TIME-TEMPERATURE INTERACTION ON POSTHARVEST RIND COLOUR DEVELOPMENT OF *CITRUS*

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature:……. Date:……..

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SUMMARY

Rind colour is one of the most important external quality characteristics of citrus fruit and plays an important role in purchasing decisions by consumers. Consumers perceive brightly-coloured fruit to be sweet and mature, whereas citrus with a green rind is perceived to be sour and immature. However, there is a poor correlation between rind colour and internal quality, contradicting what is generally assumed by the fruit-buying public. In general, a bright orange rind colour improves consumer acceptance. Thus, it is important to ensure that the rind of citrus fruit is well-coloured on arrival at the market.

Various pre-harvest cultural practices and postharvest techniques can be applied to improve rind colour. Degreening with ethylene gas is the most commonly used postharvest technology to improve rind colour, but has various negative side-effects. Degreened fruit are more prone to decay, have rinds which appear dull and flaccid, have been reported to develop off-flavours and have a shorter shelf-life period. Therefore, it is necessary to find alternatives to ethylene degreening and to extend shelf-life of citrus fruit.

Under normal orchard conditions, rind colour development is associated with low night temperatures, usually experienced during autumn or following the passing of a cold front. To simulate cold front conditions, a hydrocooler and cold room were used to rapidly drop fruit temperature to 4 ºC for 6 hours, and then fruit were incubated at 20 to 22 ºC for 72 hours. This “cold shock” treatment of ‘Nules Clementine’ mandarin improved rind colour to a level similar to that of degreened fruit in the 2002 season due to a decrease in chlorophyll content and increase in carotenoid content. However, this result could not be repeated.

Storage temperature is one of the most important postharvest factors affecting rind colour. Citrus fruit shipped to export markets requiring low temperatures (-0.6 ºC) for pest disinfestations purposes have been reported to arrive with poor rind colour. Shipping under low temperatures results in poor rind colour of fruit on arrival in the market. To comply with the USA's phytosanitary requirement for imported citrus, fruit is held at -0.6 ºC for a minimum of 22 days. The effect of shipping at various temperatures (-0.6 ºC or 4.5 ºC), durations and the influence of initial rind colour, "orange" or "yellow", on fruit colour upon arrival in the market was evaluated. Fruit shipped at a higher temperature (4.5 ºC) had a marginally better rind colour than fruit shipped at -0.6 ºC. The perceived loss of rind colour following shipping at sub-zero temperatures is probably due to carotenoid degradation. Therefore, initial rind colour plays a critical role in final product quality. Depending on market destination and shipping temperature, pale-coloured fruit should not be packed for markets sensitive to rind colour.

Holding temperature after shipping can be effectively used to improve the rind colour of fruit arriving in the market with undesirable rind colour. An intermediate holding temperature of between 11 and 15 ºC resulted in the greatest improvement in rind colour after 2 weeks. A high holding temperature (20 ºC) caused colour degradation, whereas a low holding temperature (4.5 ºC) resulted in the maintenance of rind colour. By selecting the correct holding temperature, even after shipping at sub-zero temperatures, final colour can be improved.

OPSOMMING

Tyd-temperatuur interaksie op na-oes skilkleur ontwikkeling by sitrus

Skilkleur is een van die belangrikste eksterne kwaliteitseienskappe van die sitrusvrug en spëel 'n belangrikke rol in wat verbruikers koop. Verbruikers verwag dat heldergekleurde vrugte soet en ryp sal wees, terwyl sitrus met 'n groen skil geassosieer word met onrypheid en 'n suur smaak. In teenstelling hiermee is daar egter 'n swak korrelasie tussen skilkleur en interne kwaliteit. Aangesien 'n heldergekleurde oranje skil verbruikersaanvaarding verbeter, is dit dus belangrik om te verseker dat die sitrusvrug 'n goeie skilkleur het teen die tyd wat dit die mark bereik.

Verskeie voor-oes bestuurspraktyke en na-oes tegnieke kan toegepas word om die skilkleur te verbeter. Ontgroening met etileen gas is die tegnologie wat mees algemeen gebruik word om skilkleur na oes te verbeter, maar dit het egter verskeie newe effekte tot gevolg. Ontgroende vrugte is meer vatbaar vir bederf en verwelkde skille met 'n dowwe voorkoms. Afsmaake kan voorkom en 'n verkorte rakleeftyd is al gerapporteer. Dit is dus noodsaaklik om 'n alternatief vir etileen ontgroening te ontwikkel en die rakleeftyd van sitrusvrugte te verleng.

Onder normale boordomstandighede word skilkleur ontwikkeling geassosieer met lae nag temperature wat gewoonlik in die herfs of na 'n kouefront ondervind word. Om soortgelyke omstandighede na te boots, was 'n "hydrocooler" en koelkamers gebruik om die temperatuur vinnig te laat daal tot by 4 °C en dit vir 6 uur daar te hou. Die vrugte was dan by 20 tot 22 °C geinkubeer vir 72 uur. Hierdie "koueskok" behandeling van 'Nules Clementine' mandaryn het skilkleur verbeter tot 'n vlak vergelykbaar met ontgroende vrugte in die 2002 seisoen wat

ontstaan het weens ’n verlaging in chlorofil en ’n toename in die karotinoïed inhoud van die skil.

Opbergingstemperatuur is een van die belangrikste na-oes faktore wat skilkleur beinvloed. Sitrusvrugte wat verskeep word na uitvoermarkte wat lae temperature (-0.6 °C) vir disinfestasie vereis arriveer soms by die mark met ’n swak skilkleur. Om die fitosanitêre vereistes vir die invoer van sitrus deur die VSA na tekom, was vrugte vir ’n minimum van 22 dae by -0.6 °C gehou. Die effek van verskeping by verskeie temperature (-0.6 °C of 4.5 °C), tydperke en die invloed van aanvanklike skilkleur, “oranje” of “geel” was geevalueer by aankoms in die mark. Vrugte wat by hoër temperature (4.5 °C) verskeep was het ’n effens beter skilkleur as vrugte by -0.6 °C getoon. Die verlies in skilkleur wat waargeneem word na verskeping onder vriespunt kan moontlik toegeskryf word aan karotenoiëd afbraak. Daarom speel aanvanklike skilkleur ’n kritieke rol in finale produk kwaliteit. Die finale mark bestemming en verskepingstemperatuur sal bepaal of swakgekleurde vrugte verpak kan word.

Opbergingstemperatuur na verskeping kan effektief gebruik word om die skilkleur van vrugte wat swak gekleur was met aankoms by die mark te verbeter. Matige temperature tussen 11 en 15 °C het na 2 weke die beste verbetering in skilkleur gelewer. Hoër temperature (20 °C) het skilkleur nadelig beinvloed, terwyl lae temperature skilkleur behou het. Deur die korrekte temperatuur te kies, selfs na verskeping by temperature onder vriespunt, kan uiteindelike skilkleur steeds verbeter word.

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CHAPTER 1

INTRODUCTION

Rind colour plays a central role in consumer acceptance of fruit of *Citrus* spp., as colour affects flavour perception. Red colour is associated with increased sweetness and the darker the red, the sweeter the product was judged (Clydesdale, 1993). Affluent consumers have shown a preference for deep orange rind colour for sweet oranges (*C. sinensis* [L.] Osbeck) and mandarins (*C. reticulata* Blanco) as evidenced by tighter industry fruit quality specifications (CRI, 2004a; b). Moreover, consistency of rind colour within a carton of fruit is possibly as important as colour intensity since between fruit variation in rind colour may result in consumer resistance.

Citrus is indigenous to South East Asia where the climate is subtropical and the average yearly temperature is 18 ºC (Reuther and Rios-Castano, 1969). Under these climatic conditions fruit do not develop an orange colour, but turn pale green after colour break. Under Mediterranean conditions pronounced fluctuations between day and night temperatures occur, leading to the development of a characteristic orange colour (Stearns and Young, 1942). This sudden drop in temperature occurs during autumn or early winter and leads to a visible colour break (Eilati et al., 1975).

During the onset of fruit maturity or colour break, chlorophyll in the rind breaks down to reveal the underlying carotenoids (Thomson, 1966). The fruit rind then changes from green to orange. Ethylene is the phytohormone responsible for chlorophyll degradation. Trebitsh et al. (1993) found that ethylene induces an increase in chlorophyllase activity by *de novo* synthesis of the enzyme. Rind colour in mature citrus fruit is a result of a combination of

various carotenoid pigments and chlorophyll. Chloroplasts can undergo partial degradation where the thylakoids are uncoupled, and be transformed into chromoplasts containing carotenoids (Goldschmidt, 1988). Transformation is reversible and regreening of fruit can occur under warm conditions later in the season (Saks et al., 1988) when thylakoid structures are re-assembled and chlorophyll is synthesised. The change in rind colour resulting from the transformation of chloroplasts to chromoplasts, seems to be controlled by various interrelated mechanisms viz. environmental, nutritional, hormonal and genetic (Goldschmidt, 1988).

Temperatures prior to harvest have a crucial effect on eventual rind colour. Night temperatures below 13 ºC are required for fruit to develop orange colour (Stearns and Young, 1942). Carotenoids are highly temperature sensitive and pigment degradation occurs under conditions where temperatures rise above 30 ºC or fall below 8 ºC (Eilati, 1975). Even small deviations from ideal temperatures lead to sub-optimal colour development (Erickson, 1960). Under natural conditions, rind colour development is often initiated by a sudden drop in air temperature. This temperature drop is colloquially referred to as a cold snap and is often the result of a passing cold front.

Various cultural practices are used to ensure that well-coloured fruit are harvested and marketed. These include controlled water deficit stress, summer girdling (Peng and Rabe, 1996), judicious nutrient application (Collado et al., 1996), ethephon sprays (El-Otmani et al., 1996), pruning (Morales et al., 2000) and selective harvesting of fruit that have reached a certain colour. Under conditions where poorly-coloured fruit have been harvested, post-harvest colour improvement techniques are applied. Fruit are then exposed to ethephon packhouse dips (Gilfillan and Lowe, 1985), ethylene degreening (Wheaton and Stewart, 1973) or altered shipping and holding temperatures (Le Roux, 1997).

To simulate a “cold front” this principle was carried out under laboratory conditions (Oberholster, 2001) on rind disks. Rind disks were floated in petri dishes containing water at 4 ºC and kept at this temperature for various periods. The greatest effect on rind colour development was detected in rind disks kept at 4 ºC for 6 hours.

Citrus fruit shipped to the United States of America need to be kept at -0.6 ºC for 22 days to meet the phytosanitary requirement. This sub-zero shipping temperature must be maintained for the entire period to ensure that false codling moth (*Cryptophlebia leucotreta* Meyr.) and fruitfly (*Ceratitis* spp.) in their various developmental stages are killed (Maritz, 2000). Fruit shipped at sub-zero temperatures were found to become paler, while fruit shipped at 4.5 ºC maintained consistent colour (Le Roux, 1997). Fruit shipped under sub-zero shipping conditions often arrive at the destination with unfavourable colour as low temperature causes degradation of carotenoids.

The main objectives of this study were to maximise rind colour development of sweet oranges and mandarins and correlate this with differences in carotenoid concentrations, and to study the effects of various temperature-duration combinations on rind colour and carotenoid levels. Pre-harvest conditions ideal for rind colour development were simulated postharvest by means of hydrocooling to cause rind colour development through carotenoid synthesis and accumulation. The effect of sub-zero shipping temperature was evaluated as well as the remedial effects of various post-shipping holding temperatures on rind colour.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Flavedo anatomy

The fruit of *Citrus* spp. are classified as a hesperidium, a fleshy, specialised berry and the product of one pistil (Bain, 1958; Monselise, 1986). The fruit is surrounded by a tough, leathery rind (pericarp) and has a fleshy heterogenous internal texture divided into segments by septa. The rind is usually coarse as a result of small indentations or, less often, protrusions which affect the brightness of the perceived fruit rind colour (Soule and Grierson, 1986).

The wall of a *Citrus* fruit is known as the pericarp and is composed of the exocarp (flavedo), mesocarp (albedo, inter-segmental membranes and central axis) and endocarp (segments containing juice vesicles) (Bain, 1958; Schneider, 1968; Holtzhauzen, 1969).

The flavedo gradually blends into the outer mesocarp or albedo and oil glands below (Albrigo and Carter, 1977) as the transition from one cell type to another is unclear (Fig. 2.1). The epicarp is covered with a multilayered cuticle usually above a pectin layer (Esau, 1965; Soule and Grierson, 1986) and is composed of compact tissue with small intercellular spaces (Roth, 1977). The flavedo is the external pigmented layer of rind composed of a few layers of small, compact polygonal parenchyma cells (Albrigo and Carter, 1977) containing chloroplasts and chromoplasts (Holtzhausen, 1969; Monselise, 1986) (Fig. 2.2).

The epicarp cells of green *Citrus* rind contain various plastids including highly organised chloroplasts with an extensive grana-fretwork system (Purvis, 1980). The inner flavedo cells contain fewer plastids than the outer flavedo cells (Spiegel-Roy and Goldschmidt, 1996).

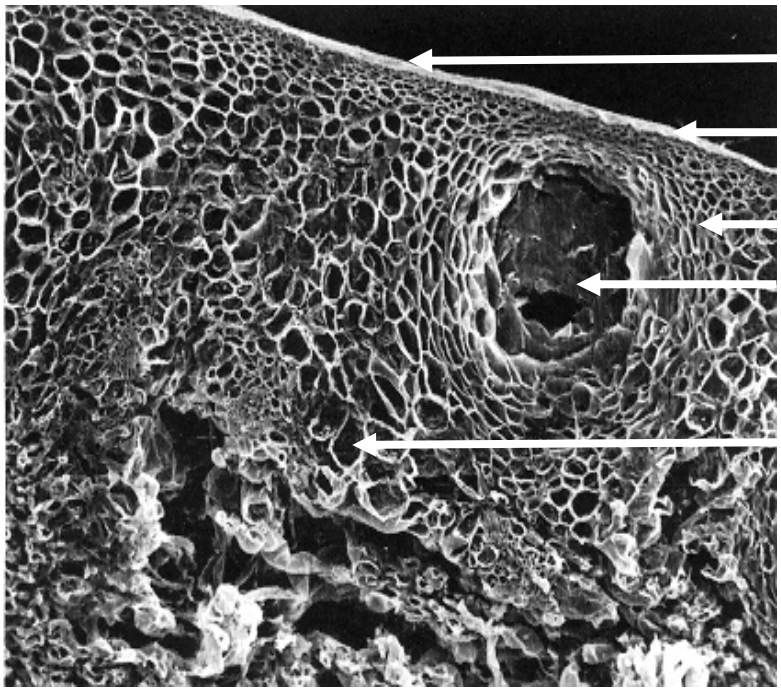

Fig. 2.1. SEM (x66) cross section of *Citrus* rind from mature 'Murcott' tangor fruit. There is a gradual transition from the densely packed flavedo cells to the loosely congregated, greatly degenerated albedo cells (Spiegel-Roy and Goldschmidt, 1996).

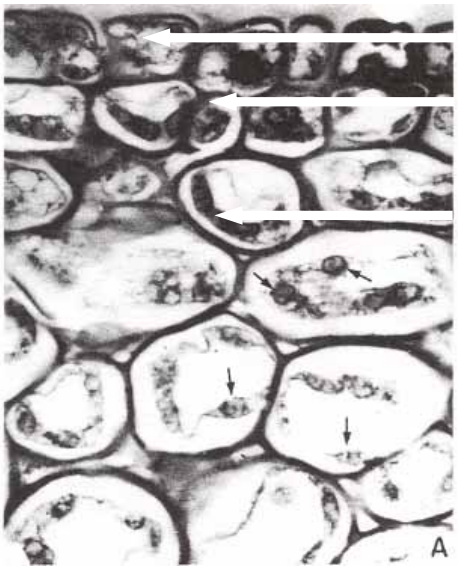

Fig. 2.2. Micrograph of *Citrus* flavedo and albedo cells from mature 'Murcott' tangor fruit (Spiegel-Roy and Goldschmidt, 1996).

Mature *Citrus* fruit have an orange coloured flavedo with epicarp cells containing an increased number of chromoplasts with carotenoids and a reduced number of chloroplasts with chlorophyll once transformation of chloroplasts to chromoplasts has taken place.

2.2 Rind pigments

2.2.1 Chlorophylls

2.2.1.1 *Structure*

Chlorophylls all have a tetrapyrrole (porphyrin) ring structure with a Mg ion chelated at the centre and joined by ligand at four positions to N atoms of the pyrrole ring, giving the pigment the observed green colour significant in photosynthesis (Fig. 2.3a). Most bonding with apoproteins occurs with the polar head group of chlorophyll (Prézelin and Nelson, 1998). Chlorophyll *a* and *b* have a phytol tail, which is a long-chain, lipophilic diterpenoid attached to the porphyrin ring. This tail extends through into the thylakoid membrane (Salisbury and Ross, 1992; Bramley, 1997). Various conformational characteristics of the porphyrin ring, while bound to proteins, give the different chlorophyll-protein complexes their particular absorption characteristics (Prézelin and Nelson, 1998). If the aldehyde group (CHO) replaces the methyl group (CH_3) attached to one of the pyrole rings of chlorophyll *a*, the new molecule is chlorophyll *b* (Fig. 2.3b).

2.2.1.2 Location

Chlorophylls are integral to plant metabolism and are housed in chloroplasts which are disk-shaped plastids containing light-harvesting structures known as thylakoids. Chloroplasts are found in all photosynthetic tissues, although not always green in appearance. Chlorophyll could be masked by other, darker pigments, but remains functional under these conditions. Chloroplasts form from small proplastids which are almost colourless and have no internal

membranes. Proplastids develop from a single unfertilised egg cell and then divide during embryo development. When chloroplasts develop from proplastids the grana are composed of vesicles which are pinched off from the plastid membrane (Thomson et al., 1967). Young chloroplasts divide especially actively when the plant tissue they are housed in is exposed to light. All chlorophylls and most carotenoids are embedded in the thylakoids in chloroplasts and are attached to proteins with non-covalent bonds.

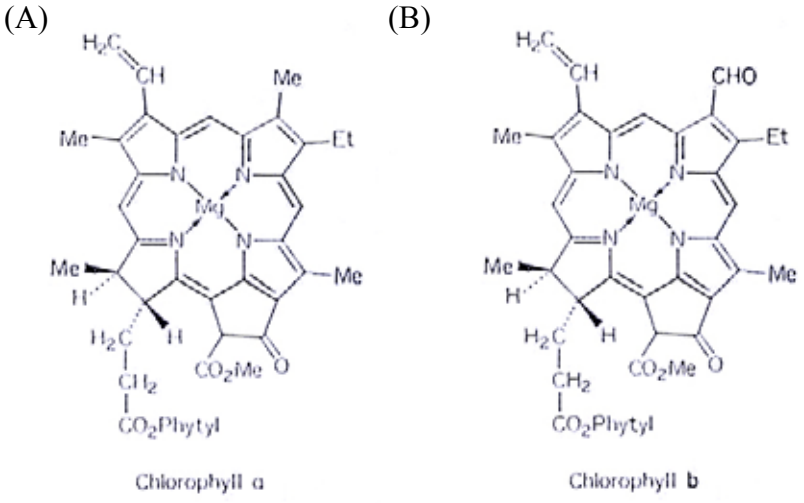

Fig. 2.3. Structure of (A) chlorophyll *a* and (B) chlorophyll *b* (Harborne, 1973).

Chloroplasts are surrounded by a double membrane, which controls the movement of substances in and out of the plastid. The chloroplast is filled with a gelatinous matrix, known as the stroma, containing ribosomes, DNA and enzymes that catalyse reactions converting CO_2 to carbohydrates. A stack of thylakoids is known as a granum and the position where one granum thylakoid is attached to another is called an appressed region (Figs. 2.4 and 2.5). The lumen is the area between thylakoid membranes and plays an important role in photosynthesis. Stroma thylakoids (frets) connect one granum to another at irregular intervals and are lengthened structures extending through the stroma (Thomson, 1966; Salisbury and Ross, 1992).

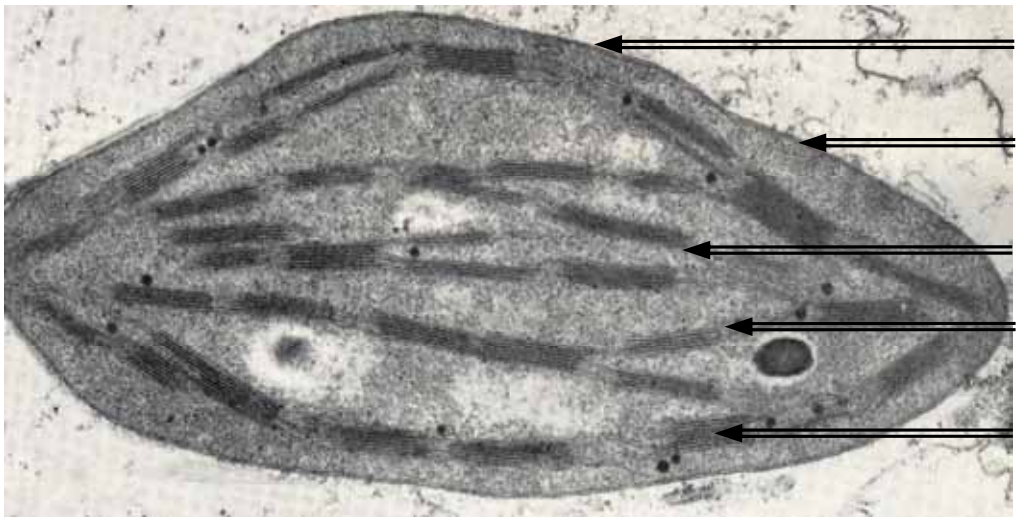

Fig. 2.4. Micrograph showing chloroplast structure (Highkin et al., 1969).

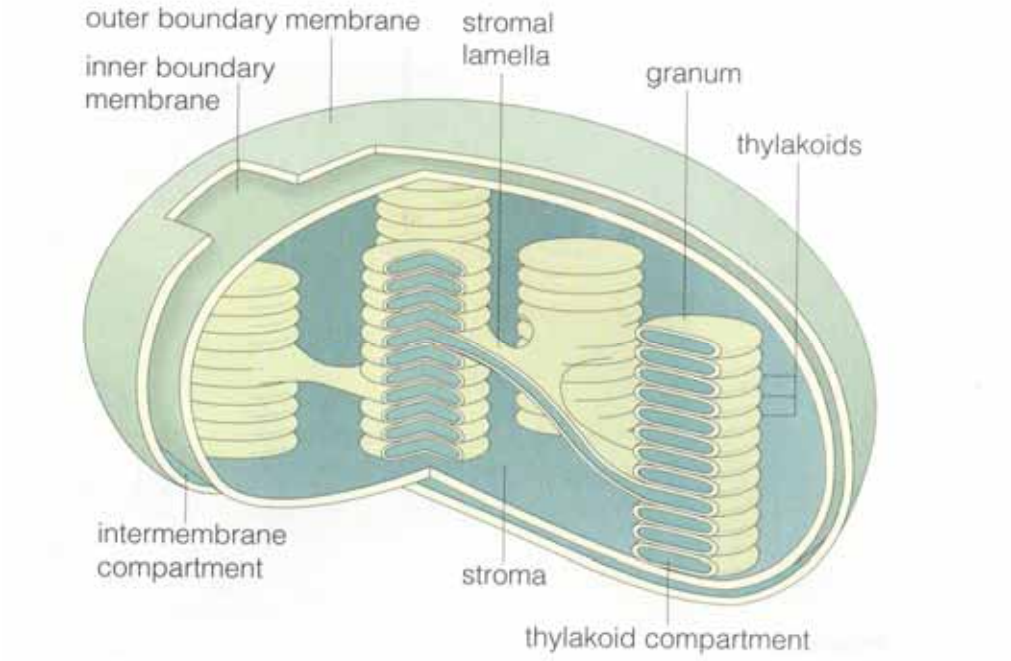

Fig. 2.5. Schematic representation of a chloroplast with major structural features (Fairbanks and Andersen, 1999).

2.2.1.3 Function

Chlorophyll *a* is the primary photosynthetic pigment and is the only chlorophyll type found in all plants (Prézelin and Nelson, 1998). Chlorophyll *b* functions as a light-harvesting pigment, and absorbs light energy, passing it on to chlorophyll *a*, thus contributing towards

photosynthesis (Prézelin and Nelson, 1998). Chlorophyll synthesis is stimulated by light (Moore et al., 1998). Light energy is used in the thylakoids to oxidise water in the metabolic processes which produce energy (adenosine triphosphate [ATP]) and reducing power (nicotineamide adenine dinucleotide phosphate [NADPH]) needed in the stroma for carbohydrate formation. Chlorophyll absorbs maximally at wavelengths of 400 to 500 nm and 600 to 700 nm (Moore et al., 1998).

2.2.2 Carotenoids

2.2.2.1 Structure

Most carotenoids have an all-E (all-trans) configuration, but structural changes can occur under specific natural or induced conditions causing the formation of Z-isomers (cis-isomers). These configurations make the initial carotenoid difficult to identify and are often present in small quantities. Conditions under which artefacts form, include high temperatures, acidic conditions and bright light. The all-trans configuration results in strong colour, whereas if the conformation changes to all-cis, the colour becomes gradually weaker as there is a shift in the absorption maxima towards shorter wavelengths.

The most abundant oxygen-containing carotenoids are leaf xanthophylls, lutein being most common. β-carotene forms the basis of zeaxanthin and xanthin, this β-ring allows epoxide formation across the ring double bond, reshuffling the epoxide and forming a furanoid, and allowing the formation of a new group of compounds from lutein, which contains one epoxide and zeaxanthin containing mono- and di-epoxides. When epoxide formation occurs, the length of the chain decreases leading to a shift in the absorption spectrum to shorter wavelengths and a paler observed colour.

