired orange/yellow color.

Relative humidity

One of the most important considerations is to maintain fruit quality and to prevent the softening and shriveling caused by excessive water loss during the 3-5 days of degreening treatment at relatively high temperatures of 20-29°C. Therefore, it is mandatory to maintain a high humidity of approximately 90-95% RH in degreening rooms, preferably by using automated controlled humidifiers. Cohen (1978b) showed that variations in RH levels between 70 and 90% did not affect fruit color change, but low RH increased weight loss. For example, after 48 h degreening of 'Shamouti' oranges, fruit weight losses at 90 and 70% RH were 0.4 and 1.2%, respectively. In addition to its direct effects on weight loss, maintaining a high-humidity atmosphere in degreening rooms prevents the enhancement of physiological blemishes. For example, fruits degreened at 74% RH suffered from three times as much stem-end rind breakdown than those degreened at 94% RH (Hopkins and McCornack 1958). High humidity during degreening may also enhance healing of wounds and, thereby reduce decay development caused by the green mold pathogen Penicillium digitatum. On the other hand, degreening under excessive humidity, that wets the fruit and causes water condensation, may severely enhance decay development.

Air circulation

It is important to ensure that there is adequate air movement and circulation inside degreening rooms. Air circulation is needed: 1) to make sure that the ethylene concentration, humidity and temperatures will be uniform throughout the room, and 2) to force the air flow to reach the surfaces of all fruit in the room. Inadequate air circulation results in uneven and poor degreening. For practical advice on how to design and load degreening rooms, including construction of wall ducts, air tunnels, insulation materials and optimum air flow rates, readers should refer to the review by Ritenour (1999).

Air replacements

During respiration, citrus fruit consume oxygen (O_2) and produce carbon dioxide (CO_2) which, at excess levels, inhibits ethylene action (Saltveit 1999). Therefore, it is crucial to ventilate degreening rooms to avoid possible build-up of CO_2 . It is not so clear what is the exact threshold concentration of CO_2 for inhibition of ethylene action in degreening rooms. Some studies found that even 0.1% CO_2 may inhibit the degreening process (Ritenour 1999), whereas others indicated 1% (Grierson and Newhall 1960). However, laboratory tests indicated that only higher CO_2 concentrations of 2.5-5% directly inhibited degreening, and that low oxygen levels inhibit degreening only below 10% (Cohen 1973).

In light of this lack of agreement regarding the exact detrimental levels of CO₂, it is also somewhat unclear how many air replacements per hour are necessary in commercial degreening rooms. In Florida, the official recommendation is to perform one air replacement per hour (Ritenour 1999; Wardowsky et al. 2006). However, other studies showed no differences in the efficacy of the degreening processes performed throughout the range of 0.25-4.0 air replacements per hour (Cohen 1973). Furthermore, too frequent air replacements are undesirable, since they lower the humidity and are costly in terms of energy and ethylene wastage (Cohen 1977). Nowadays, in modern degreening facilities equipped with computerized monitoring systems, it is common to automatically replace the air in the degreening room as soon as the CO_2 level exceeds the threshold of 0.25%. Achieving these conditions obviously depends on the temperature and fruit load in the degreening room.

Waxing

It is well known that waxing (coating the fruit with a polymeric film) drastically reduces the responsiveness of the fruit to ethylene and impedes the degreening process (Grierson and Newhall 1960; Aharoni *et al.* 1973). For example, if 'Navel' oranges were degreened for 48 h under 20 ppm ethylene and afterwards waxed and stored at 17°C, they developed a uniform yellow color after 1 week and full orange color after 3 weeks. However, if fruit were first waxed and afterwards degreened with ethylene they remained green even after 3 weeks (Aharoni *et al.* 1973). Therefore, it is necessary to first degreen the fruit in harvest bins or boxes, and only afterwards to commercially sort, grade, and apply wax on the packing line.