When a carotenoid undergoes oxidative fission and carbon atoms are removed from the chain, which is then shortened, an apocarotenoid is formed, e.g. β-citraurin. C_{30} carotenoids are commonly treated as apocarotenoids and classified accordingly. Large quantities of apocarotenoids are commonly present in *Citrus* spp. (Britton, 1997)

The basic carotenoid structure is composed of a hydrocarbon chain with a completely conjugated system of double bonds called a chromophore, which gives the plant tissue colour. This structure gives carotenoids the ability to absorb light and makes the molecule highly sensitive to oxidation (Britton, 1996). A carotenoid with several double bonds is classified as polyene and is made up of a repetition of isoprene structures, which allow it to be classified as a specific polyene. Carotenoid molecules are composed of repeated isoprene (monomethylbutadiene) units and typically consist of a C_{40} chain made up of eight or more basic isoprene units making them tetraterpenoid. All carotenoids can be formed from polyenelycopene, which is a conjugated acyclic carotenoid with the formula $C_{40}H_{56}$. Various carotenoids form from this structure by means of hydrogenation, dehydrogenation, cyclization, addition of oxygen, migration of double bonds, migration of methyl groups as well as chain lengthening and shortening (Goodwin, 1980). The greater the number of double bonds in the carotenoid chain, the greater the intensity of the observed colour contributed by the carotenoid. A chain with a minimum of seven conjugated bonds is required for observable colour, which would be pale yellow. A higher level of hydrogenation leads to a paler coloured compound. A greater concentration of carotenoids in a specific cell leads to more intense colour. Carotenoids can be divided into two groups, namely carotenes which are carotenoid hydrocarbons and xanthophylls which include an oxygen molecule in the form of a hydroxyl-, keto-, epoxy- or methoxy of carboxylic acid (Britton, 1996; Prézelin and Nelson, 1998). Xanthophylls are often present in chromoplasts as mixtures of fatty acyl esters.

2.2.2.2 Location

Carotenoids and chlorophylls are contained in chromoplasts and chloroplasts, respectively, and both pigments are fat-soluble (Britton, 1996). Carotenoids are located in the chromoplasts of non-photosynthetic and photosynthetic tissues including the chloroplasts of photosynthetic tissue, although their colour is masked by the high concentration of chlorophylls present. In chloroplasts, carotenoids are present in the thylakoid membranes in the pigment-protein complexes of photosystems I and II. Most leaves contain similar carotenoid combinations with β-carotene and lutein being present in the highest concentrations of 25 to 30% and 45%, respectively (Britton, 1996). In chromoplasts, carotenoids are widely distributed in non-photosynthetic tissue giving a distinct yellow to red colour. Carotenoids accumulate at very high concentrations in chromoplasts to provide intense colour to plant organs (Bartley and Scolnik, 1995). Chromoplasts contain specialised structures made up of carotenoids, proteins and lipids which function to seize large amounts of carotenoids. These structures are either classified as globular, membranous, fibrillar, crystalline or tubular (Marano et al., 1993).

2.2.2.3 Function

Carotenoids are able to absorb visible light as a result of the presence of conjugated bonds in their structure. Carotenoids absorb light in the blue region of the spectrum (400 to 600 nm) and this energy can then be transferred to chlorophylls (Bartley and Scolnik, 1995).

In photosynthetic tissue the xanthophylls function as accessory light-harvesting pigments during photosynthesis and prevent photo-oxidation. Photo-protection of carotenoids also occurs in non-photosynthetic tissue, but by means of a different mechanism. Carotenoids absorb light and then transfer this light to chlorophyll. β-carotene is much more effective at

transferring light energy than xanthophylls (Goodwin, 1980). In photosynthetically-active chloroplasts, carotenoids are part of the pigment-protein complexes in the thylakoid membranes. Carotenes are associated with photosystem I whereas xanthophylls are more abundant around photosystem II. The reaction centre of photosystem I is the chloropyll *a*-protein complex in plants and absorbs light at 700 nm, whereas photosystem II absorbs light at 680 nm (Prézelin and Nelson, 1998). As fruit mature, the development of chromoplasts and synthesis of carotenoids are important features of maturation. During maturation, genes involved in carotenoid biosynthesis specific to maturation are activated. The primary carotenoids that develop in chromoplasts, and not those which are present in chloroplasts, are functional in determining or playing a role in the colour of flowers and fruits and, thus, the attraction of pollinating insects (Britton and Hornero-Méndez, 1997). Carotenoids are also thought to be structural determinants in plastid pigment-protein complexes (Bartley and Scolnik, 1995).

Carotenoids have an important function in preventing photo-oxidation by singlet oxygen ($1O_2$). This becomes necessary when more light than can be used during photosynthesis is absorbed by chlorophyll molecules. Under these conditions, excited chlorophyll molecules will cross over to a slightly lower energy level but more long-term triplet excited state. This excessive energy can then be transferred to oxygen to yield highly reactive singlet oxygen, which could damage molecules and tissues by sequestering hydrogen atoms from proteins, lipids and other macromolecules. β-carotene is especially effective in absorbing this excess energy and preventing singlet oxygen formation (Britton, 1996). This protection mechanism of carotenoids is also effective in non-photosynthetic tissues. When coloured carotenoid pigments are absent plants suffer damage caused by photo-oxidation.

2.2.3 Pigment changes

During the period of continuous growth of green citrus fruit, chlorophyll content increases and then shows a rapid decrease. Carotenoids decrease to a minimum of 2μg.cm^{-2} before the typical carotenoid accumulation begins (Fig. 2.6) (Eilati et al., 1975). Carotenes and xanthophyll fractions decreased to this minimum concentration at "colour break" and the yellowing of the peel was as a result of a low carotenoid concentration and a rapid drop in chlorophyll. Following this period, there was the typical accumulation of carotenoids, mainly due to an increase in xanthophylls, while carotenes continued to decrease and the β-carotene fraction disappeared (Eilati et al., 1975).

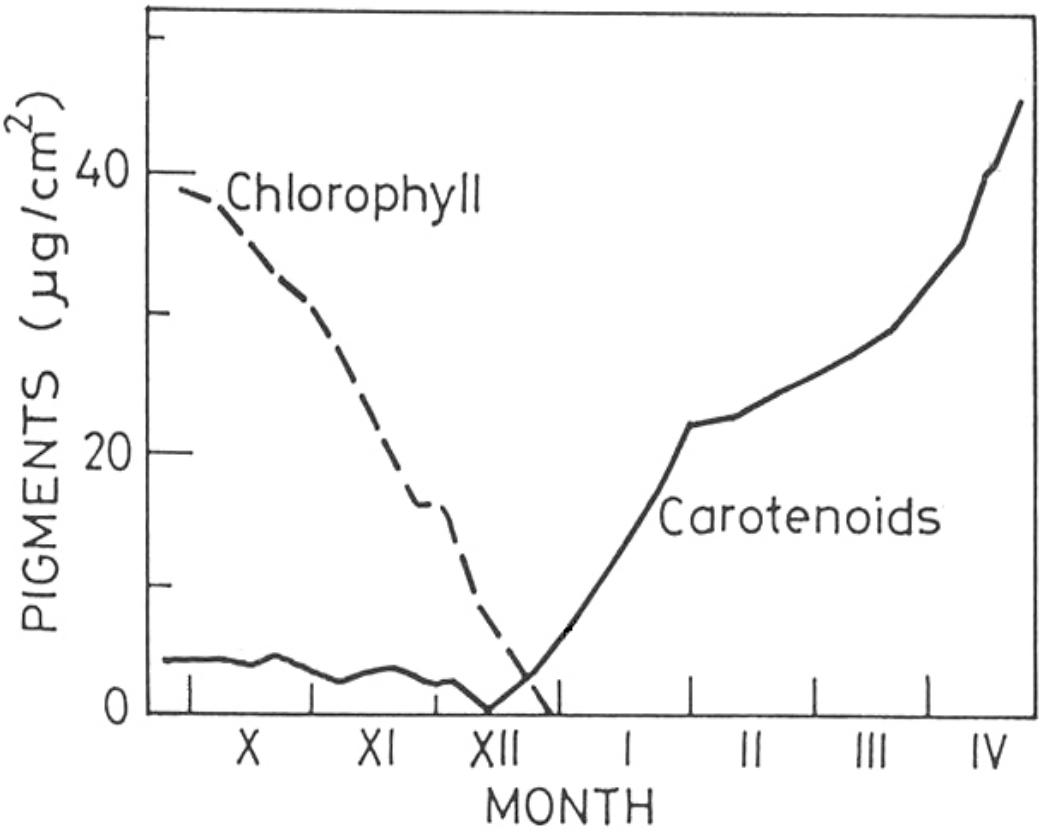

Fig. 2.6. Changes in chlorophyll and carotenoid pigment concentrations during fruit development and maturation (Spiegel-Roy and Goldschmidt, 1996).

A hypsochromic shift of the entire absorption spectrum towards lower absorbance values (of 6 to 8 nm in the absorption peaks) of the carotenoid spectrum was detected during the transition from green to orange coloured fruit rind. The three major peaks shifted to lower absorption spectra (Eilati et al., 1975). Chlorophyllase levels increased approximately in proportion to chlorophyll levels when kept in darkness for 6 days (Aljuburi et al., 1979).

The opposite is, however, true under conditions of natural degreening and regreening, where chlorophyllase content increases and decreases with chlorophyll content (Aljuburi et al., 1979).

More chlorophyll *a* than chlorophyll *b* is present in green *Citrus* fruit, but this is reversed when maturation and colouring occurs and the chlorophyll *b* levels fall below that of chlorophyll *a* (El-Zeftawi, 1977). These changes in chlorophyll levels appear to be related to increasing light intensities and hormonal control signals from seeds and roots (El-Zeftawi, 1977). During regreening in *Citrus* an increase in chlorophyll coupled with a reappearance of the thylakoid structure in chromoplasts was found, which was induced by natural or fluorescent white light (Saks et al., 1988).

The rinds of immature *Citrus* fruit are green. While the fruit mature, photosynthetic activity decreases as a result of chlorophyll degradation (Bean and Todd, 1960; Wardowski et al., 1986). Chloroplasts become structurally altered and are transformed into chromoplasts (Thomson, 1966; El-Zeftawi and Garrett, 1978; Camara and Brangeon, 1981; Mayfield and Huff, 1986). The underlying carotenoids are then unmasked and further synthesis of carotenoids occurs, resulting in the characteristic orange colour of *Citrus* fruit (Fig. 2.6). A rapid degradation in chlorophyll can be correlated visually with the loss of green colour and

the ensuing yellow colour of the fruit rind preceding colour break in *Citrus* fruit (Oberholster, 2001).

Transformation between chloroplasts and chromoplasts can occur in either direction, including the reversion of chromoplasts to chloroplasts (Thomson et al., 1967; Camara and Brangeon, 1981; Mayfield and Huff, 1986; Goldschmidt, 1988). During the reversion of chromoplasts to chloroplasts, chlorophyll accumulates and new thylakoid membranes are formed in the chromoplast. The modified plastid is similar to chloroplasts of immature green fruit, but many of the plastoglobuli formed during differentiation of chlorplasts to chromoplasts are still present in the redifferentiated chloroplasts (Mayfield and Huff, 1986). The breakdown of chlorophyll is an enzymatic process made up of various steps (Fig. 2.7), which are regulated by stage of development and environmental signals affecting plant growth regulators (Jacob-Wilk et al., 1999).

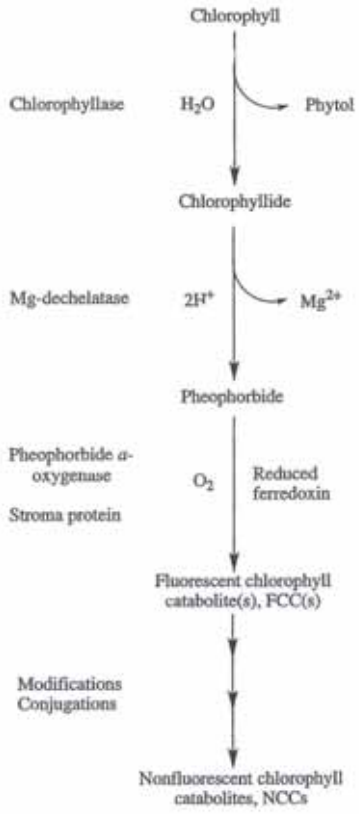

Fig. 2.7. Steps of chlorophyll degradation (Matile et al., 1996).

During fruit maturation, the internal structure (grana and thylakoids) of chloroplasts breaks down and the number and size of globules increases. It may be that a reduction in granal and fretwork membranes and the resulting reduction in lipid binding sites results in excess lipids accumulating in small globules bordering the remaining membranes. When the granal fretwork system has disappeared completely, the small globules also disappear as they may fuse to form large globules and are probably derived from dissolved products of the granal membrane system (Thomson, 1966). A high volume of the carotenoids is synthesised in the globules during maturation (Thomson, 1966). As chromoplasts develop, the internal membranes are associated with large globules. Synthesis of carotenoids may take place in association with the plastid membranes and as pigments are synthesised they accumulate in globules.

During maturation in peppers (*Capsicum annuum* L.) grana start to undergo degradation and eventually complete lysis of the grana when the fruit become orange (Camara and Brangeon, 1981). The inner envelope membranes bud into vesicles, which then develop into lamellar sheets. Large globules containing carotenoids are found in close association with these membranes, which result from *de novo* synthesis partly derived from inner envelope membranes of the plastid (Thomson et al., 1967; Camara and Brangeon, 1981). There is a correlation between chlorophyll loss and the replacement of photosynthesising granal membranes by a non-chlorophyll lamellar system. The membranes form a parallel arrangement during fruit maturation and colour change (Camara and Brangeon, 1981). The resulting chromoplast or chloroplast resulting from transformation depends on the conditions experienced by the tissue housing these plastids. Under conditions of illumination, high N levels and limited sugar levels plastids in fruit accumulate chlorophyll and chloroplast proteins, while developing thylakoid membranes (Marano et al., 1993).

Regreening is more severe in years when temperatures are high and increase during late spring and early summer (Caprio, 1956; Coggins et al., 1981). Regreening may be due to an increase in light intensity as fruit reach maturity, especially since regreening takes place in the brightest season. This may also explain why fruit do not colour in tropical countries (El-Zeftawi and Garrett, 1978b).

When *Citrus* fruit regreen, the photosynthetic membranes regenerate, and grana develop while plastids containing electron dense globules are still present and associated with the fretwork systems. The structure of the chloroplast closely resembles that of chloroplasts in immature green fruit (Thomson et al., 1967). The plastid colour correlates well with that of regreened fruit during the series of transitions between chromoplasts and chloroplasts, which suggests that chromoplasts revert to chloroplasts during regreening (Thomson et al., 1967). Large amounts of chlorophyllide *a* accumulate *in vivo* in senescing *Citrus* peel (Amir-Shapira et al., 1987). Chlorophyllase is the only well-defined enzyme system indicated as being involved in chlorophyll catabolism (Amir-Shapira et al., 1987). Chlorophyllase is present in green plant tissue before senescence while it is unclear how chlorophyll catabolism is activated during senescence (Amir-Shapira et al., 1987). Chlorophyllase activity appears to be stimulated by ethylene (Hirschfeld and Goldschmidt, 1983). Initial degradation of thylakoid membranes may be necessary to expose chlorophyll to chlorophyllase hydrolysis or that of other enzyme systems (Amir-Shapira et al., 1987). Chlorophyll degradation by ethylene in *Citrus* fruit is correlated with increased chlorophyllase activity (Amir-Shapira et al., 1987). It is not known whether the increase in chlorophyllase levels arises by *de novo* synthesis or by means of another form of activation (Amir-Shapira et al., 1987).

During colour development in *Citrus* rind there is a change in the ultrastructure of chloroplasts as well as the metabolism of carotenoids and chlorophylls (Gross, 1981). During maturation of *Citrus* fruit on the tree, chloroplasts are transformed into chromoplasts, accompanied by an initial decrease in carotenoid synthesis and an increase in chlorophyll breakdown followed by carotenoid biosynthesis (Fig. 2.6) (Eilati et al., 1975; Gross et al., 1983).

2.2.4 Carotenoid biosynthesis

Carotenoids are terpenoids that consist of eight isoprenoid units and are biosynthesised from acetyl coenzyme-A from mevalonic acid as a branch of the isoprenoid pathway (Fig. 2.8). The linkage order at the centre of the C_{40} isoprenoid molecule is reversed, making the whole molecule symmetrical (Bramley, 1985; Bartley and Scolnik, 1995). Isopentenyl pyrophosphate (IPP) is the initial C_5 terpenoid precursor of carotenoids. Mevalonic acid (MVA), a C_6 compound, is the first specific carotenoid precursor and is made up of three acetate units from acetyl-CoA. Geranylgeranyl pyrophosphate (GGPP) is formed by three condensation reactions catalysed by geranylgeranyl pyrophosphate synthase (GGPS), the first being that of dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPPP) (Bartley and Scolnik, 1995).

Carotenoid biogenesis occurs by means of steps of dehydrogenation starting with the initial, colourless phytoene formed from a two-step conversion of two molecules of GGPP first to prephytoene pyrophosphate (PPPP) and then phytoene with phytoene synthase as the catalyst (Bartley and Scolnik, 1995). Phytoene has the basic C_{40} carotenoid skeleton from which succeeding reactions result in a variety of carotenoids converted from the basic structure (Fig. 2.9).

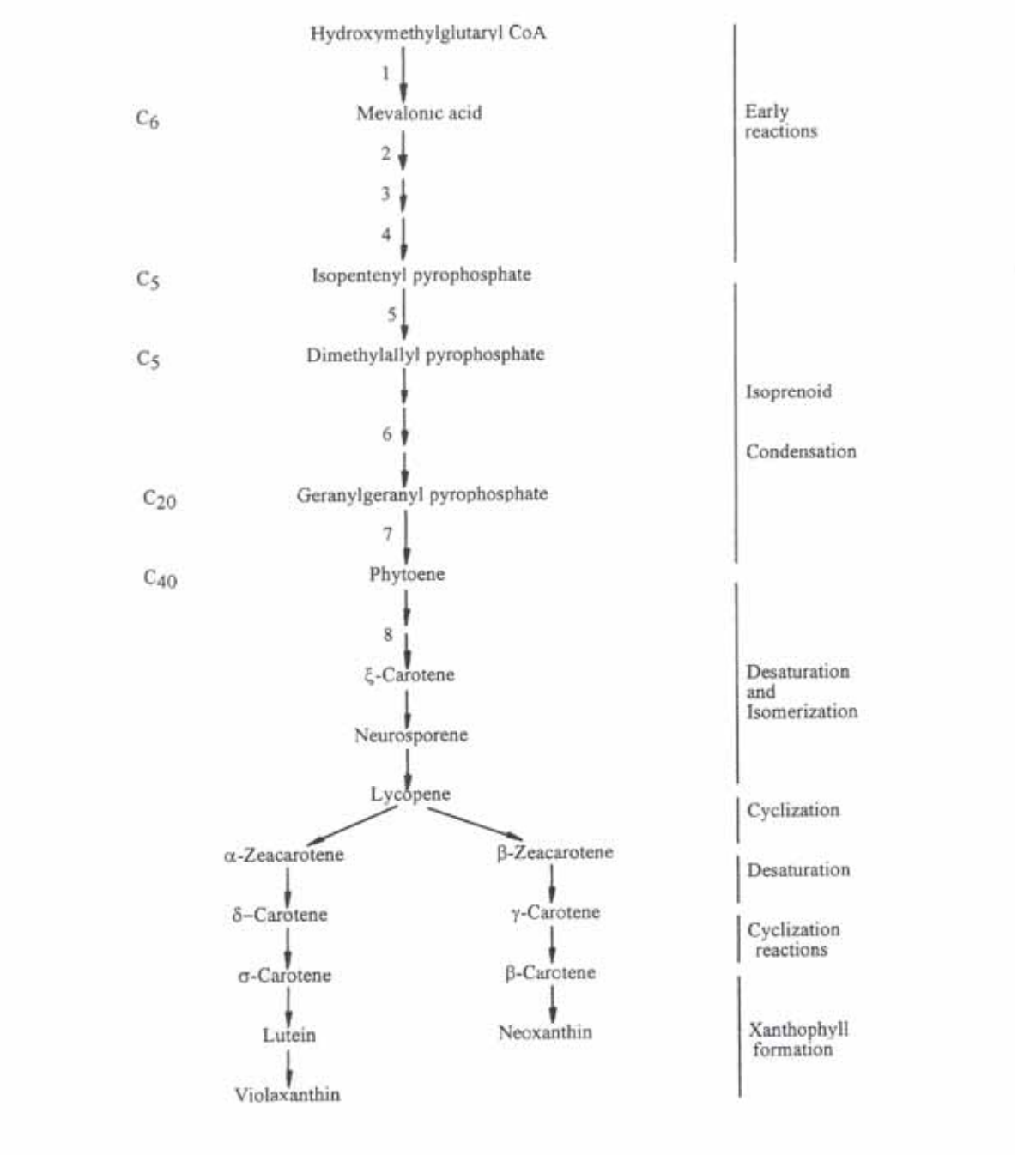

Fig. 2.8. General scheme of carotenoid biosynthesis (some of the intermediates have been excluded). Enzymes catalyzing the central reactions up to ζ-carotene are indicated by numbers: 1: 3-hydroxy-3 methylglutaryl coenzyme A reductase; 2: mevalonate kinase; 3: phosphomevalonate kinase; 4: mevalonate pyrophosphate decarboxylase; 5: isopentenyl pyrophosphate isomerase; 6: geranylgeranyl pyrophosphate synthase; 7: phytoene synthase; 8: phytoene desaturase (Marano et al., 1993).

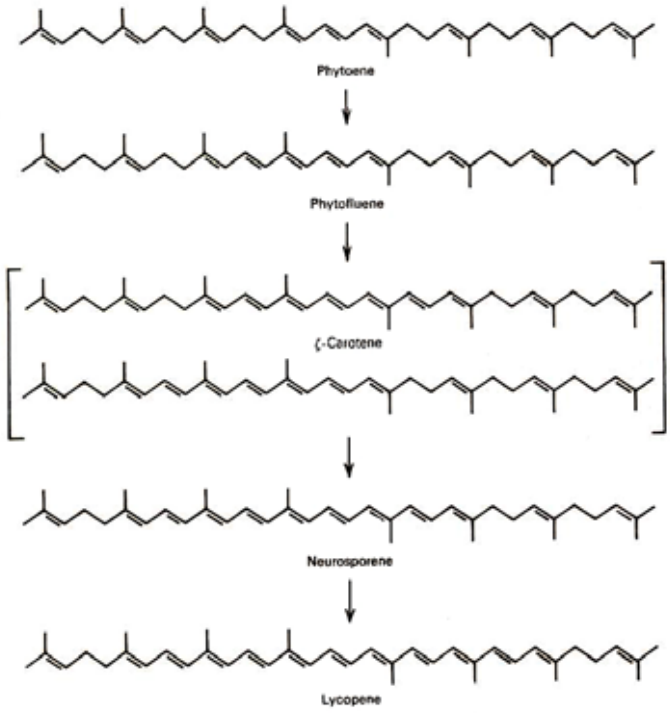

Fig. 2.9. Carotenoid dehydration reactions.

Dehydration reactions, which introduce double bonds, convert colourless phytoene into yellow, orange and red carotenoids (Fig. 2.9). Dehydration reactions result in a succession of carotenoids, namely phytofluene, ζ-carotene, neurosporene and finally lycopene with 11 conjugated double bonds. Each dehydration reaction causes a shift in the light absorption maximum towards longer wavelengths and deeper colour, i.e. lycopene with 11 conjugated double bonds absorbs maximally at 470 to 500 nm (Britton and Hornero-Méndez, 1997). Lycopene is converted to the cyclic carotenoid, β-carotene, by lycopene cyclase, or neurosporene via the same enzyme to form α-carotene (Bartley and Scolnik, 1995).

The majority of carotenoids are xanthophylls which are formed from cyclic carotenoids like α-carotene which then undergo addition of hydroxyl groups, followed by epoxidation at specific positions in the ring which introduces oxygen functions to the pigment late in the biosynthesis pathway (Bramley, 1985; Bartley and Scolnik, 1995).

2.3 Factors affecting carotenoid biosynthesis and degradation

Environmental, nutritional and hormonal factors play a central role in chloro-chromoplast interconversion (Fig. 2.10) (Goldschmidt, 1988). As long as soil temperatures are high enough to permit root growth, hormones produced in the roots, viz. gibberellins and cytokinins, and nitrogenous compounds are translocated to the canopy and delay chlorophyll degeneration and general senescence (Goldschmidt, 1988). Rind senescence occurs when root growth stops during autumn as a result of temperature drop and the decline in the export of root-produced hormones. There appear to be two principal temperature effects on rind colour, viz. i) the requirement for optimum temperature favouring biochemical processes affecting chlorophyll catabolism, and ii) whole-tree effects, which in turn, affect hormone levels in the fruit via the roots (Goldschmidt, 1988).

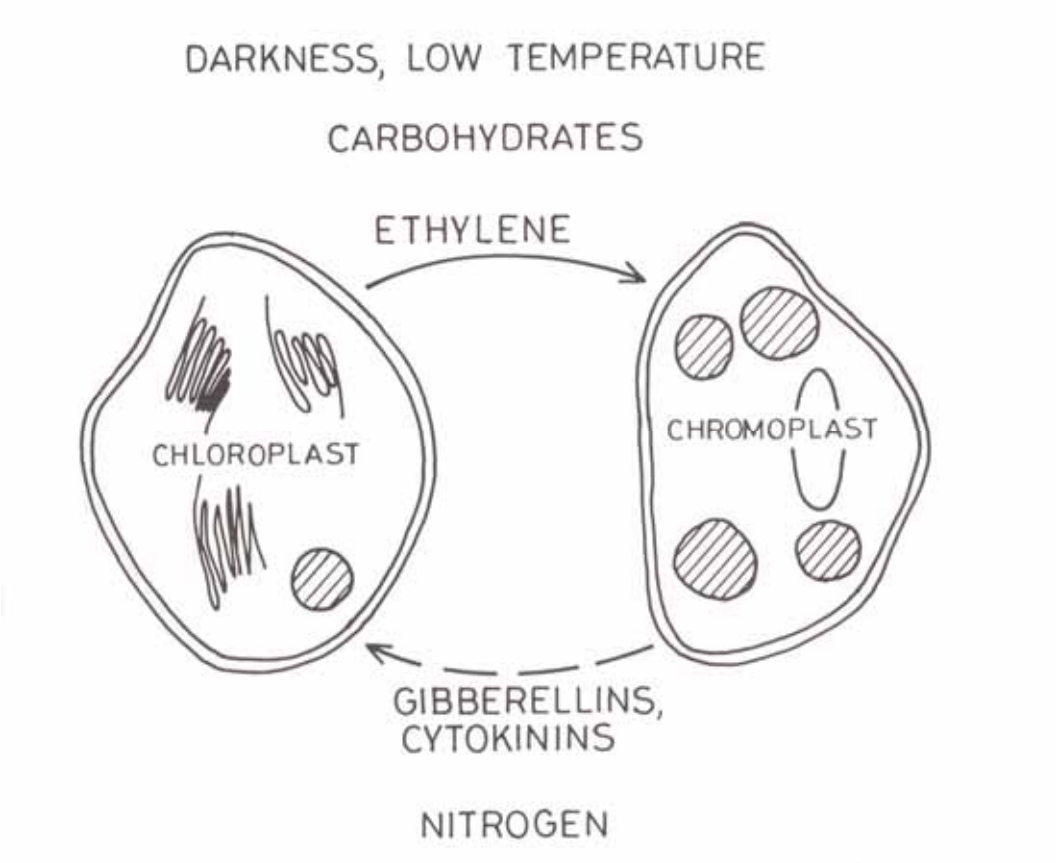

Fig. 2.10. Chloroplast-chromoplast interconversion (Spiegel-Roy and Goldschmidt, 1996).

2.3.1 Environmental

Citrus originated in southeast Asia, and is currently commercially produced in the subtropical regions between 20 and 40° N and S of the equator (Webber et al., 1967; Reuther, 1973). Climate is the major factor which determines internal and external fruit quality. This can be seen in fruit grown under tropical conditions where the rind does not become fully coloured, but changes from a dark, intense green to a pale green colour, coinciding with the onset of maturity or natural degreening on the tree, known as "colour break" (Samson, 1980). In contrast, fruit grown under Mediterranean-type conditions with large day-night fluctuations in temperature result in bright, orange coloured fruit.

2.3.1.1 Temperature

Natural colour development in *Citrus* rind is stimulated by a sudden drop in temperature during autumn and winter (Stearns and Young, 1942). Endogenous ethylene production is triggered by low night temperature which induces colour break (Eaks, 1977).

Growing seasons with good rind colour development consist of an early spring, with above average temperatures during the spring and summer period, followed by a colder than average winter and spring in the following season. The opposite conditions result in growing seasons with poor colour (Caprio, 1956).

Young and Erickson (1961) tested various combinations of night and day air temperatures and soil temperature and concluded that the combination with the lowest temperatures resulted in the most brightly coloured fruit in which no chlorophyll was visible in the rind. As air temperature increased, more poorly coloured fruit were obtained. Stearns and Young (1942) showed that when minimum temperatures dropped below 12.8 °C significant rind colour

changes occurred. Colour development continued and the magnitude of these changes are dependent on the intensity of the minimum temperatures experienced. Air temperature below 12.8 °C is thought to cause degradation of chlorophyll, revealing the underlying carotenoids and giving fruit an orange colour (Young and Erickson, 1961; Sinclair, 1984; Reitz and Embleton, 1986). However, it has not been conclusively shown whether this is a direct or indirect effect.

In experiments conducted by Cooper et al. (1969) on 'Robinson' mandarin (*C. reticulata* Blanco) it was found that at day temperatures of 20 °C and night temperatures of 5 °C (20/5 °C), 100 $\mu g.g^{-1}$ of ethylene was produced, compared to 4 $\mu g.g^{-1}$ for fruit held at temperatures of 25/20 °C. Fruit held for 14 days at the 20/5 °C temperature combination lost their chlorophyll content and developed a yellow rind. Fruit held at the 25/20 °C temperature combination remained green for 2 months. This shows the effect of temperature variation on ethylene evolution and the effect of ethylene on chlorophyll degradation.

The exposed colour depends on the composition and concentration of the carotenoids present in the flavedo cells. The most favourable temperature combinations for chlorophyll degradation and carotenoid synthesis are cool days (20 °C), cold night-air temperatures (7 °C) and cool soil temperatures (12 °C), whereas if the temperature of the soil or air is increased a greener fruit results (Young and Erickson, 1961).

Young and Erickson (1961) found that at the lowest soil temperature (12.8 °C) little or no tree growth took place, whereas at the highest soil temperature (20 °C) tree growth was favoured. Trees exposed to the highest temperatures produced fruit with the poorest rind colour.

The various carotenoids have different temperature sensitivities. Lowering the temperature to which 'Redblush' grapefruit (*C. paradisi* Macf.) had been exposed from a day/night temperature of 42 °C/36 °C to 32 °C/21 °C lead to a high level of lycopene production (Meredith and Young, 1971). Carotene production increased below the temperature combination of 32 °C/21 °C under natural conditions (Meredith and Young, 1971). Maximum rind colouration occurred at 15 °C, which was also associated with the highest increase in carotenoids and most chlorophyll degradation (Agusti, 1999). Carotenoids are highly temperature-sensitive and small variations from the optimum temperature (1 °C) may negatively affect colour development (Young and Erickson, 1961). Fruit exposed to a day temperature of 20 °C and a night temperature of 5 °C developed a uniform orange colour (Erickson, 1960). In contrast, a day temperature of 30 °C and a night temperature of 10 °C produced less well-coloured fruit. It is thought, that a high day temperatures may impede colour development even if night temperatures are adequately low. Higher day temperatures produced fruit with lower carotenoid and higher chlorophyll levels (Erickson, 1960).

The effects of temperature on endogenous hormone levels and root activity may provide an explanation for the effects of temperature and nutrition on regreening (Coggins et al., 1981). Soil moisture and temperature conditions modify the time and intensity of shoot growth in certain areas. During warm winters a vegetative growth flush coupled with increased levels of gibberellins (GA) could occur (Cooper and Peynado, 1958).

When soil temperature exceeds the lower threshold for root activity (12.8 °C), root growth and the synthesis and translocation of root-derived phytohormones is favoured. The uptake and translocation of N-containing compounds is greater under temperatures which favour vegetative growth than when below the threshold for root activity (Table 2.1).

Table 2.1. Modes of control in the chloro-chromoplast system of citrus fruit rind.

	Promoters of	
Mode of Control	**Chloroplast**	**Chromoplast**
Environmental	Light	Dark
	High temperature	Low temperature
Nutritional	Nitrogen	Carbohydrates
Hormonal	Gibberellins	Ethylene
	Cytokinins	Auxins
	Abscisic acid	

High levels of GA are associated with juvenile characteristics, including vigorous growth and the synthesis of chlorophyll, which leads to the retardation of senescence. Under these conditions the change in *Citrus* rind colour from green to orange is inhibited. Whereas, under low soil temperatures (<12.8 °C) there is cessation of root growth, leading to reduction in the uptake of N-containing compounds and production of hormones (which are antagonistic to chlorophyll degradation and carotenoid biosynthesis) by the roots. Low root temperatures may stop cytokinin and or GA production by the roots, or its translocation to the shoots and fruit, in this way preventing these inhibitors of maturation from reaching the fruit.

Low temperature may also cause rind colour development by enhancing the accumulation of ethylene in intercellular spaces under the *Citrus* rind (Cooper et al., 1969). Gas chromatographic evidence suggests that chilling causes ethylene production in grapefruit and sweet orange (*C. sinensis* [L.] Osbeck) (Cooper et al., 1969). Low temperatures may have a dual effect, by causing a reduction in the import of maturation inhibitors and by promoting ethylene production and in this way favouring senescence.

2.3.1.2 Light

Light is an important factor that affects rind colour. Fruit harvested from the inside of trees, where the lowest light levels occur have the poorest colour, whereas the most intensely coloured orange fruit are found in the outer, most well-lit sections of the tree (Sites and Reitz, 1949). Pruning trials on 'Orlando' tangelo (*C. paradisi* Macf. x *C. reticulata* Blanco.) showed that fruit harvested from the bottom-inside of the canopy had the poorest colour (highest hue angle; H=73.3°), whereas the most well-coloured fruit (H=65.6°) were harvested from the top-outer part of the canopy (Morales et al., 2000).

Citrus fruit growing under low light conditions have a pale colour as chloroplasts do not develop normally and pigment levels remain low. Unpruned trees allow little light penetration into the canopy and thus fruit inside the canopy develop a pale colour. Trees with high vigour produce many strong growth flushes leading to dense foliage and, hence, shaded fruit. Rind colour deteriorated with increased distance from the canopy surface and as relative light intensity decreased below 40%, rind colour became poorer (Iwagaki and Kudo, 1977). Light-exposed fruit were found to have higher rind carotenoid contents than those of shaded fruit (Mzini, 2002).

Exposure to sunlight results in vivid yellow colour expression and higher carotenoid concentrations in 'Hort16A' kiwifruit (*Actinidia deliciosa* var. *deliciosa* [A. Chev.] C.F. Liang et A.R. Ferguson) than shaded fruit. There is an increase in production of chloroplasts containing carotenoids and chlorophyll under bright light conditions (Currie et al., 2002). These pigments increase as a result of their role in photoprotection of plant tissues under conditions of high irradiation (Salisbury and Ross, 1992).

2.3.1.3 Water

Water indirectly affects rind colour of *Citrus*. Moist soils favour root activity, increasing the uptake of N-containing compounds and synthesis of root-produced hormones. Increased N uptake and GA production leads to favoured vigorous vegetative growth and the inhibition of senescence and thus poor colouration of *Citrus* fruit rind. Irrigation increases the production of green fruit (Koo, 1988). Flood irrigated *Citrus* trees produced fruit with higher rind carotenoid and lower chlorophyll levels than trees exposed to trickle irrigation (Huff et al., 1981). Trickle irrigation increases leaf-N levels by improving N uptake and utilisation and thus maintains chlorophyll levels. Peng and Rabe (1996) found that drier soils (-70 kPa) under deficit irrigation resulted in a greater proportion of the *Citrus* crop (45%) with an acceptable level of rind colour development at harvest. Under wetter soil conditions (-30 kPa), only 25% of the crop had reached an acceptable level of colour development by harvest.

2.3.2 Nutritional

The principal mineral nutrients affecting rind pigments are N, P and K, although N has the greatest effect (Reitz and Embleton, 1986). N deficiencies are enhanced by high sucrose concentrations (Mayfield and Huff, 1986). Sucrose and N levels appear to be correlated and have an effect on chlorophyll content and will, therefore, also be discussed in this section.

2.3.2.1 Nitrogen (N)

Nitrogen is the mineral nutrient which has the greatest effect on rind colour (Ritenour et al., 2003). Nitrogen has a strong inhibitory effect on rind colour (Iglesias, 2001). Nitrogenous fertilisation is one of the modifiable factors that most influences rind colour of *Citrus* (Sala et al., 1992). High N inhibits chlorophyll degradation, but has a minimal effect on chlorophyll synthesis (Huff, 1984). It appears that a warm shoot environment leads to increased

vegetative and reproductive growth and the resulting translocation of N to physiological sinks, causing a reduction in the content of rind N. High N increased the proportion of green fruit by 65% (Koo and Reese, 1977). Low N levels resulted in more orange-coloured fruit, which may be a lower N concentration than ideal (2.5 to 2.7% leaf N) for maximum yield (Ritenour et al., 2003). It is thus difficult to achieve an ideal N concentration, which supports maximum production and good colour development.

High N (1.1 kg per tree per year) applications were found to delay fruit maturity, delay colour break and reduce colour development at harvest as well as promote regreening (Reuther and Smith, 1952; Reitz and Koo, 1960; Coggins et al., 1981). Under environmental conditions favourable for plant growth, high N nutrition increases the degree of regreening (Coggins et al., 1981).

The type of N nutrition also appears to be of importance, as rind colour of fruit from cuttings irrigated with ammoniacal-nitric solution were more orange than fruits from cuttings treated with nitric solutions. Nitric nutritive solution, enriched with Ca also resulted in more well-coloured fruit than fruit from cuttings irrigated with a nitric nutritive solution (Sala et al., 1992). The fruit from cuttings irrigated with the ammoniacal-nitric nutritive solution (5 meq N per L) had a greater (8.75% of fresh mass) sugar content and had more well-coloured flavedos.

Differentiation of chloroplasts into chromoplasts is promoted on media with high sugar concentrations and low N levels, while media with low sugar concentrations and a N source promote redifferentiation of chromoplasts into chloroplasts (Mayfield and Huff, 1986). Nitrogen levels in the rinds of sucrose supplemented trees tended to decrease (Iglesias et al.,

2001). Under conditions of low N levels, sucrose increased colour development, probably by means of ethylene action as ethylene inhibitors counteracted this effect of sucrose (Iglesias et al., 2001).

2.3.2.2 Phosphorus (P)

Phosphorus is an important component of many plant compounds including energy producing intermediates of photosynthesis and respiration (Salisbury and Ross, 1992). High P (2.2 to 2.7 kg per tree) levels cause a delay in colour break, delay the ensuing development of a full orange colour (Smith et al., 1963), and increase the percentage of green fruit at harvest (Koo, 1988). Phosphorus deficiencies are rare, but result in good rind colour (Reitz and Embleton, 1986; Ritenour et al., 2003).

2.3.2.3 Potassium (K)

Trees fertilized with high K levels produced more late-maturing, poorly coloured fruit (Reuther and Smith, 1952; Sites and Deszyck, 1952; Reitz and Koo, 1960; Koo, 1988). A low concentration of K fertilisation produced a high percentage of early-maturing, well-coloured fruit (Reuther and Smith, 1952). Nitrogen and K were found to have an additive effect on rind colour, resulting in greener fruit. High N levels were especially disadvantageous to good rind colour, whereas K levels above that which is yield-limiting only had a moderate effect on rind colour (Rietz and Embleton, 1986). The effect of K was greater at high N levels (Reuther and Smith, 1952). Trees treated with high concentrations of K (1.7% K in the leaf behind the fruiting terminal) did not develop good rind colour, even at harvest, whereas fruit from low K (0.8% K in the leaf behind the fruiting terminal) plots were well-coloured at harvest. Trees from plots with high N (1.4 kg per year) and low K (0.11 kg per year) levels had a redder orange colour on the exposed sides of fruit than fruit which came

from low N (0.34 kg per year) and low K plots (Reuther and Smith, 1952). Increased K gave 14% more green fruit (Koo and Reese, 1977).

2.3.2.4 Micronutrients

Most micronutrients, except Fe, were found to not have an effect on rind colour (Koo, 1988). However, Mn, Zn, Cu and B have been shown to have a slight effect on rind colour (Table 2.2). It appears that only low levels of Fe has an inhibitory effect on rind colour (Koo, 1988).

Table 2.2. Effect of micronutrients on percentage of green fruit at harvest (Koo, 1988).

Manganese		Zinc		Copper		Boron		Iron	
+	–	+	–	+	–	+	–	+	–
26.7%	23.7%	25.4%	22.7%	25.4%	16.2%	25.4%	19.8%	13.1%	21.3%

2.3.2.5 Reducing and non-reducing sugars

Total sugar concentration in the centre of the epicarp appeared to be low when chlorophyll content was high in that portion of rind and high when chlorophyll levels were low (Huff, 1984). There is a close parallel between the loss or gain of chlorophyll and sugar concentrations indicating that sugars are important in affecting epicarp chlorophyll levels. Sucrose inhibits chlorophyll synthesis and promotes chlorophyll degradation (Huff, 1984). A parallel between sugar content of the flavedo and rind colour has been observed. The greater the flavedo sugar content, the greater the a/b Hunter value and the more well-coloured the fruit (Sala, 1992). In maturing fruit the transport rate of sugars into the fruit seems to decrease, which may lead to minor mobilisation of starch in the rind and the renewal of chlorophyll synthesis (El-Zeftawi and Garrett, 1978b).

The content of total sugars, glucose and fructose increases with fruit maturity. The reducing sugars/non-reducing sugars ratio increased progressively as fruit from cuttings irrigated with any of the nutritive glucose or fructose solutions matured, although it was greater in the flavedo of fruit from cuttings irrigated with nitric nutritive solutions (Sala et al., 1992).

After the removal of the epicarp of mature sweet orange fruit from a media culture with sucrose, degreening did not occur. If the fruit had previously degreened, then regreening did occur. Sucrose solutions added to the growth media prevented regreening of the flavedo (Huff, 1984). There is a parallel between chlorophyll loss and sugar accumulation. The coupled re-accumulation of chlorophyll and loss of sugar in the epicarp of sweet orange fruit shows that carbohydrate metabolism plays a role in the regulation of chloroplast-chromoplast interconversion and high sugar concentration in the flavedo causes the transformation of chloroplasts to chromoplasts (Huff, 1984). A high concentration of N in the epicarp decreases the effects of high sugar concentrations and causes the transformation of chromoplasts to chloroplasts which thus retards degreening and promotes regreening (Huff, 1984).

The removal of *Citrus* leaves from the tree resulted in the halting of sucrose build-up, a decline in N and retarded colour change. Fruit on completely defoliated trees never achieved an orange colour, remaining green, whereas the fruit rinds of trees having received a sucrose supplement, generally had a more orange colour (Iglesias et al., 2001). Sucrose treatments increased the sucrose contents of citrus rind, decreased N levels and favoured colour break (Iglesias et al., 2001).

2.3.3 Hormonal

Chlorophyll degradation and carotenoid biosynthesis take place simultaneously in chloroplasts and have different responses to exogenous hormones (Garcia-Luis et al., 1986). The control between chloroplast and chromoplast interconversion is largely hormonal, resulting from a balance between gibberellins, which maintain functional chloroplasts, and ethylene, which leads to the development of chromoplasts (Eilati et al., 1969). Chlorophyll degradation was retarded as GA_3 concentration increased within a range of 0 to 240 μM, but was insensitive to cytokinins (Garcia-Luis et al., 1986). Gibberellins antagonise natural and ethylene-induced chlorophyll degradation in *Citrus* rind, whereas cytokinins are only antagonistic towards the effect of ethylene (Garcia-Luis et al., 1986).

Ethephon [(2-chloroethyl) phosphonic acid] is a preharvest spray or postharvest dip which evolves ethylene at above pH 4 and is a substitute for ethylene gas used in postharvest degreening (Fishler and Monselise, 1971). Fruit treated with ethephon at 600 $mg.l^{-1}$ changed colour more rapidly than untreated fruit (Fishler and Monselise, 1971). Ethephon-induced chlorophyll degradation is antagonised by gibberellins and cytokinins, which may indicate that ethylene is not the primary catalyst for natural *Citrus* colour change (Apelbaum et al. 1976).

2.3.3.1 Ethylene

Ethylene (C_2H_4) is commonly used in *Citrus* production to induce chlorophyll degradation in *Citrus* rind, a postharvest handling practice referred to as degreening. Ethylene treatment causes an increase in chlorophyllase activity and a decrease in chlorophyll content in various plant tissues (Barmore, 1975). Natural ethylene evolution is low in *Citrus* fruit, which are non-climacteric, and a direct correlation between ethylene evolution and colour development

has been difficult to establish (El-Otmani and Ait-Oubahou, 1999). Chlorophyllase, which catalyses the hydrolysis of chlorophyll *in vitro* during exposure to ethylene, increases considerably during rind colour development.

The internal structure of the chloroplast breaks down during ethylene degreening and natural colour development. Membranes appeared to dilate and take on irregular spacing after 48 hours of ethylene exposure (Purvis, 1980). Exposure to ethylene caused the disappearance of grana and stroma lamellae which were concentrated around the fringes of the chloroplasts and were the last chlorophyll containing membranes to disappear. The degraded membranes developed into vesicles. Plastoglobuli accumulated and seeped from the chloroplasts into the cytoplasm and vacuoles (Purvis, 1980; Gross et al., 1983). These changes within the chloroplast only took place in fruit which had been exposed to ethylene.

Chlorophyllase is initially involved in the disruption of chloroplast membranes during rind colour development. In calamondin (*C. madurensis* Lour.) fruit, no visible change in colour or loss of chlorophyll occurred until ultrastructural changes in chloroplasts had taken place (Purvis, 1980). During degreening, chlorophyllase converts lipid-soluble chlorophyll into chlorophyllide, which is more water-soluble and is enzymatically bleached by peroxide activity (Aljuburi et al., 1979).

During ethylene degreening an inverse relationship was noted between chlorophyllase activity and chlorophyll content (Purvis, 1980). However, in rind of naturally degreened fruit there was not a consistent correlation between chlorophyllase levels and chlorophyll content (Purvis, 1980). Chlorophyllase activity in chlorophyll-free 'Valencia' oranges increased much more rapidly when exposed to ethylene than air. The ethylene-induced chlorophyllase

increase in chlorophyll-free rind indicates that this regulatory system is chlorophyll-independent (Hirschfield and Goldschmidt, 1983). Green calamondin fruit often have higher chlorophyllase levels than fruit which have lost half of their chlorophyll content, indicating that a high level of chlorophyllase alone may not cause degreening. Chlorophyllase and chlorophyll may be spatially separated until the chloroplast envelope becomes permeable, resulting from an ethylene-mediated process (Purvis, 1980).

Ethylene causes a rapid reduction in chloroplast size and eventually causes its complete disappearance, initiated by breakdown of the inner membrane system before other structures (Shimokawa, Shimada and Yaeo, 1978). Ethylene-activated enzymes degrade inner membranes, while chlorophyllase activity is stimulated by ethylene and leads to a decrease in chlorophyll content (Barmore, 1975). Ethylene treated fruit showed a complete loss of chlorophyll after 24 hours, while there was little change in the rind colour of control fruit, even after a period of 96 hours (Shimokawa et al., 1978b). Citrus fruit rind is less susceptible to ethylene treatment during specific periods, especially just before maturity or 'colour break' (Eilati et al., 1969) and after regreening when GA levels are high (Rasmussen, 1973).

Ethephon was also effective in increasing carotenoid production in fruit that had regreened on the tree (El-Zeftawi and Garret, 1978b). Chlorophyllase activity remained low during degreening, but increased after a 6 hour lag phase, and increased until 96 hours from the start of incubation. Control fruit had a consistently low level of chlorophyllase activity throughout the 96 hour period (Shimokawa et al., 1978).

Ethylene treatment to improve rind colour becomes ineffective under conditions of high CO_2 concentration, as CO_2 is a competitive inhibitor of ethylene (Shimokawa et al., 1978).

Cycloheximide (CHI), a protein inhibitor, reduced the effect of ethylene on chlorophyll degradation. The effect of ethylene on chlorophyll degradation was reduced by 80% and its effect on chlorophyllase was reduced by 60%. Ethylene can stimulate the *de novo* synthesis of chlorophyllase and thus cause chlorophyll degradation (Trebitsh et al., 1993).

Ethephon-treated fruit did not regreen and produced plastids with low proportions of lamellae and did not produce chlorophyll at the rate produced in chloroplasts of regreened fruit (El-Zeftawi and Garrett, 1978a). Ethephon reduced chlorophyll levels and increased starch levels in the plastids (El-Zeftawi and Garrett, 1978b).

Physiological conditions may lead to enhanced endogenous ethylene production after maturation. This may result from infestation of *Citrus* fruit by Mediterranean fruit fly (*Ceratitus capitata*) as these fruit become orange coloured prior to other fruit (Jacob-Wilk, 1999). Ethylene is able to overcome the inhibitory effects of GA_3 on colour development as these phytohormones act antagonistically (Eilati et al., 1969b).

The stimulation of colour development and chlorophyll degradation in *Citrus* by sucrose is mediated by ethylene, which indicates a link between nutritional and hormonal regulation in plastid conversion (Iglesias et al., 2001).

2.3.3.2 Gibberellins

Gibberellins were found to inhibit carotenoid and enhance chlorophyll synthesis during regreening, while ethephon had the opposite effect on carotenoids (El-Zeftawi and Garrett, 1978). Gibberellins favour vegetative growth with long, vigorous shoots and well-developed thorns as seen in juvenile plants (Cooper and Peynado, 1958), which coincides with immature

fruit with high chlorophyll levels, and remain greener than fruit from trees not exposed to high levels of GA (Cooper, 1958). The influence of gibberellins on carotenoids may be indirect, as they may promote the maintenance and synthesis of chlorophyll (Mackinney, 1961). Gibberellic acid (GA_3) delays colour development in *Citrus*, even at very low (0.1 $mg.L^{-1}$) concentrations (Eilati et al., 1969b; Coggins and Henning, 1988; Goldschmidt, 1988). The timing of GA_3 application is also important. When GA_3 was applied 2 weeks before harvest, there was a significant negative effect on colour development. When GA_3 was applied after acceptable colour development had occurred there were no negative effects on rind colour development (Coggins, 1981; Coggins and Henning, 1988). GA_3-treated fruit reached satisfactory colour levels only 5 weeks after untreated fruit (Eilati et al., 1969b).