Recently, it was reported that application of an edible fruit coating based on sucrose laurate ester inhibited the degreening of *nagato-yuzukichi* citrus fruit much more than other edible sugar-fatty acid ester coatings. Further investigation revealed that this phenomenon was attributed to the direct inhibitory effect of laurate on chlorophyllase and chlorophyll degrading peroxidase enzyme activities (Yamauchi *et al.* 2008).

EFFECTS OF DEGREENING ON DECAY DEVELOPMENT

Exposure to ethylene during degreening stimulates *Diplodia* stem-end rot caused by *Diplodia natalensis* and anthracnose

caused by Colletotrichum gleosporioides (Zhang and Ritenour 2002). Diplodia natalensis infects the fruit from the button at the stem-end of the fruit, and proceeds quickly through the core leading to a soft, brown to black decay. Anthracnose is visually characterized by the appearance of irregular brown to gravish black, sunken dry lesions. In both cases, the fungus grows and sporulates on citrus deadwood, and spores are transmitted to mature fruit surfaces by water splashes after rain or irrigation. In the case of Diplodia, the fungus colonizes under the buttons (calyx), and penetrates the fruit after abscission of the buttons (Barmore and Brown 1985; Brown and Lee 1993). In the case of anthracnose, the fungus proliferates and forms appressoria on the surface of the fruit. These appressoria remain latent, and after ripening germinate to form infections, especially under commercial degreening conditions (Brown 1975, 1992). In both cases, it was reported that exposure to ethylene directly stimulated fungus growth and reduced natural fruit resistance against these fungi (Barmore and Brown 1985; Brown and Lee 1993; Zhang and Ritenour 2002). Furthermore, for both pathogens, decay incidence and severity increased with increases in the duration of degreening and the concentration of ethylene used (Cohen 1978a).

Methods to control *Diplodia* stem-end rot and anthracnose decay include: 1) application of good cultural practices that minimize the amounts of deadwood on the trees; 2) preharvest spraying with benomyl; 3) postharvest drench treatments with thiabendazole before degreening; 4) reduction of degreening time and ethylene concentrations as far as possible; 5) application of thiabendazole on the packing line after degreening; and 6) cooling the fruit immediately after the degreening treatment.

In addition to stem-end rot and anthracnose, it was noted that the environmental conditions during degreening (2-5 days at 20-25°C and 95% RH) were optimal for development of green mold caused by Penicillium digitatum, which is the main postharvest pathogen of citrus fruit (Eckert and Brown 1986; Smilanick et al. 2006). Therefore, it is crucial to protect the fruit from development of green mold decay by either preharvest or postharvest applications of fungicides. Smilanick et al. (2006) recently reported that preharvest application of thiophanate methyl and postharvest applications of thiobendazole, with or without addition of 3% sodium bicarbonate, prior to degreening efficiently reduced green mold decay development. Furthermore, Zhang (2007) suggested that a new fungicide, fludioxonil, could be applied in commercial drench systems before ethylene degreening, to reduce both Diplodia stem-end rot and green mold decay. Another possible approach to reduction of green mold decay is to integrate curing at a high temperature of 40°C into the first 24 h of the degreening process (Plaza et al. 2004).

EFFECTS OF DEGREENING ON DEVELOPMENT OF PEEL DISORDERS

Exposure to ethylene for 3 to 5 days at relatively high temperatures of 20-29°C transiently increases respiration rates (Eaks 1970; McCollum and Maul 2007), and enhances senescence (physiological aging) of the peel tissue and in certain instances causes the appearance of peel disorders. The degreening enhancement of development of peel disorders can be attributed to two different factors: 1) the enhancement of weight loss and desiccation of cells on the peel surface during the degreening process; and 2) the direct effect of ethylene in induction of peel senescence (ethylene damage).