Ethephon-treated fruit were found to have no chlorophyll, while GA-treated fruit did (El-Zeftawi and Garrett, 1978b). GA_3 application reduced, but did not prevent chlorophyll loss (Rasmussen, 1973) and reduced the rate of carotenoid accumulation resulting in fruit with a pale orange rind colour, contrasting with brightly coloured rinds of untreated fruit (Coggins, 1981). Gibberellins did not influence starch levels of green fruit, but increased the rate of starch loss in coloured fruit when compared to untreated fruit. Ethephon maintained higher starch levels (El-Zeftawi and Garrett, 1978b). GA_3 appeared to counteract the effect of sucrose on chlorophyll breakdown and was ineffective in the absence of sucrose (Iglesias et al., 2001).

Exogenously applied ethylene increases chlorophyllase activity leading to the disappearance of chlorophyll, while GA_3 partially counteracts the ethylene induced increase in chlorophyllase, causing a delay in chlorophyll breakdown and degreening (Trebitsh et al. 1993; Cooper and Henry, 1968). The application of GA_3 to trees resulted in fruit with a green

rind colour at harvest, whether sprayed alone or in conjunction with para chlorophenoxyacetic acid (PCPA) or benzyladenine (BA) (Cooper and Henry, 1968). GA_3 was shown to reduce the sucrose induced effect on colour (Iglesias, 2001).

The increase in chlorophyll and decrease in carotenoids and starch in regreening fruit correlates with an increase in plastid lamella (El-Zeftawi and Garrett, 1978a). Ethephon treatment increased carotenoids and decreased chlorophyll, while GA did the opposite and 2,4-D, an auxin, had no effect (El-Zeftawi, 1978). Potassium gibberellate was found to enhance the accumulation of chlorophyll, where treated fruit had a minimum of three times more chlorophyll *a* + *b* than control fruit (Coggins and Lewis, 1962). Chlorophyll *a* was more strongly influenced and showed a greater increase in concentration than chlorophyll *b*. GA has a marked effect on pigment level and inhibits carotenogenesis (El-Zeftawi, 1978). GA_3 is antagonistic to the ethylene-induced loss of chlorophyll in mature 'Valencia' oranges (Garcia-Luis et al., 1986).

Chlorophyll and carotenoid changes during chloroplast-chromoplast transformation are a loosely paired parallel series (Garcia-Luis et al., 1986; Goldschmidt, 1988). GA_3 retards senescence of *Citrus* fruit, especially the subepidermally situated chloroplasts and favours regreening (Cooper and Henry, 1968). Gibberellin-like substances decrease rapidly in the flavedo in mature green fruit when treated with ethylene (Goldschmidt, 1976). This may be an indication that ethylene may not trigger colour change, but simply increases the rate of processes which already occur (Purvis, 1980; Garcia-Luis et al., 1986). GA-treated fruit showed regreening at approximately the same rate as untreated fruit (El-Zeftawi and Garrett, 1978b). GA did not affect the starch content of green fruit, but accelerated the loss of starch in coloured fruit compared to untreated fruit (El-Zeftawi and Garrett, 1978b).

Colour change in non-climacteric fruit, coupled with low levels of ethylene throughout maturation, appears to be controlled by a dual hormone system made up of ethylene (an activator) and GA (an inhibitor). After natural reduction of endogenous GA, colour change may be stimulated by low levels of endogenous ethylene as a result of *de novo* synthesis of chlorophyllase (Iglesias et al., 2001). GA plays a regulatory role by inhibiting chlorophyllase synthesis and controlling the timing of chlorophyll degradation (Iglesias et al., 2001). Gibberellins play a major role in regulating the conversion of chloroplasts to chromoplasts and the reversion of chromoplasts to chloroplasts, and the speed of plastid conversion in either direction is temperature dependent (Coggins and Jones, 1977).

Paclobutrazol, a gibberellin biosynthesis inhibitor, showed a marked improvement in rind colour at harvest, compared to fruit from control trees (Gilfillan and Lowe, 1985). Paclobutrazol restricts vegetative growth via the inhibition of GA biosynthesis and thus improves rind colour development (Aron et al., 1985). In turn, this would reduce cytokinin and gibberellin production, known antagonists of chlorophyll degradation, and favour senescence and carotenoid biosynthesis, thereby resulting in improved rind colour development.

2.3.3.3 Auxins

α-Naphthalene-acetic acid (NAA), applied at concentrations between 8 and 80 $mg.l^{-1}$ to green harvested 'Shamouti' oranges had almost no effect on rind colour development (Eilati et al., 1969a). In another experiment, ethylene evolution was stimulated by NAA application, although auxin is not required for ethylene production. This may be an explanation for the similarity in physiological responses of NAA and ethylene (Abeles and Rubinstein, 1964).

Immature tomato (*Lycopersicon esculentum* Mill.) fruit produced increased levels of ethylene after auxin (NAA) treatment, but the opposite was seen in mature fruit (Abeles and Rubenstein, 1964).

Ethychlozate (Figaron®) is an auxin compound shown to accelerate rind colour development and cause significantly increased carotenoid concentrations, while rapidly decreasing chlorophyll content (Kamuro and Hirai, 1981). Ethychlozate treatment caused a slow increase in ethylene production, but the positive effect on colour development was maintained over a longer period (Kamuro and Hirai, 1981). 2,4-dichlorophenoxyacetic acid (2,4-D), a synthetic auxin, causes a delay in the loss of chlorophyll and has little or no effect on carotenoid synthesis or accumulation (Coggins and Jones, 1977). Carotenoid content appeared to be favoured by 2,4-D at 50 $mg.L^{-1}$ although results were inconsistent (Mzini, 2002).

2.3.3.4 Abscisic acid

Abscisic acid (ABA) may stimulate ethylene production (Abeles and Rubenstein, 1964) and in this way lead to rind colour development. ABA accumulated in *Citrus* rind under natural and ethylene-induced maturation (Goldschmidt et al., 1973). *Citrus* fruit that matured on the tree, followed a pattern of ABA accumulation similar to that of green ethylene-treated fruit (Goldschmidt et al., 1973). Therefore, it appears that there is a relationship between ABA and ethylene evolution and interaction of these plant hormones affects rind colour development.

ABA concentrations in mature *Citrus* flavedo are amongst the highest reported (1 to 2 $\mu g.g^{-1}$) and occur mainly in the bound form (Goldschmidt et al., 1972; Goldschmidt, 1976). ABA increased throughout the period of colour development and reached a maximum concentration

at colour-break, inhibiting full colour development (Richardson and Cowan, 1995). The content of ABA in flavedo increases throughout *Citrus* fruit development and maturation, and reaches a high level by autumn as ABA accumulates under short-day conditions (Kefeli and Kadyrov, 1971; Brisker et al., 1976). Full colour development only occurs after a decrease in ABA levels, probably resulting from increased ABA metabolism and the formation of ABA conjugates (Harris and Duggar, 1986). Carotenoid concentration of *Citrus* flavedo can increase four-fold during colour development (Gross, 1981), and there is a close relationship between ABA content and colour development in the *Citrus* flavedo (Goldschmidt, 1976). Rind disks treated with 0.1 μM ABA had significantly higher carotenoid levels than untreated disks 96 hours after treatment (Oberholster, 2001).

2.3.3.5 Cytokinins

Benzyladenine (BA) caused a delay in orange colour development in *Citrus* rind and maintained chlorophyll in detached leaves, delaying senescence (Eilati et al., 1969b). Cytokinins play an important role in root-canopy relations, which affects maturation and changes in rind colour (Eilati et al., 1969b). High cytokinin levels favour vegetative growth and juvenile characteristics leading to fruit which remain green and growth flushes in the canopy. Low cytokinin levels result in the opposite tree characteristics. GA-treated and BA-treated fruit did not develop as high carotenoid concentrations as untreated or ABA treated fruit (Rasmussen, 1973).

2.4 Postharvest manipulation of rind pigments

2.4.1 Ethylene degreening

Degreening causes the artificial breakdown of chlorophyll and simulates natural colour break. This process allows fruit to be harvested earlier than what would be possible under natural

conditions and advances the harvesting period (Poole and Gray, 2002). During degreening chlorophyll levels decreased sharply with time, while total carotenoid content remained almost constant (Eilati et al., 1969b; Jimenez-Cuesta et al., 1981). The aim of degreening is to reduce the period a producer has to wait for internally mature fruit to reach an acceptable colour for harvest and reach market acceptability (Krajewski and Pittaway, 2001). Degreening should only be applied once the fruit has reached an acceptable level of internal maturity, preventing the consumption of poor quality fruit (Pool and Gray, 2002).

Fruit can be safely harvested once colour-break has taken place and degreening can be implemented to break down the chlorophyll, resulting in an orange rind colour. However, degreening accelerates senescence by increasing the rate of respiration, transpiration, button dehydration and increases decay (Cohen, 1977). As a result of the consumer preference for orange coloured fruit, the industry encourages degreening although this is often at the expense of shelf-life and internal quality (Poole and Gray, 2002).

Degreening is brought about by a rapid colour change and is confirmed by the decrease in absorbance values of the flavedo of fruit treated with ethylene. This was found after a 3 day ethylene treatment although green patches were still visible. A further 2 day ethylene treatment resulted in further colour change, but did not remove all green colour. Further storage resulted in very little colour change (Oberbacher, 1962).

Degreening with ethylene causes an increase in the respiration rate of the fruit, promotes senescence and increases susceptibility to decay (McGlashan and Potgieter, 2002). The respiration rate of degreened fruit was higher than that of non-degreened fruit. At the end of

the degreening treatment the respiration rate dropped sharply, but remained higher than non-degreened fruit (Cohen, 1977).

The temperature of the degreening process appears to influence the removal of green colour and the development of orange rind colour (Cohen, 1978). Ethylene gas degreening is temperature sensitive and careful control is necessary (Gilfillan, 1988). A higher degreening temperature results in a more rapid change in rind colour (Cohen, 1978; McGlashan and Potgieter, 2002) and increases the risk of a pale eventual rind colour, while a more natural, brilliant colour is achieved under lower temperatures (McGlashan and Potgieter, 2002). The opposite effect was found by Cohen (1978) with results indicating that fruit degreened at 25 °C reached an orange rind colour, whereas fruit degreened at 20 °C became yellow-coloured. At high temperatures (35 °C) fruit reached a pale yellow colour after 60 hours of degreening and further colour development did not take place after degreening whereas this did take place in fruit degreened at 25 °C (Cohen, 1978).

In fruit picked at the same stage of maturity, when still green, and degreened at either 25 °C or 30 °C, it was found that a final orange colour was only reached in fruit degreened at 25 °C. It appears that temperatures of 30 °C and higher retard carotenoid synthesis (Cohen, 1978).

Chlorophyll degradation and colour improvement of 'Robinson' mandarin rind increased during continuous exposure to 7.7 $\mu l.l^{-1}$ ethylene. Colour change was greater after the first 24 hour period and degreening continued for 24 hours after removal from the degreening room (Purvis and Barmore, 1981).

Carbon dioxide levels must be kept below 0.3% as high concentrations slow the rate of degreening as CO_2 is a competitive inhibitor of ethylene by competing for the same binding sites as ethylene (Salisbury and Ross, 1992), and could reduce the shelf life of sensitive fruit (Krajewski and Pittaway, 2001).

2.4.2 Shipping temperature

Shipping temperature has a strong effect on eventual fruit colour and is cultivar and colour dependent (Capespan Technology Development, 2001). Shipping temperature is one of the final interventions which can be used to improve *Citrus* rind colour. Typical shipping temperatures of 4.5 °C and 11 °C are used by the South African citrus industry. The temperature used depends on the fruit colour at packing and the destined market. Higher shipping temperatures result in a greater improvement in rind colour (Gilfillan, 1988).

Certain export markets require sub-zero shipping temperatures to satisfy the phytosanitary requirements of those countries. When exporting fruit from South Africa to the United States of America it is necessary that the fruit undergo cold-sterilisation at -0.6 °C for a minimum of 22 days to eradicate possible infestation of fruit fly (*Ceratitus capitata*) or false codling moth (*Cryptophlebia leucotreta*) larvae (Maritz, 2000).

Fruit shipped at -0.5 °C showed no colour development (Le Roux, 1997). Some export companies have stricter rind colour requirements for fruit shipped at sub-zero temperatures as colour development is inhibited (Maritz, 2000). Fruit shipped at 4.5 °C eventually developed a yellow rind colour, while fruit shipped at 11 °C had an orange rind colour at arrival in the market (Le Roux, 1997). Shipping at 8.5 °C or 11 °C instead of 4.5 °C when 'Satsuma' mandarin (*C. unshiu* Marc.) fruit were slightly green resulted in the best colour development

on arrival at the market (Gilfillan, 1988). It may be favourable to ship early season fruit with a poorer rind colour at 11 °C, while late season fruit with a better rind colour should be shipped at 4.5 °C to maintain internal fruit quality and external rind colour (Le Roux, 1997). At sub-zero shipping temperatures fruit sometimes become yellower instead of the desired orange colour (Koch, personal communication). In some cases, fruit shipped with an orange colour arrive at their destination with a pale yellow appearance. It is hypothesised that carotenoid breakdown occurs at sub-zero shipping temperatures resulting in pale fruit after shipment.

2.4.3 Storage temperature

Carotenoid synthesis in *Citrus* is temperature sensitive. An optimum range for synthesis and accumulation appears to be between 15 and 25 °C, whereas fruit kept at 30 °C showed less colour development (Wheaton and Stewart, 1973). The colour of cool-stored fruit was better than fruit kept on the tree towards the end of the season (Wheaton and Stewart, 1973). This was mainly due to higher carotenoid and lower chlorophyll levels, whereas fruit held on the tree had much higher chlorophyll and lower carotenoid levels (El-Zeftawi, 1976).

Le Roux (1997) showed that it was advantageous to expose fruit to a post-shipping holding temperature of 20 °C to improve rind colour. Carotenogenesis was apparently enhanced and chlorophyll synthesis was inhibited when fruit were stored at 15 °C, whereas the opposite occurred at 25 °C. Carotenoid levels of fruit stored at 5 °C were higher and chlorophyll levels were equal to fruit stored at 25 °C. Over time, the highest temperature maintained chlorophylls and inhibited carotenogenesis in stored fruit.

2.5 Quantification of rind colour

The perception of colour involves the interaction between the light incident on an observed object, the spectral reflectance of the object, and the spectral sensitivity of the human eye. If any of these factors were to change, this could result in a change in observed colour (Voss and Hale, 1998). Rind colour of *Citrus* fruit can be measured subjectively or objectively. Subjective measurement of rind colour generally involves some form of rating system, but is subject to human perception. Objective measurement of rind colour requires instrumentation to measure colour variables or to determine the quantity of specific pigment components, and is a more accurate method of measurement. An objective colour measurement is necessary to account for perceived colour. The measured value needs to relate to perceived colour to be understood. When relating the two concepts, it is critical to understand how the human eye sees colour (Francis, 1980).

2.5.1 Subjective measurements: Colour rating

A colour rating chart is a set of colour prints, made up of a series of photos of citrus fruit ranging in degree of colour development from intense, fully coloured fruit (T1) to poorly coloured, dark green fruit (T8) (CRI, 2004a; b; Appendix 1; Appendix 2). When using the South African colour rating chart, rind colour is classified from dark intense green (T8) ranging through fruit at colour break (T7) (where the chlorophyll has started to break down and reveal the underlying orange colour) to brightly coloured orange fruit without any visible chlorophyll (T1) (Table 2.3).

A colour rating chart is a more reliable method to quantify rind colour than a description of colour. Actual fruit can in this way, be compared to a specific colour print depicting a specific degree of colour development. Descriptors can be unreliable and inconsistent as a

measurement of rind colour since a decision must be made. Different users may interpret a specific colour description in various ways.

Factors that are inherent to a colour chart can affect the perceived colour, i.e. the paper a chart is printed on and the exact colour of ink used may vary including, external factors such as lighting and angle of view. These factors can lead to an inaccurate judgement of rind colour, which may vary under differing conditions.

Table 2.3. Descriptors used for colour ratings in the South African rind colour chart (adapted from Capespan, 2001) (also see Appendix 1 and 2).

Rating	**Descriptors**
8	Blue green/avocado green. Dark green immature colour with no colour break.
7	Colour break has taken place.
6	Green and yellow/orange is in equal ratios. Typically a green fruit streaked with yellow/orange.
5	Yellow/orange is becoming dominant over the green.
4	Yellow/orange strongly dominant but patches of green or strong green undertone still present.
3	Yellow/orange with a slight green tinge.
2	Light yellow/orange with no green tinge at all.
1	Fully coloured fruit at its deepest intensity

2.5.2 Objective measurements

The Commission Internationale d'Eclairage X Y Z (CIELAB L*, a*, b*) and Hunter (L_L a_L b_L) are the scales popularly used, and rely on the mathematical relationship between the parameters for a specific colour index (McGuire, 1992). These systems both locate a point in a three-dimensional colour space. The measured colour is then related to visually perceived colour. Instrumental measurement provides an easily replicated description of colour as it avoids the effects of the spectral characteristics of changing daylight and artificial light sources (Voss, 1992).

2.5.2.1 Colorimeter

Colour is made up of three components, viz. lightness (L), chroma (c) and hue angle (h°). The a* parameter measures the difference of light reflected by the object in the red and green zones of the spectrum (Ihl et al., 1994). Positive values of "a" indicate red colours, and negative values indicate green colours. Negative values of "b" indicate blue colours and positive values indicate a yellow colour (Fig. 2.11) (Jimenes-Cuesta et al., 1981). Tristimulus measurement of colour is widely used because it is a non-destructive method of measurement and the same area of rind can be measured repeatedly. Plant material with a specific colour falls within a certain range of hue values and a specific quadrant in the three dimensional colour space.

Hue angle (h°), calculated from the a* and b* values ($H = \tan^{-1} b^*/a^*$), refers to the angle formed by the line from the origin of the intercept on the a* and b* co-ordinates on an x- and y-axis, where 0°=red, 90°=yellow, 180°=green and 270°=blue (McGuire, 1992).

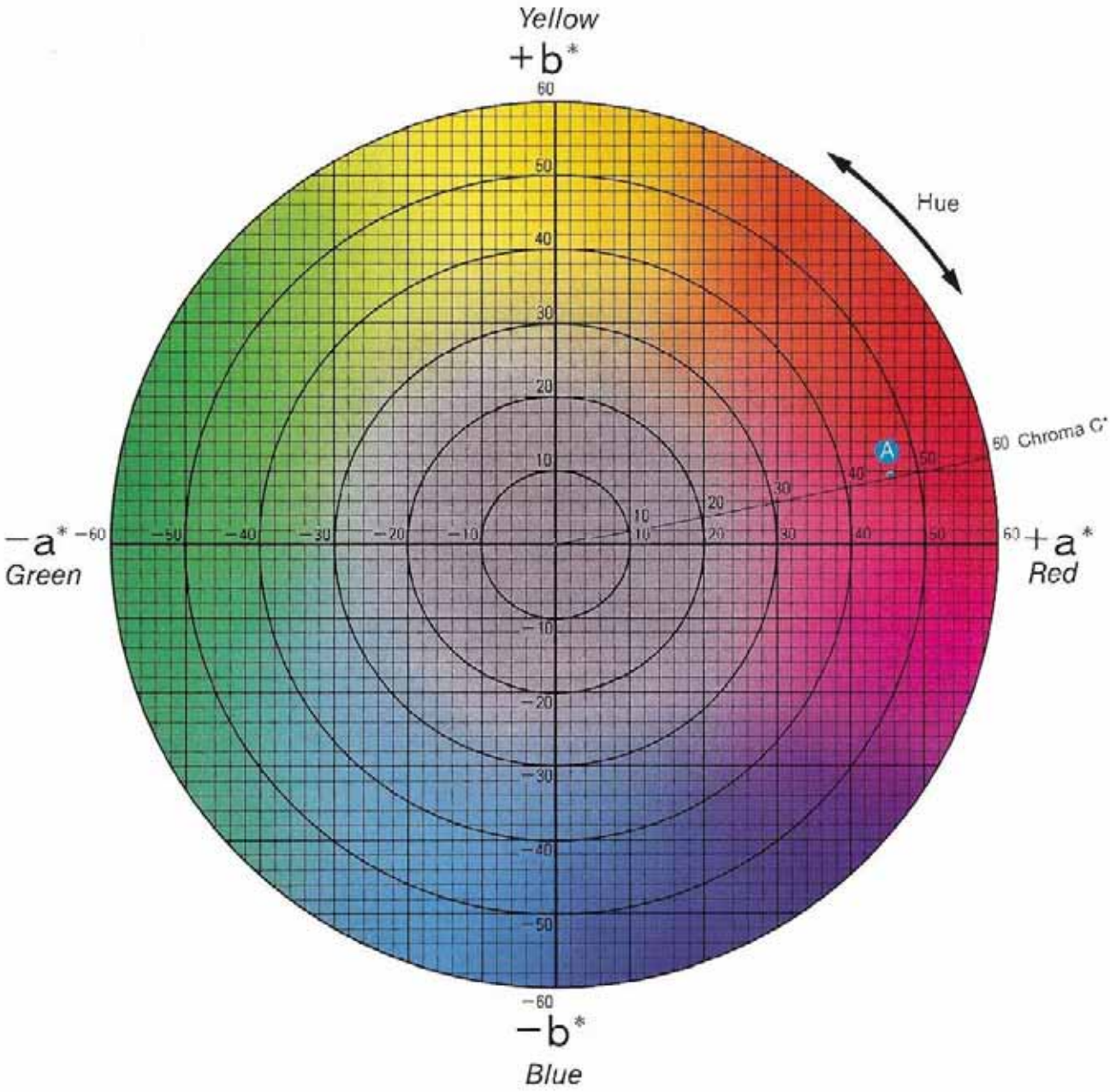

Fig. 2.11. Schematic representation of the three-dimensional colour space.

Hue angle decreases as citrus rind colour changes from green to orange. When interpreting h-values, it is expected that as fruit mature rind colour changes from green (180°) moves through yellow (90°) and approaches orange, moving away from 90° (yellow) towards 0° (red), orange is thus somewhere between yellow and red depending on the shade, i.e. 60 to 70°. Hue represents actual perceived colour, i.e. orange or green, and is the primary variable in changes in orange colour, while chroma and lightness are less important (Stearns and

Young, 1942). Chroma or saturation index quantifies the intensity or saturation of the measured colour or the movement away from grey towards a pure colour in the three-dimensional colour space. Lightness refers to the proportion to light reflected from the object on a scale of zero (black) to 100 (white) (Little, 1976).

A direct relationship between measured colour and pigment content is expected. However, in cases where more than one pigment is present in the plant material a good correlation between pigment content and colour measurement does not exist (Lancaster et al., 1997). This would also be true in the case of *Citrus* rind where carotenoids and chlorophyll are responsible for the observed colour. Lancaster et al. (1997) showed a linear relationship between log (chlorophyll content) and L-value.

2.5.2.2 Pigment content

A spectrophotometer is used to measure total pigment concentration by measuring pigments at their absorption maxima, i.e. carotenoids at 447 nm, chlorophyll *b* at 642 nm and chlorophyll *a* at 664 nm.

The quantitative determination of carotenoids is very complicated because of the innate properties of carotenoids. Carotenoids are subject to isomerisation and decomposition and need to be handled in a way that minimises their exposure to air, light, high temperatures and reactive chemicals (Stewart, 1977).

By using Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) techniques, quantitative pigment analysis can accurately differentiate and quantify individual rind pigments (Britton, 1997). TLC is used to separate carotenoids depending on

their inherent characteristics. The various bands can then be isolated to be identified and quantified by means of HPLC. HPLC provides the most sensitive, precise and reproducible method for quantitative analysis of carotenoids (Britton, 1997).

2.6 Integration of factors affecting rind colour

Changes in rind pigments appear to be largely under hormonal control and greatly enhanced or retarded by the levels of specific hormones. Ethylene is central to rind colouration, causing chlorophyll degradation and the synthesis and accumulation of carotenoids. Ethylene is linked to senescence, which is closely coupled with rind colour development by means of chlorophyll degradation. Increased auxin levels allow fruit to compete more effectively for metabolites and appear to be linked to increased ethylene evolution and rind colour development.

Colour change in non-climacteric fruit, coupled with low levels of ethylene throughout maturation, appears to be controlled by a dual hormone system made up of ethylene (an activator) and GA (an inhibitor). GA and cytokinins are associated with juvenility, the maintenance of the vegetative phase and chlorophyll synthesis and thus delay fruit maturation. Gibberellins favours regreening of *Citrus* under environmental conditions favourable for vegetative growth, viz. high temperatures, sufficient soil moisture, high light and N levels. Under higher soil temperatures (>12.8 ˚C), root activity is stimulated leading to the uptake and translocation of N-containing compounds and production of phytohormones (which are antagonistic to chlorophyll degradation and carotenoid biosynthesis) by the roots. Whereas, under low soil temperatures (<12.8 ˚C) there is cessation of root growth, leading to reduction in the uptake of N-containing compounds and inhibition of hormones associated with juvenility and chlorophyll production.

The orange colour of *Citrus* rind can only be exposed once the masking chlorophyll pigments, housed in chloroplasts, have undergone degradation. Chlorophyll degradation is favoured by low light intensity, low temperatures, high carbohydrate (sugar) and ethylene levels coupled with low GA and cytokinin levels, low N concentrations and soil water levels which are suboptimal for maximal fruit production.

There is clearly a key interaction between environmental and nutritional factors which influences the endogenous hormone levels and leads to either the promotion of senescence or juvenility in the *Citrus* tree and thus affects the specific pigment composition of the flavedo resulting in a certain rind colour.

CHAPTER 3

LOW-TEMPERATURE COLD SHOCK INDUCES RIND COLOUR DEVELOPMENT OF *CITRUS* FRUIT

Abstract

To simulate a drop in temperature resulting from a cold front, fruit were cooled post-harvest. Hydrocooling was used to rapidly reduce the rind temperature. The fruit were then subjected to 6 hours at 4 °C followed by an incubation period of between 20 and 22 °C for up to 72 hours. The effect of low temperature treatment or "cold shock" on rind colour development was evaluated on various *Citrus* spp. In the 2002 season, cold shock of 'Nules Clementine' mandarin improved rind colour to a level comparable with degreening. Pigment data showed that the carotenoid concentration of cold shocked fruit was similar to that of degreened fruit and more than 50% higher than the control. Chlorophyll concentrations of cold shocked and degreened fruit were low, whereas the chlorophyll concentrations of control fruit where five times higher than that of cold shocked fruit. However, this result could not be repeated.

Introduction

Rind colour of *Citrus* spp. is largely determined by prevailing weather conditions during fruit maturation. Two temperature effects on rind colour of *Citrus* spp. are important, namely, the requirement for optimum temperature favouring biochemical processes affecting chlorophyll catabolism and carotenoid biosynthesis, and the effect of temperature on the entire tree which influences hormone levels in the fruit via the roots (Goldschmidt, 1988). The most favourable temperature combination for chlorophyll degradation and carotenoid biosynthesis leading to a bright orange *Citrus* rind are mild days, cold night air temperatures and cool soil temperatures (Young and Erickson, 1961). For example, the rinds of fruit grown under tropical conditions

do not become fully coloured, but change from a dark, solid green to a pale green colour, coinciding with the onset of maturity or "colour break" (Erickson, 1960; Reuther and Rios-Castano, 1969; Samson, 1980).

In temperature experiments, Erickson (1960) showed that fruit exposed to a day temperature of 20 °C and a night temperature of 5 °C developed a uniform orange colour. In contrast, a day temperature of 30 °C and a night temperature of 10 °C produced less well-coloured fruit. It is thought, that high day temperatures may inhibit colour development even if night temperatures are adequately low. Higher day temperatures (30 °C) produced fruit with lower carotenoid and higher chlorophyll levels. Fruit grown under more extreme conditions with large day-night fluctuations produce crops of bright, orange coloured fruit (Stearns and Young, 1942; Agusti, 1999). Air temperature below 13 °C has been observed to cause degradation of chlorophyll and thus reveal underlying carotenoids, giving fruit a bright orange colour (Young and Erickson, 1961; Reitz and Embleton, 1986). As air and soil temperatures fall below 15 °C, chlorophyll degradation takes place as chloroplasts are converted to carotenoid-containing chromoplasts.

Under orchard conditions, a rapid drop in temperature resulting from a "cold front" is often associated with the onset of so-called "colour break". A "cold front" is a boundary between air masses with different densities, usually as a result of temperature differences, and typically results in a rapid drop in temperature.

To simulate this phenomenon in the laboratory, Oberholster (2001) exposed 'Valencia' sweet orange (*C. sinensis* [L.] Osbeck) flavedo discs to 4 °C for various periods, ranging from 2 to 10 hours, and then incubated the discs at 22 °C for up to 96 hours. Rind colour was improved

after an incubation period of 24 hours and the improvement in colour was even more pronounced after 96 hours. It appears that carotenoid accumulation was enhanced in flavedo discs following cold shock. Duration of cold shock appears to be of secondary importance to the intensity of cold, but further evaluation is required. The effects of cold shock had only previously been tested on rind discs in the laboratory, making research on whole fruit necessary.

Hydrocooling is an effective technology to rapidly cool fruit (Thompson et al., 2002), whereas forced-air cooling leads to a more gradual drop in temperature (Brosnan and Sun, 2001). For example, hydrocooling took <1 hour to decrease the temperature of oranges by 88%, whereas forced-air cooling took between 1.5 and 3 hours to achieve the same decrease in temperature, depending on the position of the fruit in the package (Teruel et al., 2003). In addition, hydrocooling resulted in more uniform temperature within the package than forced-air cooling. Therefore, hydrocooling was applied to whole citrus fruit in an attempt to simulate natural "cold front" conditions. The objective of this study was to determine whether a postharvest cold shock treatment could enhance rind colour of early-maturing *Citrus* spp.

Materials and Methods

Sites and plant material. In the 2002 season, 'Nules Clementine' mandarin (*C. reticulata* Blanco) and 'Bahianinha Navel' sweet orange (hereafter referred to as 'Nules' and 'Bahianinha', respectively) were selected from the Del Monte packhouse in Gouda, Western Cape, South Africa (33°19′S, 19°03′E). This trial was repeated during November 2002 in Bakersfield, California, USA with fruit grown in Maricopa (35°04′N, 119°24′W). 'Nules' and 'Becks Early Navel' sweet orange (hereafter referred to as 'Becks'), were selected from

the Sun Pacific packhouse. In the 2003 season, only 'Nules' was used from the Del Monte packhouse in Gouda.

Treatments and experimental design. The fruit were subjected to hydrocooling using a commercial hydrocooler (MBB Engineers & York Refrigeration, Cape Town, South Africa). The thermostat of the hydrocooler was set to cool water to between 1 and 2 °C. Fruit were hydrocooled for 30 minutes, until the fruit rind temperature was <4 °C. Directly after hydrocooling, fruit were transferred to a cold room set at 4 °C for 6 hours to complete the cold shock treatment. After the cold shock period, fruit were incubated at 20 °C for approximately 72 hours or for a period equal to that of degreening to synchronise the treatments.

Degreening was applied using standard commercial practices of 95% relative humidity, 23 °C, an ethylene concentration of 2 ppm and a carbon dioxide concentration lower than 0.3% (Krajewski and Pittaway, 2002). Degreening was applied for 60 hours to 'Nules' and 72 hours to 'Bahianinha' fruit. All experimental fruit were then subjected to standard packhouse treatments, including fungicide application, waxing and packing. The fruit were drenched with 2,4-D (2,4-dichlorophenoxyacetic acid) (125 $mg \cdot L^{-1}$), Tecto® (thiabendazole) (500 $mg \cdot L^{-1}$) and Sporekill® (dimethyldidecyl ammonium chloride) (120 $mg \cdot L^{-1}$). Fruit were stored under simulated shipping conditions of -0.6 °C for an average of 28 days, complying with the phytosanitary requirement for shipping fruit to the USA which requires that fruit be kept at -0.6 ° C for a minimum period of 22 days (Maritz, 2000). Fruit were then exposed to a holding temperature of 4.5 °C for 7 days, followed by an extended shelf life at 15 °C for a minimum of 14 days.

Fruit used to monitor changes in rind and pulp temperature were selected randomly within the treatments, and thermocouple wires were inserted either into the pulp or superficially under the fruit rind and secured with insulation tape. Thermocouple wires were then connected to a data logger (Squirrel logger, 1200 series, Grant Instruments, Cambridge, England) to log temperature. Thermocouples were also attached to the water inlet and outlet.

Two hundred fruit of 'Nules' and 'Bahianinha' were sorted into two colour categories by visually selecting fruit according to colour categories T4 and T5, which are a series of photographs depicting various stages of rind colour development for oranges and mandarins (CRI, 2004a; b) (Appendix 1; Appendix 2). Within each colour category, 30 fruit were randomly allocated to six replicates which were randomly allocated to treatments in a completely randomized block design. Treatments included an untreated control, standard degreening, cold shock, and a combination of cold shock and degreening.

Data collection. Rind colour. Ten fruit were randomly selected from each of the replicates, each having a T4 and T5 initial colour category per treatment. A circle was drawn at the equatorial position of 10 fruit using a permanent marker to ensure that consecutive colour measurements were made at the same position at each evaluation date, thereby minimising variation of rind colour from one position on the rind to another. Rind colour was quantified objectively (Lightness, Chroma and Hue angle) using a colorimeter (Model NR-3000, Nippon Denshoku Kogyo, Tokyo) and subjectively according to the "CRI colour charts, set no. 34 or 36, 1997" for oranges and mandarins, respectively (CRI, 2004a; b; Appendix 1; Appendix 2). Visual observations relating to colour intensity and general appearance were also noted. Rind colour was measured directly after treatment, after 6 days at -0.6 °C, after 28 days at -0.6 °C, after 7 days at 4.5 °C, and after 14 days at 15 °C.

Rind pigments. The outer, coloured (flavedo) portion of the rind was removed with a sharp knife or kitchen peeler, ensuring that the white (albedo) section was not included in the sample. Rind samples were collected from eight fruit from each of all six replicates and combined to form one rind sample per treatment. These pooled rind samples were immediately immersed in liquid nitrogen and roughly ground in a pestle and mortar. The frozen rind was then stored at -80 °C. Once completely frozen for a minimum period of 1 day, the rind was freeze-dried at an initial temperature of -38 °C, until all moisture had been removed from the samples. This process was complete after 4 days. The dry sample was then milled with a blender (Waring, Torrington, Conn., USA) until fine and homogenous. Samples were then stored in plastic vials at -80 °C to inhibit enzymatic degradation of the carotenoids and chlorophyll. Preparation of samples was carried out under low light conditions to inhibit carotenoid and chlorophyll degradation.

A 0.2 g sub-sample of the freeze-dried rind was added to 10 ml of 95% (v/v) aqueous ethanol solvent containing butylated hydroxytoluene (BHT) (100 $mg \cdot L^{-1}$) and diethyldithiocarbamate (DDC) (200 $mg \cdot L^{-1}$) antioxidants to prevent carotenoid degradation. The samples were vortexed for two 1-minute bursts and stored at 4 °C to extract pigments for 1.5 hours. Following pigment extraction the extracts were poured through ashless filter paper (Schleicher & Schuell, Dassel, Germany) to remove rind particles. The extracts were poured into disposable plastic cuvettes, placed in a spectrophotometer (Cary 50 conc UV-visible spectrophotometer, Varian Australia (Pty) Ltd, Mulgrave, Victoria, Australia), and readings were taken at 470, 649 and 664 nm. A cuvette filled with the ethanol/antioxidant solvent was used as a standard to calibrate the spectrophotometer. Absorbance values were calculated to determine chlorophyll a (C_a), chlorophyll b (C_b), total chlorophyll (C_{a+b}) and total carotenoid

(C_{x+c}) concentration all measured as mg/g dry weight, using the Lichtenthaler equations (Lichtenthaler, 1987):

$$C_a = 13.36\ A_{664} - 5.19A_{649}$$

$$C_b = 27.43A_{649} - 8.12A_{664}$$

$$C_{a+b} = A_{664} + 22.24A_{649}$$

$$C_{x+c} = \frac{1000\ A_{470} - 2.13\ C_a - 97.64\ C_b}{209}$$

Statistical analysis. Data were subjected to analysis of variance using the general linear model (GLM) procedure of Statistical Analysis Systems (SAS Inc., Cary, N.C.), and means were separated using Fisher's LSD.

Results

Interpretation of the cooling curve. Hydrocooling rapidly reduced rind and pulp temperature from ambient (~18 °C) to <2 °C and ~5 °C, respectively, within 30 minutes (Fig. 3.1). The set-point temperature of the hydrocooler was 0 °C. After hydrocooling, rind temperature increased to ~6 °C, probably because of the higher pulp temperature and exposure to warm ambient temperatures between the hydrocooler and the cold room. During the transfer of the fruit to cold storage at 4 °C, rind temperature increased to ~9 °C within 15 minutes, then decreased gradually to ~4 °C in the cold room within ~5.5 hours.

Rind colour 2002 season. Cold shock resulted in a visible improvement in rind colour in 'Nules' (Fig. 3.2), as evidenced by significantly lower hue angle (Table 3.1; Fig 3.3) and higher lightness (Table 3.2) and chroma (Table 3.3) than the control. Hue angle values of cold shocked fruit were similar to those of degreened fruit. The hue angles of untreated fruit

remained significantly higher than all other treatments throughout the experimental period until those of initially T4 fruit were similar to all other treatments after 3 weeks at shelf life.

Large differences in pigments among the treatments supported visual observations of differences in rind colour due to treatment effects (Fig. 3.2) and colorimeter data (Tables 3.1 to 3.3; Fig. 3.3), although the rind pigment data could not be statistically analysed. Chlorophyll levels were nine times higher in untreated fruit than in any of the other treatments (Fig. 3.4). Carotenoid levels of untreated fruit were almost half that of cold shocked and degreened fruit, in both T4 and T5 fruit colour classes (Fig. 3.5). Within treatments, T4 fruit tended to have higher carotenoid and lower chlorophyll concentrations than T5 fruit.

Cold shock did not improve rind colour of 'Bahianinha', whereas degreening resulted in decreased hue angle (Table 3.4; Fig 3.6). Degreening did not have a consistent effect on lightness and chroma (Tables 3.5 and 3.6). Hue angle decreased throughout the evaluation period in all treatments (Figs. 3.6 and 3.7). The initial differences among treatments decreased during storage and the effect of initial colour at harvest was less marked by the end of shelf-life. Well-coloured fruit at harvest (T4 vs. T5) consistently resulted in better final rind colour irrespective of treatment, and was especially evident after shipping (Tables 3.4 to 3.6).

Cold shock of 'Nules' in California during November 2002 was unsuccessful in improving rind colour (Tables 3.7 to 3.9; Fig. 3.8). Hue angle of all treatments, including the control, decreased consistently during the three evaluation periods after treatment. Cold shocked and control fruit had similar hue values which were higher than the fruit from the degreened and combination treatments. Visually, cold shocked fruit were pale and yellow-coloured compared with degreened fruit, which had a more deep orange-coloured rind. On visual

observation, cold shock did not appear to increase the incidence of oleocellosis, but oleocellosis was more prevalent in degreened fruit (whether cold shocked or not). Cold shocked fruit kept at ambient conditions were firmer than degreened and control fruit. This may be a result of the exposure of cold shocked fruit to extra moisture and its absorption by the rind. Degreened fruit appeared more wilted than cold shocked fruit.

Cold shock of 'Becks' was also unsuccessful in improving rind colour (Tables 3.10 to 3.12). There was no visible improvement in rind colour of cold shocked fruit compared with other treatments. All of the treatments showed a consistent decrease in hue angle (Fig. 3.9). Throughout the evaluation period, cold shocked fruit had the highest hue angle and degreened fruit had the lowest hue angle. The rind of cold shocked fruit was more yellow-coloured than the degreened fruit which had a bright orange colour. Degreened and combination treated fruit had lower hue values than control and cold shocked fruit.

Rind colour 2003 season. Cold shock did not improve rind colour of 'Nules' fruit (Tables 3.13 to 3.15; Fig. 3.10). Cold shocked fruit had the highest hue angles whereas degreened and combination treated fruit had the lowest hue angles (Fig. 3.11). Hue angles in most treatments remained consistent during shipping and holding and decreased sharply during shelf-life at 15 °C. Cold shocked fruit were paler and less orange than degreened fruit.

Discussion and Conclusions

Cold shock of 'Nules Clementine' mandarin in the 2002 season reduced chlorophyll content to a similar extent as commercial ethylene degreening, resulting in fruit with rind colour similar to that of degreened fruit. Postharvest simulation of low orchard temperature may enhance rind colour of early-maturing *Citrus* species. If such postharvest treatment reduced

rind chlorophyll content, then duration of exposure to ethylene degreening of early maturing cultivars of *Citrus* spp. could be reduced. It appears that the improved rind colour resulted from both chlorophyll degradation and carotenoid biosynthesis and accumulation (Figs. 3.4 and 3.5). Replacing or reducing the duration of degreening with an alternative cold shock treatment would result in a longer shelf-life because of a reduction in rind senescence following prolonged exposure to ethylene, and possibly firmer fruit.

Cold shock was unsuccessful in 'Navel' oranges treated in the same season as mandarins (2002) and no colour development was observed. However, cold shock appears to have had negative effect on fruit colour leading to a slightly paler rind although chlorophyll was removed by the treatment, making the underlying colour visible. It is thought that the cold shock treatment may vary in effectiveness from one *Citrus* spp. to another and that the 'Navel' oranges used may have been less sensitive to the treatment. The lack of positive response could also have been as a result of being later in the season and cold weather may have initiated colour development before the cold shock treatment.

Data from Californian showed no difference in the effectiveness of the treatments, as each treatment resulted in similar final colour values. There could be various reasons for the poor performance of cold shocked fruit. Temperature data for the period before and during the experiment show minimum temperatures which were very favourable for good natural colour development on the tree (Fig. 3.11). Only two of the minimum recorded temperatures were above 13 °C, being the critical level below which temperature must drop to cause the initiation of colour development. These ideal temperature conditions may have initiated good colour development and negated any positive affect of cold shock on colour development. Cold shock may have a more meaningful effect during warm seasons leading to poor colour.

Furthermore, experimental cold shock temperatures in California may have been sub-optimal. The hydrocooler could only be set at 0 °C for the entire period, whereas in previous experiments fruit were cold shocked at 4 °C. This low temperature may have negatively affected carotenoid biosynthesis, leading to the pale coloured fruit observed. There may be an optimum postharvest cold shock temperature below which inhibition of carotenoid biosynthesis and accumulation occurs. Also, the incubation temperature used in California (<20 °C) was variable and may have been too low to trigger colour development.

In conclusion, cold shock did not consistently improve rind colour in 'Nules Clementine' mandarins and 'Navel' oranges. However, one attempt to improve rind colour by cold shocking fruit was successful and, if this response could be consistently achieved, cold shock could partially or completely substitute ethylene degreening.

Due to the positive results on 'Nules' in the 2002 season and the need to reduce the negative effects of degreening, further research is recommended. Cold shock may be more effective during poor-colour years, in which night temperatures remain above 13 °C. Cold shock earlier in the season, before a drop in temperature initiates a natural response on the tree, may also be more successful. This may explain why cold shock was only successful on the 'Nules' in 2002 season and not on later maturing 'Navel' oranges, which may have been exposed to natural conditions more favourable to rind colour development. It may also be necessary to increase the temperature at which cold shock was applied. The cold shocked fruit in this study appeared to have a bleached yellow appearance. This may be as a result of the destruction of carotenoids under very low temperatures of 4 °C and below (El-Zeftawi, 1976), whereas in nature temperatures below 13 °C are sufficient to cause a colouring response.

Table 3.1. The effect of cold shock on hue angle as a component of rind colour in 'Nules Clementine' mandarin during the 2002 season.

Source of variation	After Treatment	6 days @ -0.6 °C	28 days @ -0.6 °C	7 days @ 4.5 °C	21 days @ 15 °C
Initial colour (I) [z]					
T4	74.13[a x]	73.56[a]	73.44[a]	75.32[a]	68.45[a]
T5	75.92[a]	74.44[a]	74.97[a]	76.09[a]	69.84[a]
LSD	1.80	1.61	1.92	1.78	3.36
Treatments (T)[y]					
Control	92.07[a]	89.70[a]	91.63[a]	91.96[a]	76.82[a]
Deg	72.57[c]	72.98[b]	72.70[b]	73.66[b]	68.58[b]
CS	76.25[b]	72.00[b]	73.35[b]	74.64[b]	67.50[b]
CS + Deg	72.11[c]	72.07[b]	71.66[b]	73.70[b]	68.58[b]
LSD	2.99	2.67	3.19	2.95	5.57
P-value					
I x T	0.6017	0.0427	0.2560	0.1573	0.2176
I	0.0295	0.2735	0.0255	0.0734	0.0960
T	<0.0001	<0.0001	<0.0001	<0.0001	0.0411

[z] Initial rind colour category on a scale from T8 (dark green) to T1 (bright orange), where T5 is less well coloured than T4.
[y] Deg = degreening, CS = cold shock, CS + Deg = combination of cold shock and degreening.
[x] Means with the same letter are not significantly different (P>0.05).

Table 3.2. The effect of cold shock on lightness as a component of rind colour in 'Nules Clementine' mandarin during the 2002 season.

Source of variation	After Treatment	6 days @ -0.6 °C	28 days @ -0.6 °C	7 days @ 4.5 °C	21 days @ 15 °C
Initial colour (I) [z]					
T4	71.72[a x]	72.88[a]	69.47[b]	72.49[a]	70.88[a]
T5	72.29[a]	72.38[a]	70.45[a]	71.51[a]	70.91[a]
LSD	1.15	1.37	0.88	1.42	1.72
Treatments (T) [y]					
Control	66.15[d]	65.98[b]	66.75[b]	68.41[b]	69.93[a]
Deg	73.87[b]	72.55[a]	70.57[a]	72.70[a]	70.88[a]
CS	76.20[a]	72.69[a]	70.60[a]	73.17[a]	70.38[a]
CS + Deg	70.75[c]	73.96[a]	70.10[a]	71.97[a]	71.30[a]
LSD	1.91	2.27	1.46	2.36	2.86
P-value					
I x T	0.3710	0.3062	0.3220	0.1491	0.9714
I	0.7356	0.1247	0.0104	0.7148	0.9994
T	<0.0001	<0.0001	0.0015	0.0157	0.6981

[z] Initial rind colour category on a scale from T8 (dark green) to T1 (bright orange), where T5 is less well coloured than T4.
[y] Deg = degreening, CS = cold shock, CS + Deg = combination of cold shock and degreening.
[x] Means with the same letter are not significantly different (P>0.05).

Table 3.3. The effect of cold shock on chroma as a component of rind colour in 'Nules Clementine' mandarin during the 2002 season.

Source of variation	After Treatment	6 days @ -0.6 °C	28 days @ -0.6 °C	7 days @ 4.5 °C	21 days @ 15 °C
Initial colour (I) [z]					
T4	65.30[a x]	69.36[a]	66.18[a]	68.13[a]	68.20[a]
T5	65.40[a]	67.57[b]	66.71[a]	66.39[b]	67.91[a]
LSD	0.71	1.13	1.19	1.08	1.04
Treatments (T) [y]					
Control	58.84[d]	59.06[b]	57.64[b]	59.76[c]	63.24[b]
Deg	67.47[b]	68.87[a]	68.14[a]	68.68[ab]	68.35[a]
CS	68.84[a]	70.17[a]	68.39[a]	69.27[a]	69.43[a]
CS + Deg	64.41[c]	69.50[a]	66.75[a]	67.38[b]	68.35[a]
LSD	1.18	1.87	1.98	1.80	1.72
P-value					
I x T	0.0089	0.0366	0.0780	0.5384	0.1591
I	0.0986	0.0008	0.2273	0.0160	0.1700
T	<0.0001	<0.0001	<0.0001	<0.0001	0.0002

[z] Initial rind colour category on a scale from T8 (dark green) to T1 (bright orange), where T5 is less well coloured than T4.
[y] Deg = degreening, CS = cold shock, CS + Deg = combination of cold shock and degreening.
[x] Means with the same letter are not significantly different (P>0.05).

Table 3.4. The effect of cold shock on hue angle as a component of rind colour in 'Bahianinha Navel' sweet orange during the 2002 season.

Source of variation	After Treatment	6 days @ -0.6 °C	28 days @ -0.6 °C	7 days @ 4.5 °C	21 days @ 15 °C
Initial colour (I) [z]					
T4	81.57[b x]	78.25[b]	77.26[b]	77.76[b]	74.77[a]
T5	85.12[a]	81.54[a]	79.95[a]	80.72[a]	73.17[b]
LSD	1.56	1.18	1.14	1.08	1.37
Treatments (T) [y]					
Control	87.29[a]	83.66[a]	82.38[a]	81.23[b]	74.00[b]
Deg	80.77[b]	77.46[b]	75.56[b]	76.90[c]	73.86[b]
CS	88.52[a]	84.91[a]	84.06[a]	84.21[a]	77.25[a]
CS + Deg	79.47[b]	76.05[b]	74.94[b]	75.95[c]	70.79[c]
LSD	2.46	1.86	1.81	1.71	2.17
P-value					
I x T	0.0130	0.0453	0.2319	0.2114	0.0040
I	0.0107	0.0002	0.0003	<0.0001	0.2466
T	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[z] Initial rind colour category on a scale from T8 (dark green) to T1 (bright orange), where T5 is less well coloured than T4.
[y] Deg = degreening, CS = cold shock, CS + Deg = combination of cold shock and degreening.
[x] Means with the same letter are not significantly different (P>0.05).

Table 3.5. The effect of cold shock on lightness as a component of rind colour in 'Bahianinha Navel' sweet orange during the 2002 season.

Source of variation	After Treatment	6 days @ -0.6 °C	28 days @ -0.6 °C	7 days @ 4.5 °C	21 days @ 15 °C
Initial colour (I) [z]					
T4	64.46[a x]	72.03[b]	70.74[a]	74.35[a]	71.61[a]
T5	64.14[a]	73.14[a]	70.74[a]	74.45[a]	71.63[a]
LSD	1.18	0.77	0.68	0.74	1.00
Treatments (T) [y]					
Control	70.92[ab]	73.24[a]	73.94[a]	74.00[b]	71.77[b]
Deg	69.38[b]	72.35[a]	69.08[c]	73.33[b]	70.53[b]
CS	71.96[a]	72.35[a]	72.59[b]	76.55[a]	73.40[a]
CS + Deg	71.64[a]	72.10[a]	69.48[c]	73.46[b]	70.89[b]
LSD	1.93	1.22	1.07	1.17	1.5
P-value					
I x T	0.9016	0.0495	0.0115	0.0254	0.0390
I	0.9439	0.0243	0.8576	0.6963	0.9766
T	0.0104	0.1463	<0.0001	<0.0001	0.0004

[z] Initial rind colour category on a scale from T8 (dark green) to T1 (bright orange), where T5 is less well coloured than T4.
[y] Deg = degreening, CS = cold shock, CS + Deg = combination of cold shock and degreening.
[x] Means with the same letter are not significantly different (P>0.05).

Table 3.6. The effect of cold shock on chroma as a component of rind colour in 'Bahianinha Navel' sweet orange during the 2002 season.