For some as yet unknown reasons, certain citrus varieties, such as 'Villa Franka' lemons (Cohen *et al.* 1986) and 'Fallglo' tangerines (Petracek and Montalvo 1997) are more sensitive than others to ethylene, and are more prone to suffer from physiological breakdown of the peel. These ethylene injuries are characterized by random senescence of peel cells, which results in the appearance of brown sunken areas of collapsed tissue. An example of ethylene damage

Ethylene damage on 'Villa Franka' lemon



Green calyx

Abscised brown calyx

Peel bruising on

'Satsuma' mandarin





Fig. 4 Effects of degreening on clayx senescence of 'Satsuma' mandarins. Pictures were taken at time 0 (left) and after 5 days of degreening with ethylene at 1.5 ppm, at 20° C (right).

on the peel surface of 'Villa Franka' lemons is shown in **Fig. 3**.

Another detrimental effect of ethylene degreening is that it often potentates the fruit peel to becoming more sensitive to mechanical injuries, with the result that the fruit often suffers darkening and bruising after being passed over brush rollers on the packing line, after the degreening treatment. An example of ethylene-induced susceptibility of 'Satsuma' mandarins to mechanical injuries on the packing line is shown in **Fig. 3**. To avoid this problem, packers often allow the fruit to stand at the same temperature for an extra day in a separate room without ethylene before passing them to the packing line.

Another defect that often occurs during fruit degreening, known as "green islands", is characterized by the presence of residual green spots on the peel surface. These green areas appear where adjacent fruit are in contact or because of the presence of water drops on the peel which blocks the degreening effect of ethylene. **Fig. 3** shows the appearance of "green islands" on the peel of 'Navel' oranges after 5 days of degreening.

One of the most noted detrimental effects of ethylene during degreening is the enhancement of calyx senescence, resulting in calyx abscission and browning (Carvalho *et al.* 2008). **Fig. 4** shows the visual appearance of the calyx of 'Satsuma' mandarins: before degreening the calyx is green and fresh, whereas after five days degreening with 1.5 ppm ethylene at 20°C the calyx abscises and turns brown. In order to reduce abscission and retain the freshness of the calyx, packers usually treat the fruit with the synthetic auxin 2,4,-D, either as a postharvest drench treatment before degreening, or within the wax emulsion on the packing line shortly after degreening (Cronje *et al.* 2005; Carvalho *et al.* 2008).

Overall, measures that should be taken in order to eliminate, as much as possible, the development of peel disorders following the degreening treatment are: 1) termination of irrigation 1-2 weeks before harvest in order to reduce the turgor pressure in the fruit and, thereby to make it somewhat softer and less susceptible to mechanical damage on the packing line; 2) maintenance of a high humidity of 95% RH in the degreening room, to reduce peel senescence; 3) use of low ethylene concentrations, of not more than 1-5 Green islands on 'Navel' orange



Fig. 3 Development of peel disorders and defects in citrus fruit after degreening. Pictures were taken after 5 days of degreening with ethylene at 1.5 ppm, at 20°C followed by cold storage for 10 days at 5°C.

ppm; 4) if fruit are extremely sensitive to ethylene, it would be necessary to shorten the degreening period, even to ter-minate it before complete color development.

CONCLUSIONS

Degreening with ethylene is commercially applied in order to accelerate external color development and render the fruit more attractive to consumers. However, the improve-ment of fruit visual appearance by the degreening treatment may result in scarification of other fruit quality attributes, such as shorter shelf life, increased susceptibility to decay, enhanced weight loss, and acceleration of rind and calyx senescence. Thus, the decision of whether to degreen or not degreen is not simple, and relies on various circumstances, such as marketing demands, fruit quality at harvest, and estimated storage and shelf life periods (Pool and Gray 2002). Overall, because of the detrimental effects of ethy-lene, we suggest growers, packers and exporters to be cauti-ous and minimize ethylene concentrations and exposure times as much as possible, maintain a moderate temperature and high RH in degreening rooms, and apply appropriate treatments to prevent decay and peel blemishes.

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