Source of variation	After Treatment	6 days @ -0.6 °C	28 days @ -0.6 °C	7 days @ 4.5 °C	21 days @ 15 °C
Initial colour (I) [z]					
T4	64.46[a x]	73.47[a]	71.33[a]	70.72[a]	69.87[a]
T5	64.14[a]	73.08[a]	69.99[b]	70.19[a]	68.80[a]
LSD	1.18	0.77	0.59	0.57	1.15
Treatments (T) [y]					
Control	64.70[a]	73.80[a]	74.14[a]	71.43[a]	69.86[a]
Deg	64.03[a]	73.42[a]	69.35[c]	69.53[b]	67.17[b]
CS	63.80[a]	73.42[a]	71.71[b]	71.78[a]	70.77[a]
CS + Deg	64.94[a]	72.75[a]	69.76[c]	69.73[b]	69.90[a]
LSD	1.86	1.21	0.93	0.91	1.82
P-value					
I x T	0.0038	0.0017	0.3306	0.2262	0.7581
I	0.2896	0.8093	0.0002	0.0271	0.2218
T	0.4331	0.3301	<0.0001	<0.0001	0.0002

[z] Initial rind colour category on a scale from T8 (dark green) to T1 (bright orange), where T5 is less well coloured than T4.
[y] Deg = degreening, CS = cold shock, CS + Deg = combination of cold shock and degreening.
[x] Means with the same letter are not significantly different (P>0.05).

Table 3.7. The effect of cold shock on hue angle as a component of rind colour in 'Nules Clementine' mandarin in California during the 2002 season.

Treatment [z]	After 24 hrs	After 48 hrs	After 72 hrs
Control	82.34[b y]	78.76[a]	74.51[a]
Deg	81.85[b]	75.16[b]	69.39[b]
CS	84.43[a]	78.76[a]	74.74[a]
CS + Deg	85.11[a]	76.26[ab]	70.97[b]
LSD	1.94	2.67	1.67
P-value	0.0051	0.0345	<0.0001

[z] Deg = degreening, CS = cold shock, CS + Deg = combination of cold shock and degreening.
[y] Means with the same letter are not significantly different (P>0.05).

Table 3.8. The effect of cold shock on lightness as a component of rind colour in 'Nules Clementine' mandarin in California during the 2002 season.

Treatment [z]	After 24 hrs	After 48 hrs	After 72 hrs
Control	69.61[a y]	70.07[a]	70.16[a]
Deg	69.49[a]	69.70[a]	70.01[a]
CS	69.04[a]	70.15[a]	70.79[a]
CS + Deg	68.54[a]	69.09[a]	70.17[a]
LSD	1.50	1.47	1.19
P-value	0.4460	0.4435	0.536

[z] Deg = degreening, CS = cold shock, CS + Deg = combination of cold shock and degreening.
[y] Means with the same letter are not significantly different (P>0.05).

Table 3.9. The effect of cold shock on chroma as a component of rind colour in 'Nules Clementine' mandarin in California during the 2002 season.

Treatment [z]	After 24 hrs	After 48 hrs	After 72 hrs
Control	61.70[a y]	64.47[a]	66.51[a]
Deg	60.07[a]	62.94[ab]	65.73[a]
CS	60.41[a]	63.78[a]	65.59[a]
CS + Deg	60.68[a]	61.17[b]	65.23[a]
LSD	2.03	2.0233	1.60
P-value	0.3938	0.0167	0.414

[z] Deg = degreening, CS = cold shock, CS + Deg = combination of cold shock and degreening.
[y] Means with the same letter are not significantly different (P>0.05).

Table 3.10. The effect of cold shock on hue angle as a component of rind colour in 'Becks Early Navel' sweet orange in California during the 2002 season.

Treatment [z]	After 24 hrs	After 48 hrs	After 96 hrs
Control	91.06[b y]	86.11[b]	78.67[b]
Deg	89.75[b]	80.15[c]	70.49[d]
CS	95.80[a]	90.90[a]	82.42[a]
CS + Deg	94.40[a]	87.16[b]	74.29[c]
LSD	2.14	1.72	1.47
P-value	<0.0001	<0.0001	<0.0001

[z] Deg = degreening, CS = cold shock, CS + Deg = combination of cold shock and degreening.
[y] Means with the same letter are not significantly different (P>0.05).

Table 3.11. The effect of cold shock on Lightness as a component of rind colour in 'Becks Early Navel' sweet orange in California during the 2002 season.

Treatment [z]	After 24 hrs	After 48 hrs	After 96 hrs
Control	67.35[a y]	68.62[a]	69.55[a]
Deg	67.88[a]	68.66[a]	67.72[b]
CS	66.96[a]	68.21[a]	69.50[a]
CS + Deg	67.39[a]	68.21[a]	68.36[a]
LSD	2.33	1.83	1.29
P-value	0.8765	0.9499	0.0181

[z] Deg = degreening, CS = cold shock, CS + Deg = combination of cold shock and degreening.
[y] Means with the same letter are not significantly different (P>0.05).

Table 3.12. The effect of cold shock on Chroma as a component of rind colour in 'Becks Early Navel' sweet orange in California during the 2002 season.

Treatment [z]	After 24 hrs	After 48 hrs	After 96 hrs
Control	57.82[a y]	60.085[a]	63.22[a]
Deg	57.28[a]	60.677[a]	63.78[a]
CS	59.88[a]	59.015[a]	62.55[a]
CS + Deg	59.45[a]	58.220[a]	62.73[a]
LSD	3.62	2.86	1.98
P-value	0.3952	0.3082	0.5801

[z] Deg = degreening, CS = cold shock, CS + Deg = combination of cold shock and degreening.
[y] Means with the same letter are not significantly different (P>0.05).

Table 3.13. The effect of cold shock and duration thereof (i.e. 2, 4 or 6 hours) on hue angle value as a component of rind colour in 'Nules Clementine' mandarin during the 2003 season.

Treatment [z]	After Treatment	6 days @ -0.6 °C	31 days @ -0.6 °C	7 days @ 4.5 °C	14 days @ 15 °C
Control	72.49$^{b\ y}$	71.96^{bc}	71.46^{bc}	71.96^{bc}	67.39^{ab}
Deg	74.54^{a}	73.93^{a}	71.99^{bc}	73.93^{a}	64.64^{cd}
CS2	75.29^{a}	73.77^{ab}	73.66^{ab}	73.77^{ab}	67.53^{ab}
CS4	74.42^{a}	74.15^{a}	74.15^{a}	74.15^{a}	68.62^{a}
CS6	75.50^{a}	75.36^{a}	75.36^{a}	75.36^{a}	68.53^{a}
CS2DEG	71.20^{b}	71.58^{c}	70.92^{c}	71.58^{c}	63.54^{d}
CS4DEG	75.14^{a}	73.95^{a}	73.49^{ab}	73.95^{a}	67.62^{a}
CS6DEG	72.65^{b}	71.68^{c}	71.64^{bc}	71.68^{c}	65.56^{bc}
LSD	1.68	1.79	2.45	1.90	1.99
P-value	<0.0001	<0.0001	0.0163	0.0012	<0.0001

[z] Deg = degreening, CS 2, 4 or 6 = cold shock applied for 2, 4 or 6 hrs, CS + Deg = combination of cold shock and degreening, where cold shock was applied for 2, 4 or 6 hrs.
[y] Means with the same letter are not significantly different (P>0.05).

Table 3.14. The effect of cold shock and duration thereof (i.e. 2, 4 or 6 hours) on lightness as a component of rind colour in 'Nules Clementine' mandarin during the 2003 season.

Treatment [z]	After Treatment	6 days @ -0.6 °C	31 days @ -0.6 °C	7 days @ 4.5 °C	14 days @ 15 °C
Control	69.91$^{ab\ y}$	71.59^{a}	68.73^{bc}	71.59^{a}	67.51^{bc}
Deg	69.76^{ab}	71.61^{a}	68.46^{bc}	71.61^{a}	66.59^{cd}
CS2	68.74^{bc}	71.57^{a}	67.93^{c}	71.57^{a}	67.22^{bc}
CS4	70.32^{a}	70.59^{abc}	69.76^{ab}	70.59^{ab}	68.93^{a}
CS6	70.31^{a}	71.28^{ab}	70.38^{a}	71.28^{ab}	68.85^{a}
CS2DEG	68.00^{c}	69.65^{c}	68.20^{c}	69.65^{c}	65.75^{d}
CS4DEG	68.78^{bc}	70.59^{abc}	68.97^{abc}	70.59^{abc}	67.96^{ab}
CS6DEG	69.25^{abc}	70.18^{bc}	68.35^{bc}	70.18^{bc}	66.64^{cd}
LSD	1.27	1.39	1.52	1.22	0.99
P-value	0.0050	0.0272	0.0344	0.0115	<0.0001

[z] Deg = degreening, CS 2, 4 or 6 = cold shock applied for 2, 4 or 6 hrs, CS + Deg = combination of cold shock and degreening, where cold shock was applied for 2, 4 or 6 hrs.
[y] Means with the same letter are not significantly different (P>0.05).

Table 3.15. The effect of cold shock and duration thereof (i.e. 2, 4 or 6 hours) on chroma as a component of rind colour in 'Nules Clementine' mandarin during the 2003 season.

Treatment [z]	After Treatment	6 days @ -0.6 °C	31 days @ -0.6 °C	7 days @ 4.5 °C	14 days @ 15 °C
Control	67.94[a y]	68.17[ab]	69.87[ab]	68.17[ab]	65.75[a]
Deg	67.63[ab]	68.41[a]	68.71[bcd]	68.41[a]	65.85[a]
CS2	67.76[ab]	67.75[ab]	68.22[d]	67.75[ab]	66.04[a]
CS4	67.57[ab]	68.48[a]	69.18[abc]	68.48[a]	66.61[a]
CS6	67.73[ab]	67.23[ab]	70.15[a]	67.23[ab]	66.59[a]
CS2DEG	66.53[b]	66.67[b]	67.79[d]	66.67[b]	65.64[a]
CS4DEG	68.13[a]	66.94[ab]	68.28[cd]	66.95[ab]	66.38[a]
CS6DEG	68.78[a]	66.72[b]	68.63[bcd]	66.72[b]	65.82[a]
LSD	1.26	1.72	1.27	1.53	1.56
P-value	0.0736	0.0994	0.0055	0.0821	0.8225

[z] Deg = degreening, CS 2, 4 or 6 = cold shock applied for 2, 4 or 6 hrs, CS + Deg = combination of cold shock and degreening, where cold shock was applied for 2, 4 or 6 hrs.
[y] Means with the same letter are not significantly different (P>0.05).

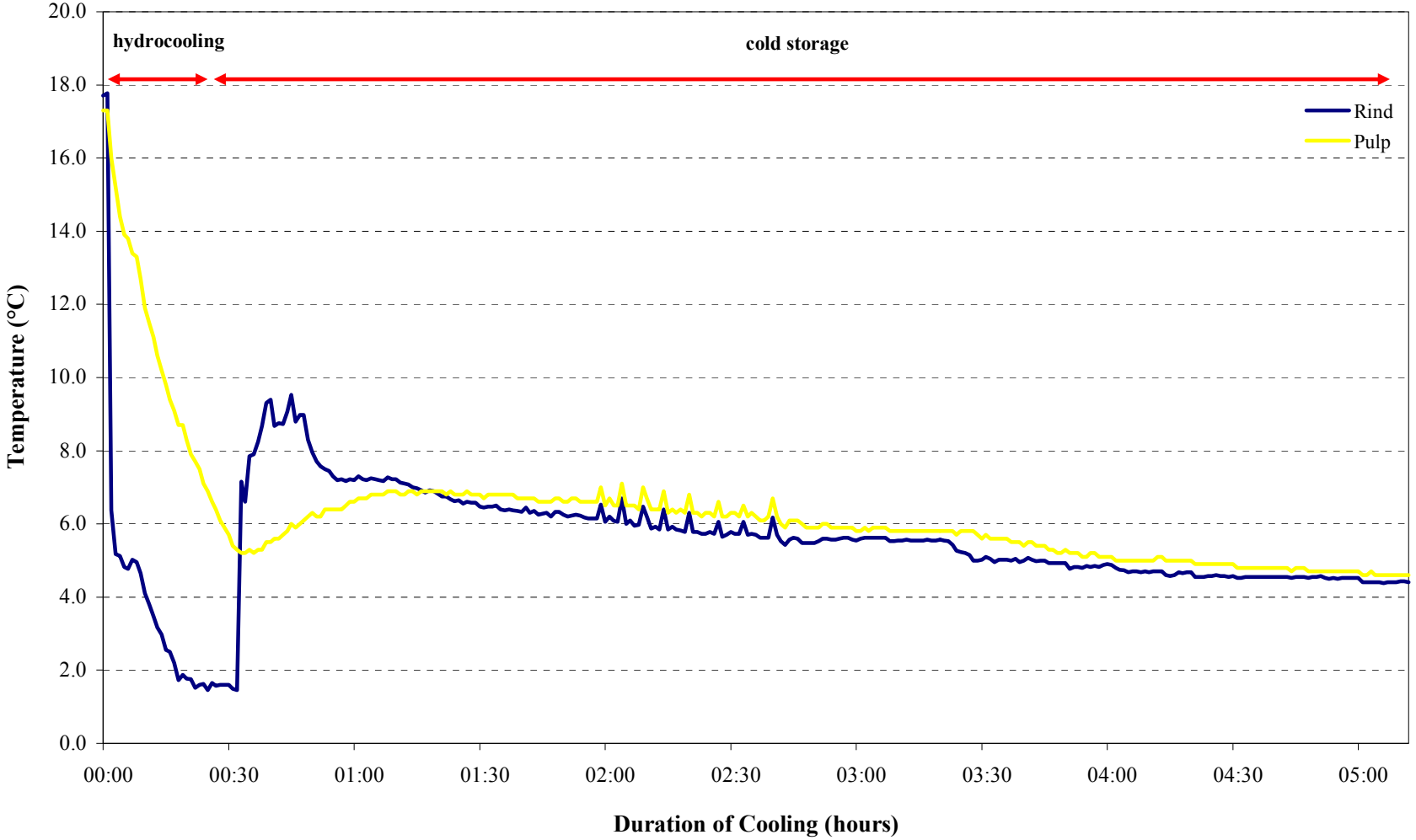

Fig. 3.1. Rind and pulp temperature of 'Nules Clementine' mandarin during hydrocooling and subsequent cold storage in the 2002 season.

Fig. 3.2. Photograph of untreated 'Nules Clementine' mandarin fruit (left) compared with cold shocked fruit (right) in the 2002 season.

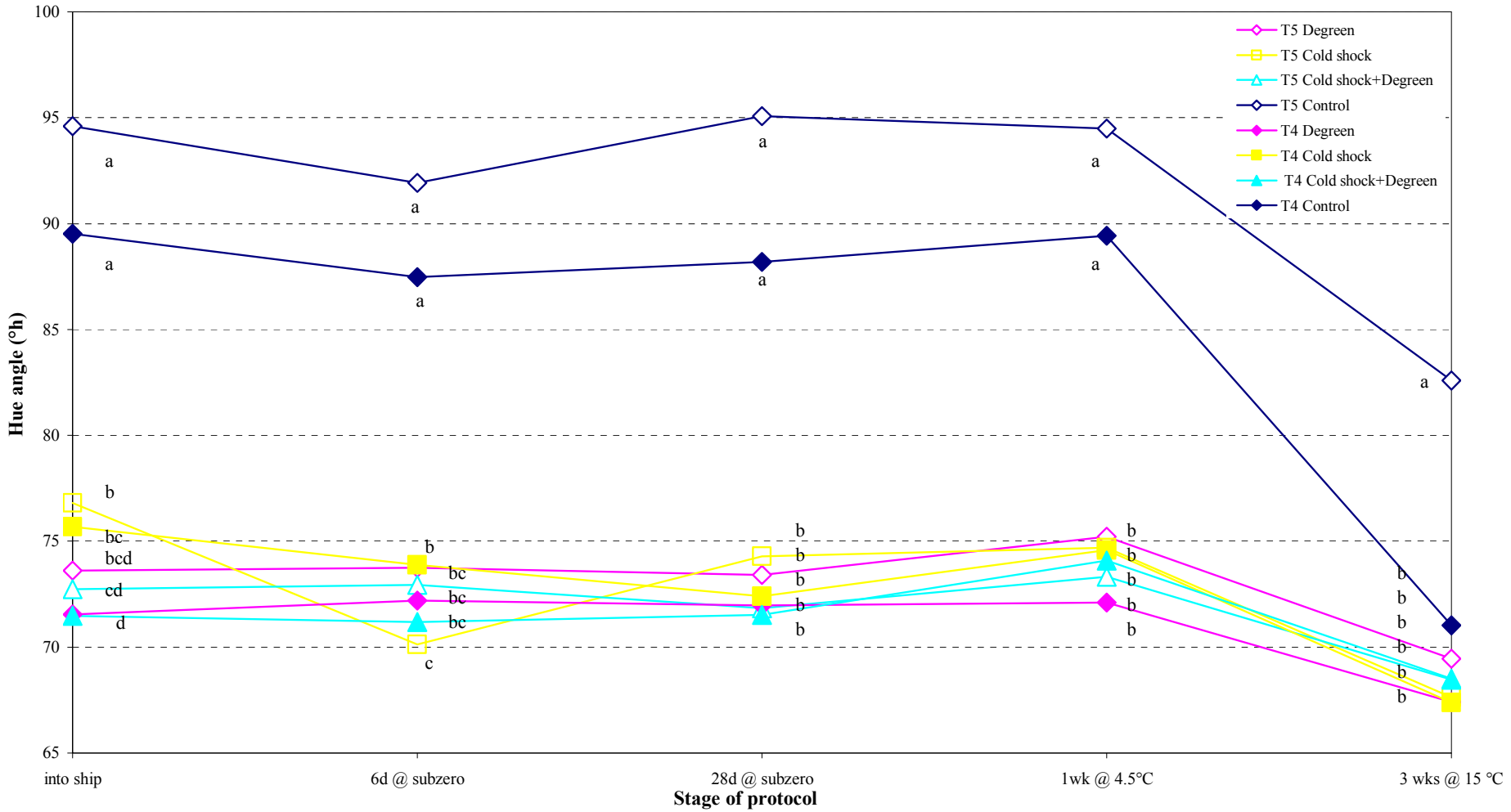

Fig. 3.3. Change in hue angle of 'Nules Clementine' mandarin at Gouda during the 2002 season during the period following a cold shock treatment (T5=yellow/orange becoming dominant over green; T4=yellow/orange strongly dominant with patches of green).

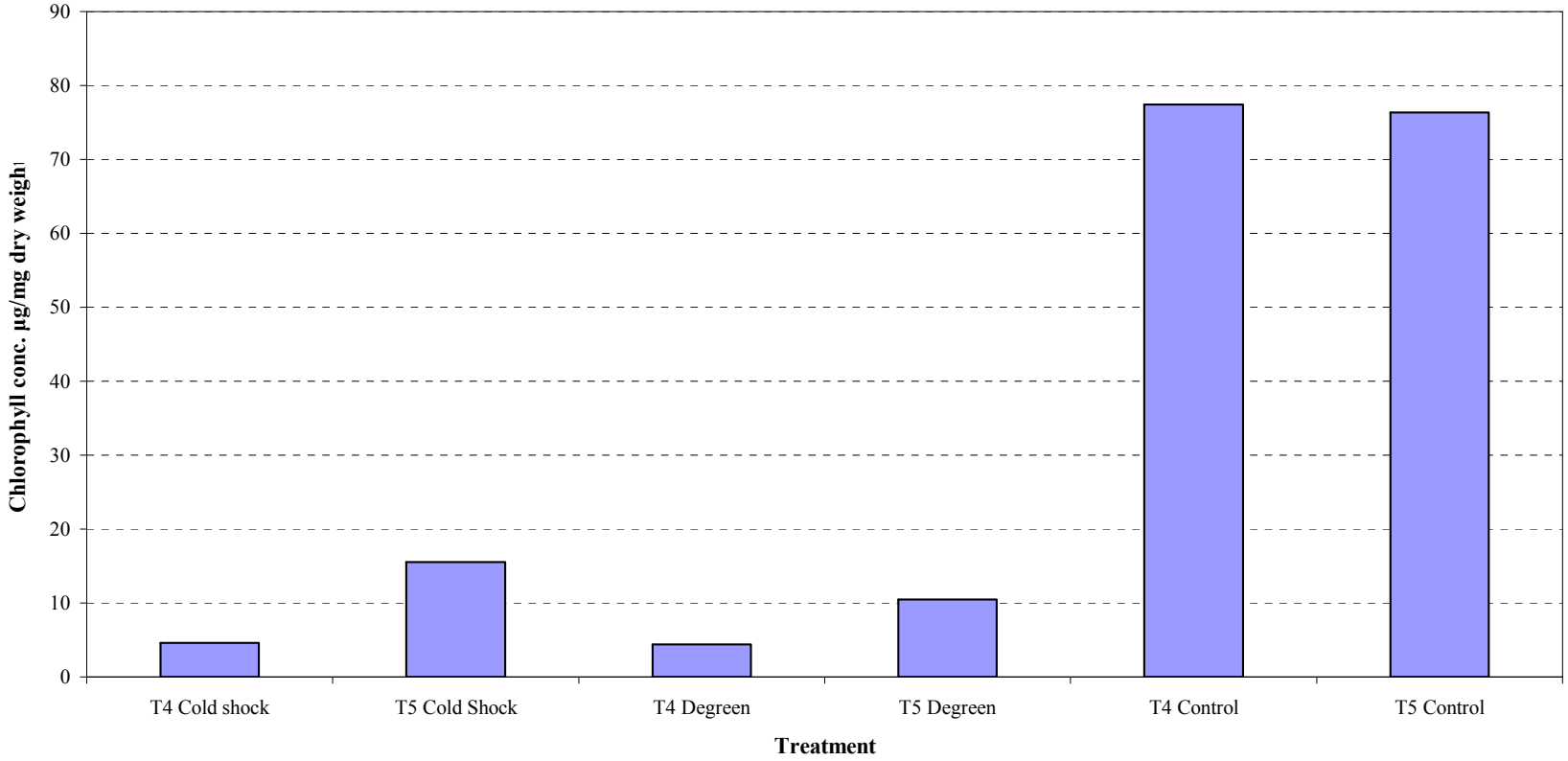

Fig. 3.4. Chlorophyll concentration of 'Nules Clementine' mandarin after cold shock treatment during the 2002 season. (Rind samples were collected from eight fruit from each of all six replicates and combined to form one pooled rind sample per treatment; T5=yellow/orange becoming dominant over green; T4=yellow/orange strongly dominant with patches of green).

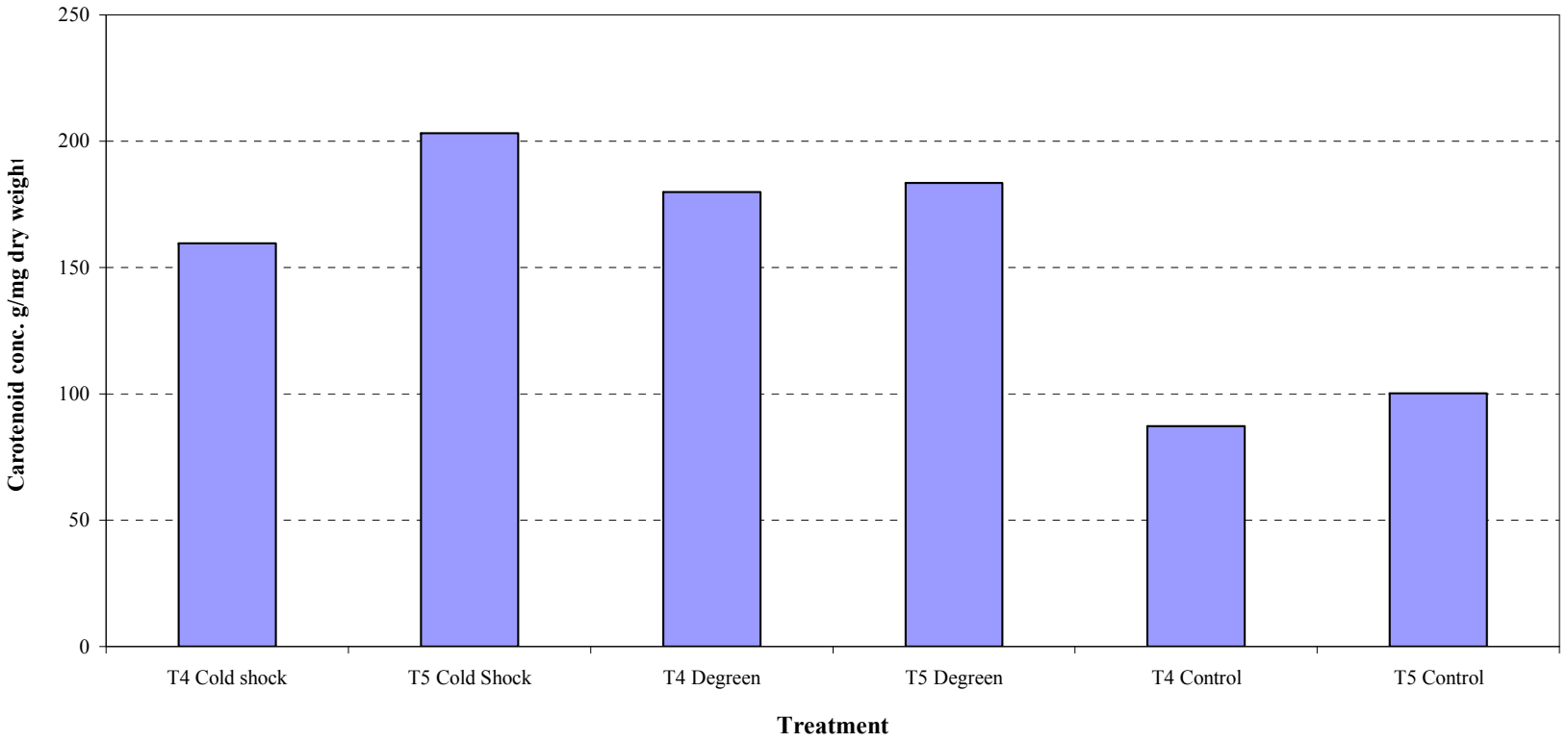

Fig. 3.5. Carotenoid concentration of 'Nules Clementine' mandarin after cold shock treatment during the 2002 season. (Rind samples were collected from eight fruit from each of all six replicates and combined to form one pooled rind sample per treatment; T5=yellow/orange becoming dominant over green; T4=yellow/orange strongly dominant with patches of green).

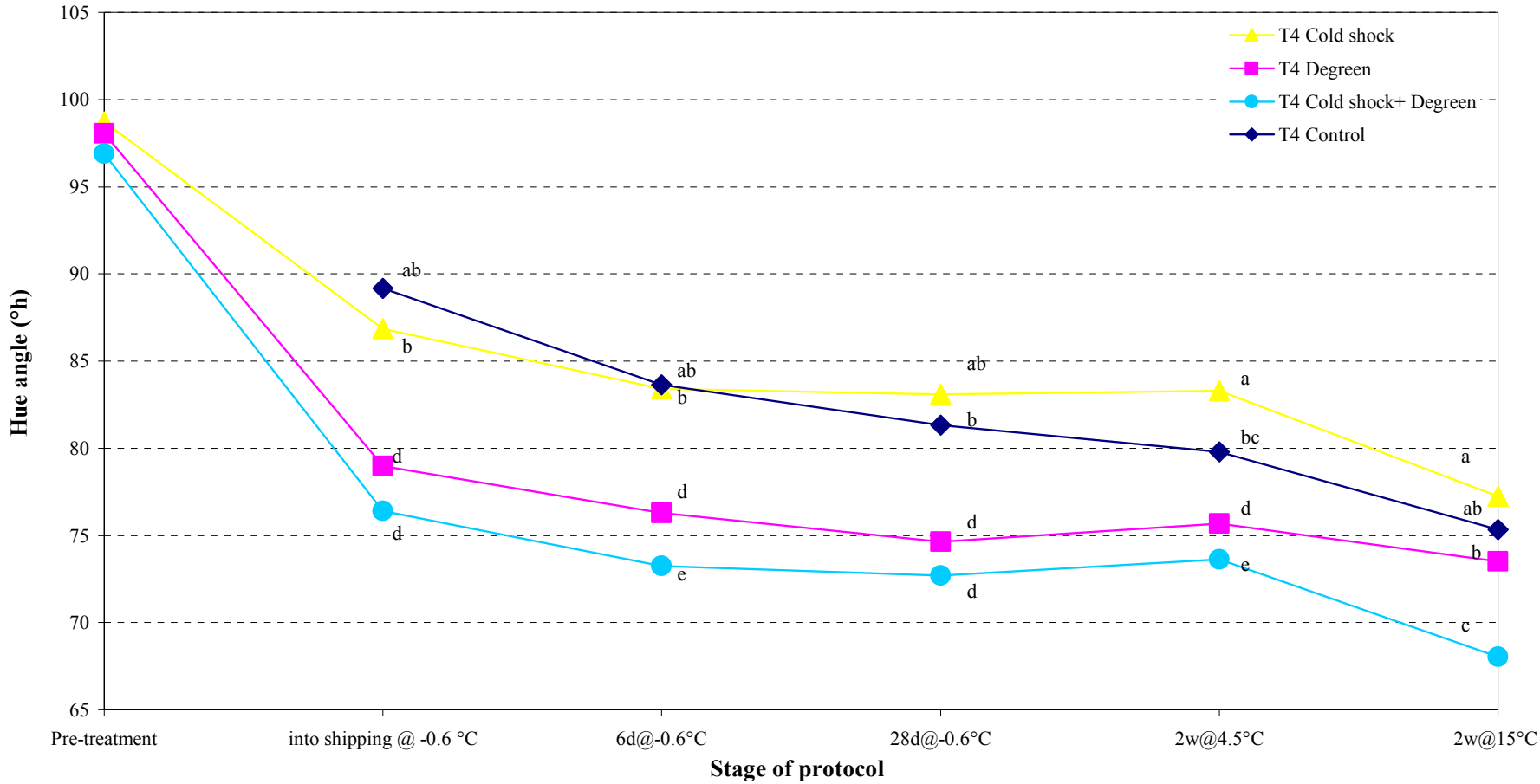

Fig. 3.6. Change in hue angle of initially T4 'Bahianinha Navel' sweet orange fruit over time in the 2002 season.

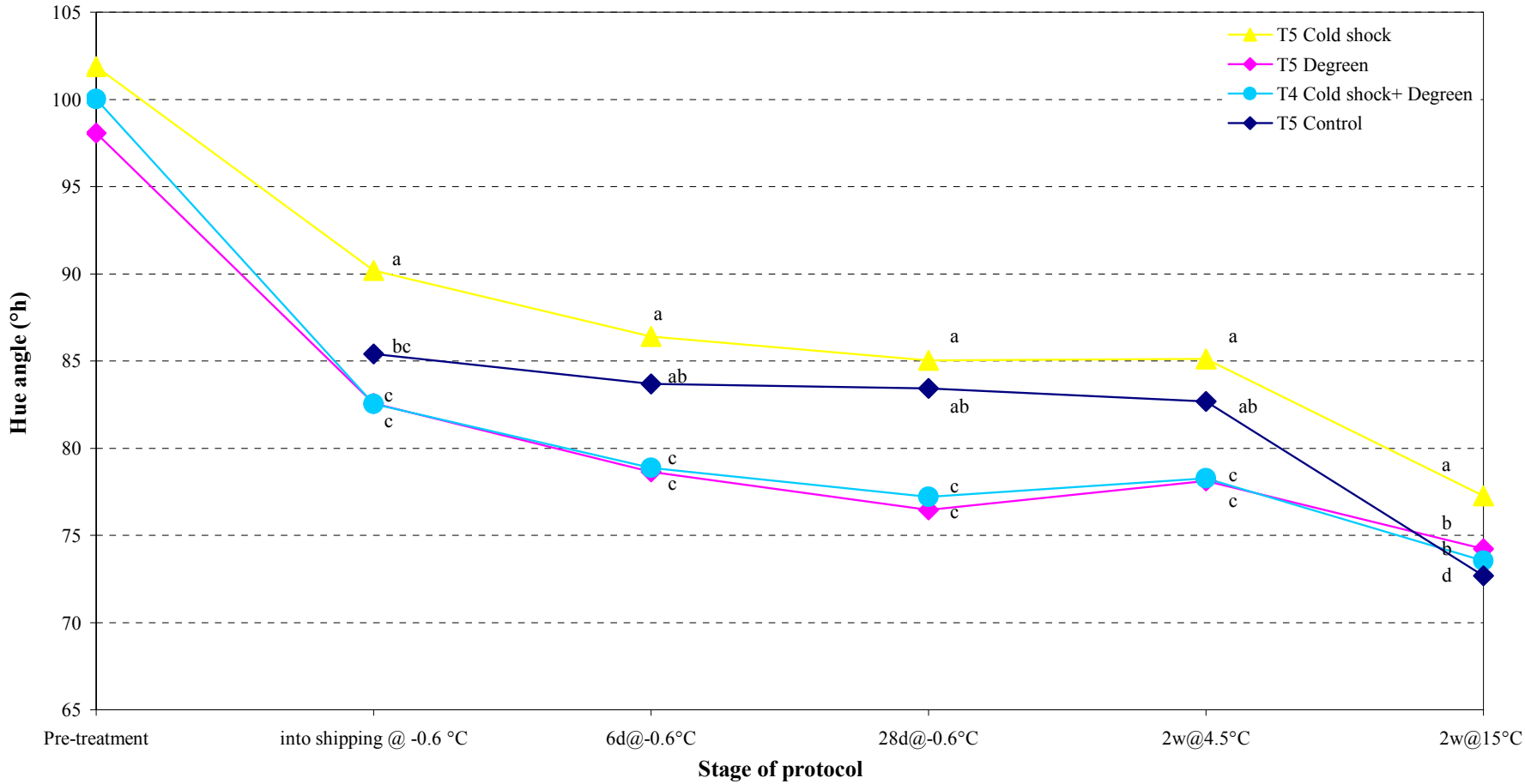

Fig. 3.7. Change in hue angle of initially T5 ‘Bahianinha Navel’ sweet orange fruit over time in the 2002 season.

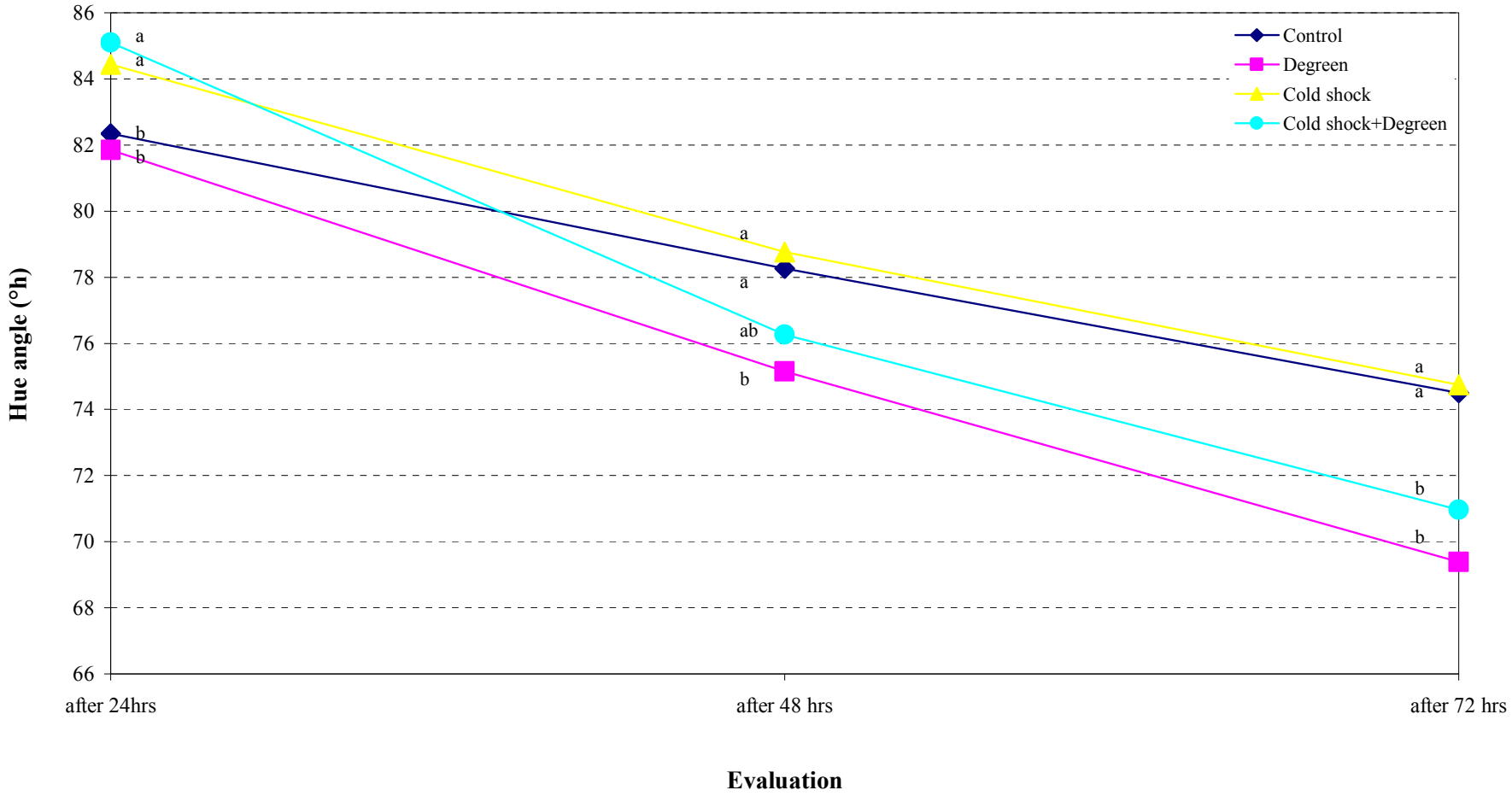

Fig. 3.8. Change in hue angle of initially T5 'Nules Clementine' mandarin over time in California during the 2002 season.

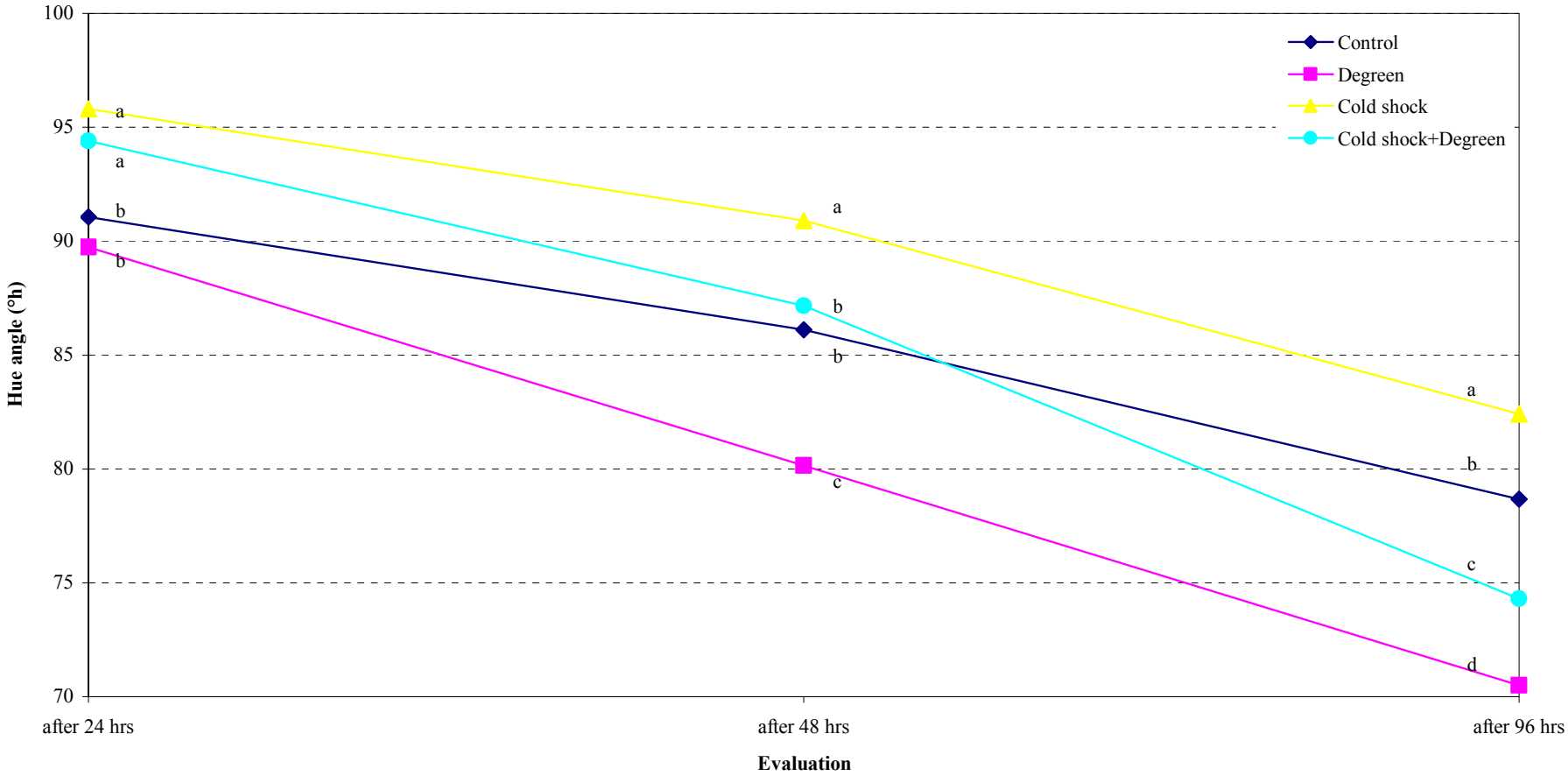

Fig. 3.9. Change in hue angle of initially T5 'Becks Early Navel' sweet orange over time in California during the 2002 season.

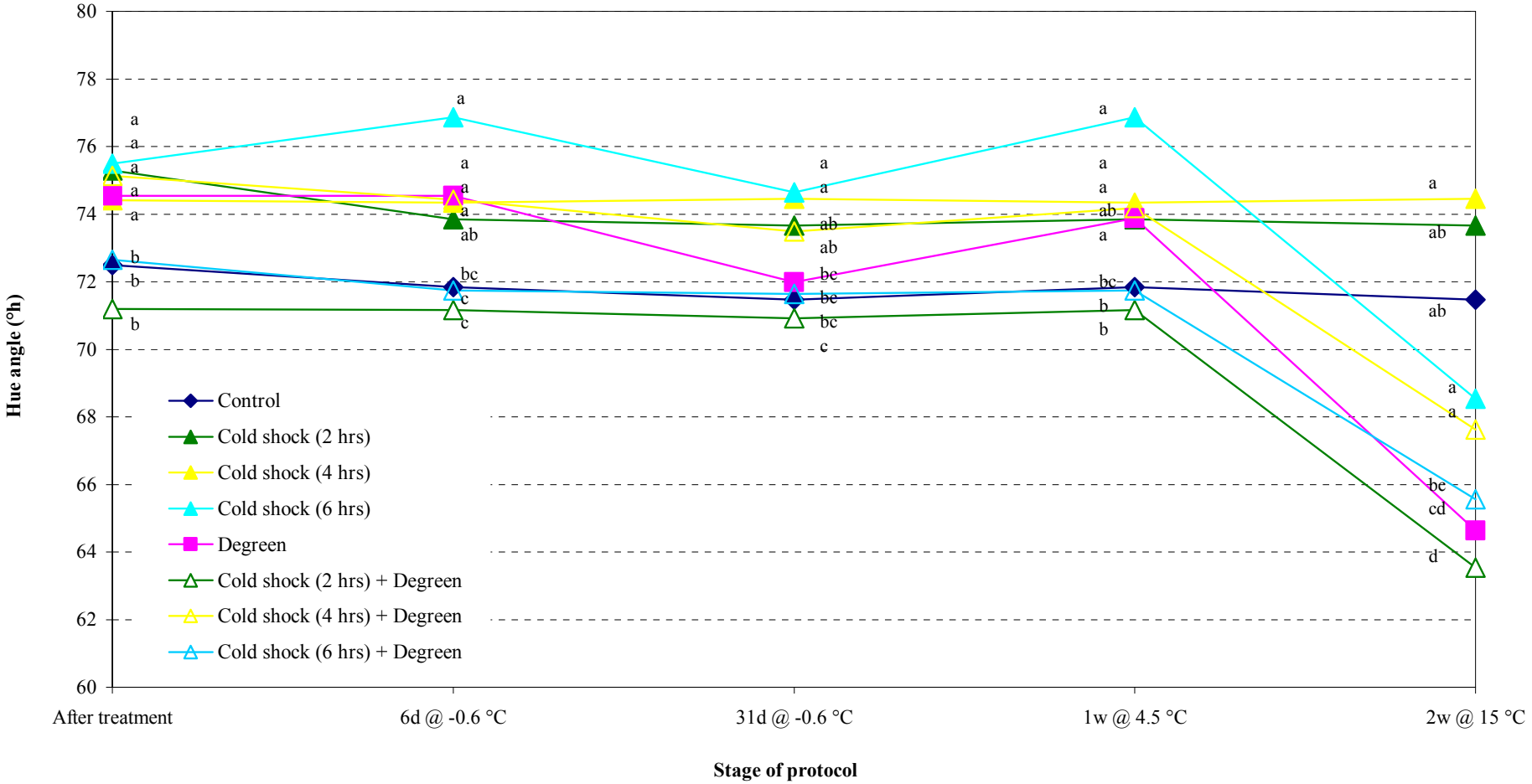

Fig. 3.10. Change in hue angle of initially T5 'Nules Clementine' mandarin over time during the 2003 season.

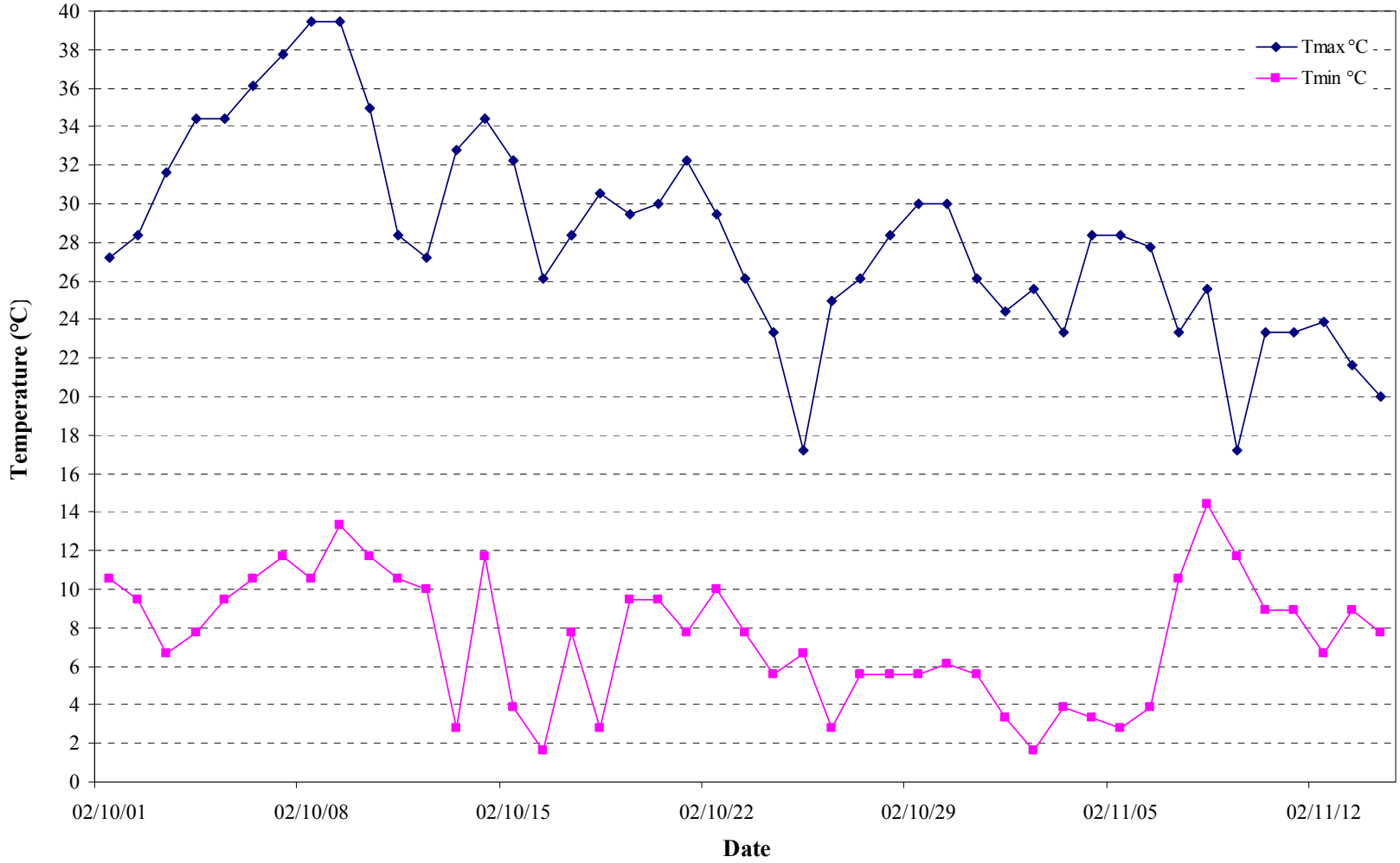

Fig. 3.11. Maximum and minimum temperatures at Maricopa flats, California, USA during October and November 2002.

CHAPTER 4

EXTENDED LOW-TEMPERATURE SHIPPING ADVERSELY AFFECTS RIND COLOUR OF *CITRUS* FRUIT

Abstract

Storage temperature is one of the most important postharvest factors affecting rind colour. Citrus fruit shipped to export markets requiring low temperatures (-0.6 °C) for pest disinfestations purposes often arrive with poor rind colour. Shipping under low temperatures results in poor rind colour of fruit on arrival in the market. To comply with the USA's phytosanitary requirement for imported citrus, fruit is held at -0.6 °C for a minimum of 22 days. The effect of shipping at various temperatures (-0.6 °C or 4.5 °C), durations and the influence of initial rind colour, "orange" or "yellow", on fruit colour upon arrival in the market was evaluated. Fruit shipped at a higher temperature (4.5 °C) had a marginally better rind colour than fruit shipped at -0.6 °C. The perceived loss of rind colour following shipping at sub-zero temperatures is probably due to carotenoid degradation. Therefore, initial rind colour plays a critical role in final product quality. Depending on market destination and shipping temperature, pale-coloured fruit should not be packed for markets sensitive to rind colour.

Introduction

Shipping temperature has a strong effect on eventual fruit colour and is cultivar and colour dependent (Capespan Technology Development, 2001). Shipping temperature is one of the final interventions which can be used to improve *Citrus* rind colour. Typical shipping temperatures of 4.5 °C and 11 °C are used by the South African citrus industry. The

temperature used depends on the fruit colour at packing and the destined market. Higher shipping temperatures result in a greater improvement in rind colour (Gilfillan, 1988).

Certain export markets require sub-zero shipping temperatures to satisfy the phytosanitary requirements of those countries. When exporting fruit from South Africa to the United States of America it is necessary that the fruit undergo cold-sterilisation at -0.6 °C for a minimum of 22 days to eradicate any possible infestation of fruit fly (*Ceratitus capitata*) or false codling moth (*Cryptophlebia leucotreta*) larvae (Maritz, 2000).

Fruit shipped at -0.5 °C showed no colour development (Le Roux, 1997). Some export companies have stricter rind colour requirements for fruit shipped at sub-zero temperatures as colour development is inhibited (Maritz, 2000). Fruit shipped at 4.5 °C eventually developed a yellow rind colour, while fruit shipped at 11 °C had an orange rind colour at arrival in the market (Le Roux, 1997). Shipping at 8.5 °C or 11 °C instead of 4.5 °C when 'Satsuma' mandarin (*C. unshiu* Marc.) fruit were slightly green resulted in the best colour development on arrival at the market (Gilfillan, 1988). It may be favourable to ship early season fruit with a poorer rind colour at 11 °C, while late season fruit with a better rind colour should be shipped at 4.5 °C to maintain internal fruit quality and reduce the incidence of decay (Le Roux, 1997). At sub-zero shipping temperatures fruit sometimes become yellower instead of the desired orange colour (Koch, personal communication). In some cases, fruit shipped with an orange colour arrive at their destination with a pale yellow appearance.

It is hypothesised that carotenoid breakdown occurs at sub-zero shipping temperatures resulting in pale fruit after shipment. The objective of this research was to quantify the effect

of sub-zero shipping temperature (2002 and 2003 seasons) and its duration (2003 season only) on rind colour retention of 'Navel' sweet orange (*C. sinensis* [L.] Osbeck).

Materials and Methods

Sites and plant material. 'Palmer Navel' sweet oranges (hereafter referred to as 'Palmer') were selected from the ALG Packhouse in Citrusdal, South Africa (32°40′S, 19°03′E) in the 2002 and 2003 seasons. The fruit were drenched with 2,4-D (2,4-dichlorophenoxyacetic acid) (125 $mg \cdot L^{-1}$), Tecto® (thiabendazole) (500 $mg \cdot L^{-1}$) and Sporekill® (dimethyldidecyl ammonium chloride) (120 $mg \cdot L^{-1}$), and then transported to Stellenbosch where fruit were subjected to various cold storage regimes to simulate commercial shipping and holding conditions.

Treatments and experimental design. In 2002, 1200 fully coloured fruit with no green patches were visually sorted into two colour classes, "orange" and "yellow" (Fig. 4.1). Fruit were stored at either 4.5 °C for 21 days or at -0.6 °C for 28 days to simulate commercial shipping conditions for Navel oranges exported to Europe or the USA, respectively. Fruit were then stored at 4.5 °C for 7 days to simulate post-shipping holding conditions, followed by an extended shelf-life at 15 °C for 21 or 28 days, depending on shipping temperature and duration.

In 2003, 1200 fruit were sorted into the two colour classes ("orange" and "yellow") using a colorimeter to ensure that the yellow (hue angle ~60 °) and orange (hue angle ~75 °) fruit were separated into two distinct colour classes. There were six replicates per treatment, each containing 40 fruit. Fruit were stored at 4.5 °C for 21 days or at -0.6 °C for periods of 26 days, 28 days, 30 days or 32 days. Fruit were then exposed to a holding temperature of 4.5 °C

for 7 days followed by an extended shelf-life at 15 °C for 21 or 28 days, depending on shipping temperature and duration.

Rind colour measurement. A circle was drawn at the equatorial position of 10 fruit using a permanent marker to ensure that consecutive colour measurements were made at the same position on the fruit, thereby minimising variation in rind colour from one position on the rind to another. Ten fruit per replicate were used in 2002, and eight fruit per replicate were used in 2003. Rind colour was quantified objectively using a colorimeter (Model NR-3000, Nippon Denshoku Kogyo, Tokyo) by measuring hue angle, lightness and chroma. Visual observations related to colour intensity and general appearance were also noted. In the 2002 season, rind colour was measured at intake (before cold-storage treatment), after 2 weeks shipping at 4.5 °C and -0.6 °C, after 3 weeks shipping at 4.5 °C or 4 weeks shipping at -0.6 °C, after 7 days holding at 4.5 °C, after 2 weeks holding at 4.5 °C and after a 3 week shelf-life period at 15 °C. In the 2003 season, rind colour was measured at intake, after shipping at 4.5 °C or -0.6 °C, after 7 days holding at 4.5 °C and after 2 weeks shelf-life at 15 °C.

Statistical analysis. Data were subjected to analysis of variance using the general linear model (GLM) procedure of Statistical Analysis Systems (SAS Inc., Cary, N.C.), and means were separated using Fisher's LSD.

Results

2002 season. Changes in hue angle followed similar trends for both shipping temperatures, irrespective of initial fruit colour class. Hue angle increased during shipping at both 4.5 °C and -0.6 °C (Fig. 4.2; Table 4.1). Hue angle decreased during the 1 week holding period at 4.5 °C and 2 week shelf-life periods; initially there was a rapid drop in hue angle followed by a slight increase on the last evaluation date (Fig. 4.2). Fruit shipped at 4.5 °C had

significantly lower hue angles after 4 weeks, i.e. fruit were more orange, than fruit shipped at -0.6 °C.

On the first evaluation date (at intake) there was a significant difference in hue angle between "orange" and "yellow" fruit (64.73 ° vs. 69.04 °). During shipping and holding "yellow" fruit had a significantly higher hue angle than "orange" fruit, i.e. less well-coloured (Fig. 4.2), but this was more consistent for fruit shipped at -0.6 °C than for fruit shipped at 4.5 °C. On the final evaluation date, "orange" fruit shipped at 4.5 °C had the best colour, whereas there was no significant difference among "yellow" fruit shipped at either 4.5 °C or -0.6 °C and "orange" fruit shipped at -0.6 °C (Fig. 4.2).

Lightness followed a similar trend to that of hue angle (Fig. 4.3; Table 4.2), i.e. lightness initially increased during shipping then decreased during the holding period. Fruit shipped at 4.5 °C tended to have lower lightness values than fruit shipped at -0.6 °C, and "orange" fruit had significantly lower lightness values than "yellow" fruit. Chroma also initially increased during shipping, then decreased during holding (Fig. 4.4; Table 4.3). Chroma was consistently lower for fruit shipped at 4.5 °C than -0.6 °C. However, initial colour class did not affect chroma.

2003 season. "Orange" fruit had significantly lower hue values than "yellow" fruit at all stages of sampling and for all shipping conditions (Fig. 4.5; Table 4.4). "Orange" and "yellow" fruit maintained their initial difference in hue angle throughout the shipping and holding periods. "Orange" fruit remained more well-coloured than "yellow" fruit irrespective of shipping regime (temperature x duration). Hue angle increased slightly during shipping, at both temperatures, remained relatively constant, then decreased slightly during shelf-life (3

weeks at 15 °C). There were no consistent differences in hue angle among fruit of the same colour class shipped at 4.5 °C or -0.6 °C for different durations, although fruit shipped at 4.5 °C tended to have lower hue angles than fruit shipped at -0.6 °C (Table 4.4).

Lightness of "orange" and "yellow" fruit remained relatively constant during the postharvest storage period although "orange" fruit had significantly lower lightness values than "yellow" fruit at all stages of sampling and for all shipping conditions (Fig. 4.6; Table 4.5). There were no consistent differences in lightness among storage regimes, although lightness of fruit shipped at 4.5 °C was significantly lower than for fruit shipped at -0.6 °C at the end of the storage period.

Chroma of all fruit increased during shipping, followed by a decrease during the holding and shelf-life periods to similar values to those measured at intake (Fig. 4.7; Table 4.6). Chroma of "orange" fruit was significantly lower than that of "yellow" fruit. After the shipping period, chroma of fruit shipped at 4.5 °C was significantly lower than fruit shipped at -0.6 °C (Table 4.6).

Discussion and Conclusions

At the last evaluation date, initially "orange" fruit shipped at 4.5 °C still had the deepest orange colour (lowest hue angle and lightness values) although there was no significant difference between "yellow" fruit shipped at either temperature and "orange" fruit shipped at -0.6 °C. Due to a high rate of decay in all treatments during the post-shipping period, most well-coloured fruit may have decayed first. Therefore, when assessing rind colour after 2 weeks at the post-shipping holding temperature, it is apparent that, irrespective of initial rind colour, fruit shipped at 4.5 °C were deeper orange in colour than fruit shipped at -0.6 °C.

“Orange” fruit shipped at -0.6 °C deteriorated to the same eventual colour level as “yellow” fruit shipped at 4.5 °C. Therefore, shipping temperature affected colour development. However, shipping temperature did not significantly improve the colour of poorly coloured “yellow” fruit and cause it to fall into the same category as “orange” fruit or to lessen the average difference in hue angle of “orange” and “yellow” fruit. Initial rind colour at packing appears to be the most important factor affecting final rind colour upon arrival in the market.

Data from the 2003 season also showed that initial rind colour is the most important factor determining rind colour on arrival in the market, irrespective of the shipping regime. There was a significant difference in rind colour between fruit shipped at 4.5 °C and fruit shipped at -0.6 °C. Fruit shipped at 4.5 °C tended to be deeper orange (lower hue angle and lightness) than fruit shipped at -0.6 °C. There were no consistent trend in changes in rind colour among fruit shipped at -0.6 °C for different durations, ranging from 26 to 32 days.

Le Roux (1997) showed that rind colour improved for fruit shipped at 11 °C compared with fruit shipped at 4.5 °C, which did not show a change in rind colour. However, fruit shipped at 11 °C developed an orange rind colour compared to fruit shipped at 4.5 °C which developed a yellow rind colour. Shipping at sub-zero (-0.6 °C) or low (4.5 °C) temperatures does not favour colour development. Thus, fruit need to be well-coloured before shipping as no further colour development occurs during shipping. Low temperature shipping regime cannot be used to improve the rind colour of poorly coloured fruit. Low-temperature (<5 °C) shipping of citrus fruit has previously been shown to limit rind colour development (Gilfillan, 1988; Le Roux, 1997). However, the current study highlights the role of low-temperature shipping on the loss of rind colour, possibly via carotenoid degradation or changes in carotenoid composition. Individual fruit with deep orange initial colour (~60 ° hue, <65 Lightness) prior

to shipping at low temperatures are better able to withstand colour loss associated with low temperature shipping, whereas fruit with pale orange initial colour (~75 °hue, >70 lightness) prior to shipping at low temperature will be pale after shipping. This response of individual fruit of varying colour to low temperature shipping results in variation in rind colour among fruit at the point of arrival in export markets.

The perceived loss of rind colour following shipping at sub-zero temperatures is not a result of visibly increased “greenness” due to chlorophyll synthesis, but rather a result of decreased “orangeness”, probably due to carotenoid degradation. Therefore, initial rind colour plays a critical role in final product quality. Depending on market destination, and hence, shipping temperature, fully coloured yet pale fruit should not be packed for markets sensitive to rind colour, especially if sub-zero shipping regimes are required.

Table 4.1. Effect of shipping temperature on hue angle of 'Palmer Navel' orange during the 2002 season. Fruit were shipped for 3 weeks at 4.5 °C or 4 weeks at -0.6 °C, held for 1 week at 4.5 °C and at 15 °C for 21 or 28 days, depending on shipping temperature and duration to simulate shelf-life.

Treatment	At intake	During shipping (both temps)	After shipping at 4.5 °C or During shipping at -0.6 °C	After shipping at -0.6 °C	Holding at 4.5 °C		After shelf-life at 15 °C
					1 week	2 weeks	
Initial colour (I)							
Yellow	69.04[a z]	70.13[a]	71.38[a]	68.75[a]	68.13[a]	64.33[a]	65.85[a]
Orange	64.73[b]	66.37[b]	68.22[b]	65.52[b]	65.22[b]	61.00[b]	62.70[b]
Shipping temp (S)							
-0.6 ˚C	67.22[a]	69.01[a]	70.11[a]	69.00[a]	69.23[a]	64.59[a]	65.18[a]
4.5 ˚C	66.56[a]	67.49[a]	69.49[a]	65.27[b]	64.12[b]	60.74[b]	63.36[b]
LSD	1.66	1.68	1.57	1.84	1.99	2.01	1.73
P values							
I x S	0.2541	0.1008	0.0603	0.4899	0.5068	0.7144	0.0709
I	<0.0001	0.0002	0.0005	0.0015	0.0063	0.0025	0.0012
S	0.4133	0.0731	0.4253	0.0004	<0.0001	0.0007	0.0402

[z] Means with the same letter are not significantly different (P>0.05).

Table 4.2. Effect of shipping temperature on lightness of 'Palmer Navel' orange during the 2002 season. Fruit were shipped for 3 weeks at 4.5 °C or 4 weeks at -0.6 °C, held for 1 week at 4.5 °C and at 15 °C for 21 or 28 days, depending on shipping temperature and duration to simulate shelf-life.

Treatment	At intake	During shipping (both temps)	After shipping at 4.5 °C or During shipping at -0.6 °C	After shipping at -0.6 °C	Holding at 4.5 °C		After shelf-life at 15 °C
					1 week	2 weeks	
Initial colour (I)							
Yellow	65.56[a z]	69.71[a]	68.86[a]	67.83[a]	68.23[a]	63.84[a]	66.51[a]
Orange	63.46[b]	66.84[b]	67.49[b]	65.93[b]	66.44[b]	61.33[b]	64.90[b]
Shipping temp (S)							
-0.6 ˚C	64.84[a]	68.61[a]	68.72[a]	67.79[a]	69.35[a]	64.17[a]	66.34[a]
4.5 ˚C	64.19[a]	67.94[a]	67.63[a]	65.97[b]	65.32[b]	61.01[b]	65.06[b]
LSD	1.23	1.39	1.22	1.30	1.56	1.41	1.24
P values							
I x S	0.6903	0.1517	0.0680	0.8414	0.9676	0.0954	0.2526
I	0.0019	0.0003	0.0289	0.0063	0.0266	0.0014	0.0136
S	0.2811	0.3239	0.0767	0.0088	<0.0001	0.0001	0.0434

[z] Means with the same letter are not significantly different (P>0.05).

Table 4.3. Effect of shipping temperature on chroma of 'Palmer Navel' orange during the 2002 season. Fruit were shipped for 3 weeks at 4.5 °C or 4 weeks at -0.6 °C, held for 1 week at 4.5 °C and at 15 °C for 21 or 28 days, depending on shipping temperature and duration to simulate shelf-life.

Treatment	At intake	During shipping (both temps)	After shipping at 4.5 °C or During shipping at -0.6 °C	After shipping at -0.6 °C	Holding at 4.5 °C		After shelf-life at 15 °C
					1 week	2 weeks	
Initial colour (I)							
Yellow	65.94[a z]	69.59[a]	71.31[a]	68.99[a]	67.24[a]	65.05[a]	66.30[a]
Orange	65.93[a]	68.73[a]	71.35[a]	68.08[a]	67.26[a]	64.73[a]	66.29[a]
Shipping temp (S)							
-0.6 ˚C	66.07[a]	70.05[a]	72.33[a]	70.63[a]	68.09[a]	65.50[a]	66.88[a]
4.5 ˚C	65.79[a]	68.26[b]	70.33[b]	66.44[b]	66.41[b]	64.28[b]	65.71[b]
LSD	0.97	1.01	1.44	1.40	1.31	1.10	1.13
P values							
I x S	0.8877	0.4162	0.2377	0.5110	0.4034	0.0564	0.3193
I	0.9746	0.0907	0.9468	0.1923	0.9802	0.5409	0.9818
S	0.5502	0.0014	0.0088	<0.0001	0.0147	0.0317	0.0438

[z] Mean with the same letter are not significantly different (P>0.05).

Table 4.4. Effect of shipping temperature and duration on hue angle of 'Palmer Navel' orange during the 2003 season. Fruit were either shipped at 4.5 ˚C for 21 days or at -0.6 ˚C for various durations and held for 1 week at 4.5 ˚C and 2 weeks at 15 ˚C.

Treatment	At intake	After shipping	1w @ 4.5°C	2w @ 15°C
Initial colour (I)				
Yellow	74.43[a z]	75.05[a]	75.28[a]	73.24[a]
Orange	58.62[b]	61.28[b]	61.57[b]	59.69[b]
LSD	0.60	0.68	0.64	0.54
Shipping regime (S)				
4.5 / 21 days	66.55[ab]	67.17[c]	-	65.43[c]
-0.6 / 26 days	66.44[ab]	69.02[a]	66.86[c]	66.78[b]
-0.6 / 28 days	66.00[b]	68.08[abc]	69.46[a]	65.50[c]
-0.6 / 30 days	66.41[ab]	67.81[bc]	68.08[b]	66.94[ab]
-0.6 / 32 days	67.22[a]	68.76[ab]	69.30[a]	67.66[a]
LSD	0.94	1.07	0.91	0.85
P-value				
I x S	0.0460	0.0448	0.0306	0.0874
I	<0.0001	<0.0001	<0.0001	<0.0001
S	0.1437	0.0075	<0.0001	<0.0001

[z] Means with the same letter are not significantly different (P>0.05).

Table 4.5. Effect of shipping temperature and duration on lightness in 'Palmer Navel' orange during the 2003 season. Fruit were shipped at either 4.5 ˚C for 21 days or at -0.6 ˚C for various durations and held for 1 week at 4.5 ˚C and 2 weeks at 15 ˚C.

Treatment	At intake	After shipping	1w @ 4.5°C	2w @ 15°C
Initial colour (I)				
Yellow	71.06[a z]	72.26[a]	71.57[a]	71.13[a]
Orange	63.82[b]	63.71[b]	63.36[b]	63.41[b]
LSD	0.44	0.50	0.47	0.41
Shipping regime (S)				
4.5 /21 days	67.75[a]	68.10[a]	-	65.84[b]
-0.6 /26 days	67.66[ab]	68.39[a]	68.11[a]	67.53[a]
-0.6 /28 days	66.97[b]	68.15[a]	68.03[ab]	67.58[a]
-0.6 /30 days	67.30[ab]	67.19[b]	67.42[b]	67.57[a]
-0.6 /32 days	67.51[ab]	68.10[a]	66.29[c]	67.83[a]
LSD	0.70	0.80	0.66	0.65
P-value				
I x S	0.0923	0.0298	0.3417	0.4287
I	<0.0001	<0.0001	<0.0001	<0.0001
S	0.1876	0.0416	<0.0001	<0.0001

[z] Means with the same letter are not significantly different (P>0.05).

Table 4.6. Effect of shipping temperature and duration on chroma in 'Palmer Navel' orange during the 2003 season. Fruit were either shipped at either 4.5 ˚C for 21 days or at -0.6 ˚C for various durations and held for 1 week at 4.5 ˚C and 2 weeks at 15 ˚C.

Treatment	At intake	After shipping	1w @ 4.5°C	2w @ 15°C
Initial colour (I)				
Yellow	67.33[a z]	73.96[a]	72.63[a]	69.23[a]
Orange	66.85[a]	72.58[b]	70.84[b]	67.73[b]
LSD	0.53	0.54	0.61	0.55
Shipping regime (S)				
4.5 /21 days	67.18[a]	71.23[c]	-	68.14[b]
-0.6 /26 days	67.29[a]	73.71[ab]	68.98[c]	68.49[ab]
-0.6 /28 days	67.00[ab]	74.37[a]	73.54[a]	69.10[a]
-0.6 /30 days	67.70[a]	73.19[b]	72.76[a]	68.55[ab]
-0.6 /32 days	66.28[b]	73.85[ab]	71.65[b]	68.14[b]
LSD	0.84	0.85	0.86	0.87
P-values				
I x S	0.0094	0.2642	0.9296	0.4603
I	0.0717	<0.0001	<0.0001	<0.0001
S	0.0225	<0.0001	<0.0001	0.1727

[z] Means with the same letter are not significantly different (P>0.05).

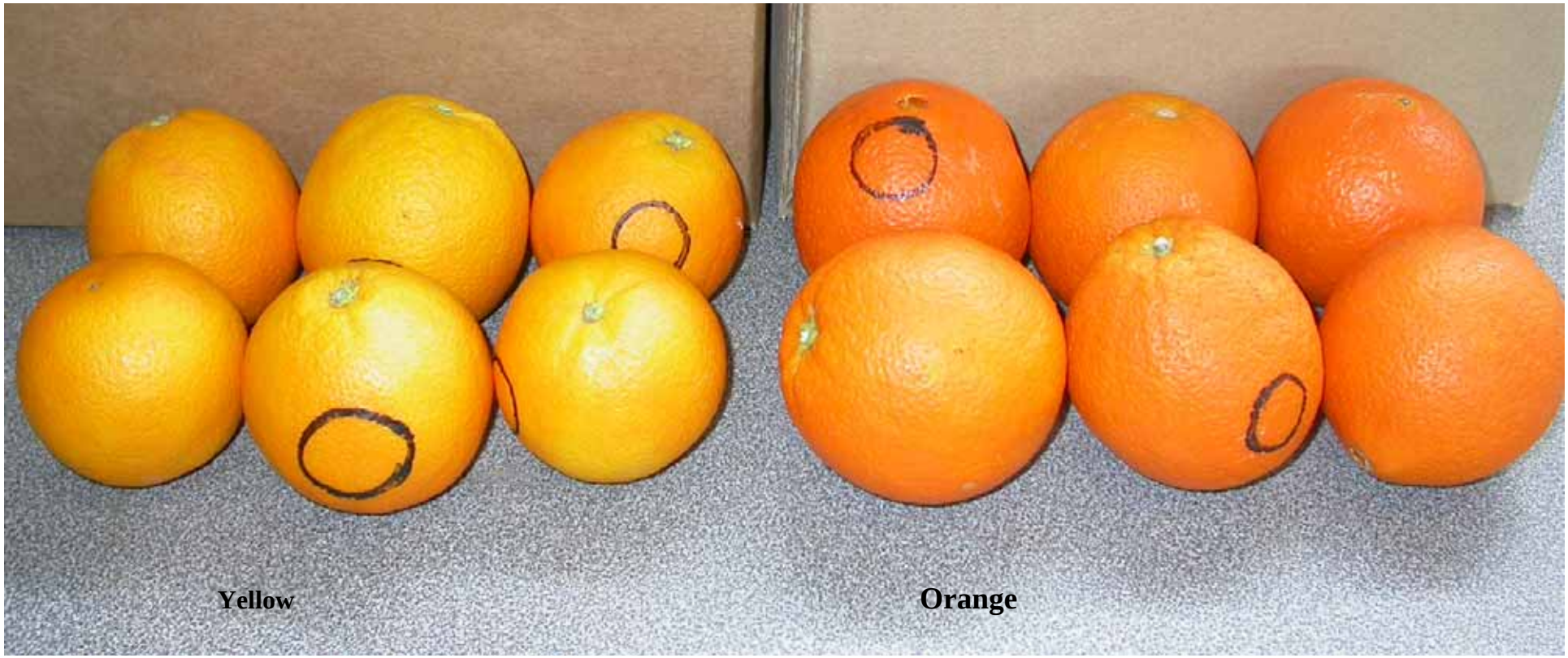

Fig. 4.1. ‘Palmer Navel’ sweet orange fruit sorted into “yellow” and “orange” colour classes prior to shipping treatments.

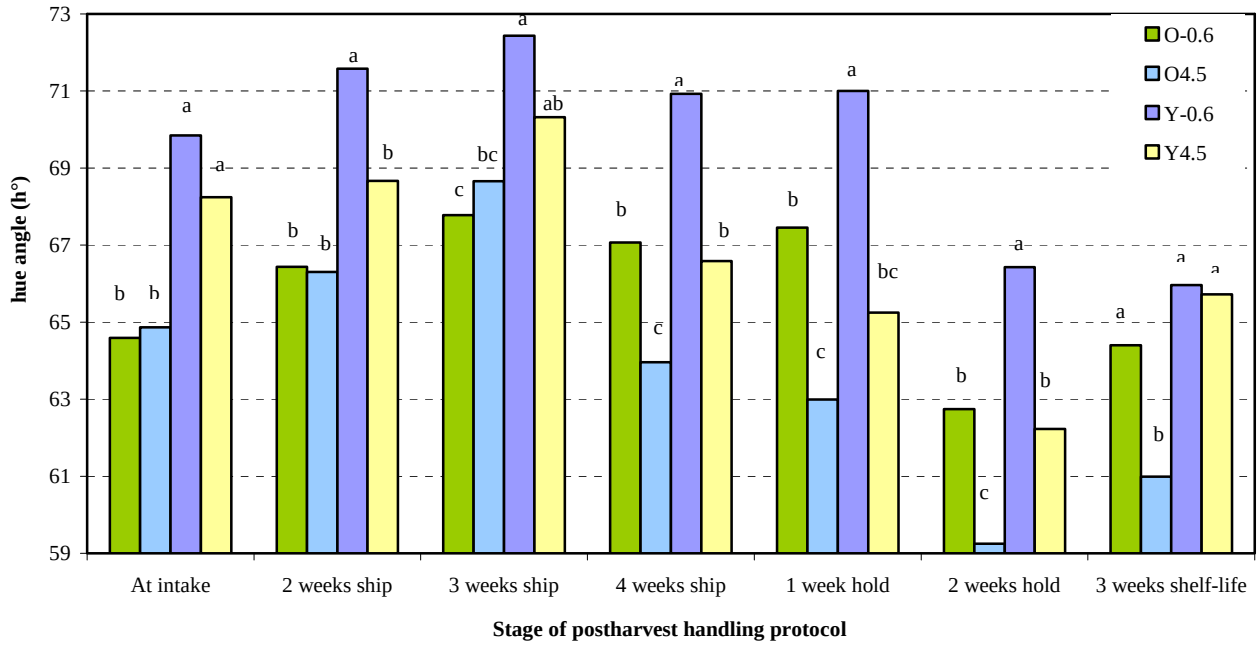

Fig. 4.2. Change in hue angle of 'Palmer Navel' orange over time in initially orange (O) and yellow (Y) fruit during shipping at -0.6 °C or 4.5 °C during the 2002 season.

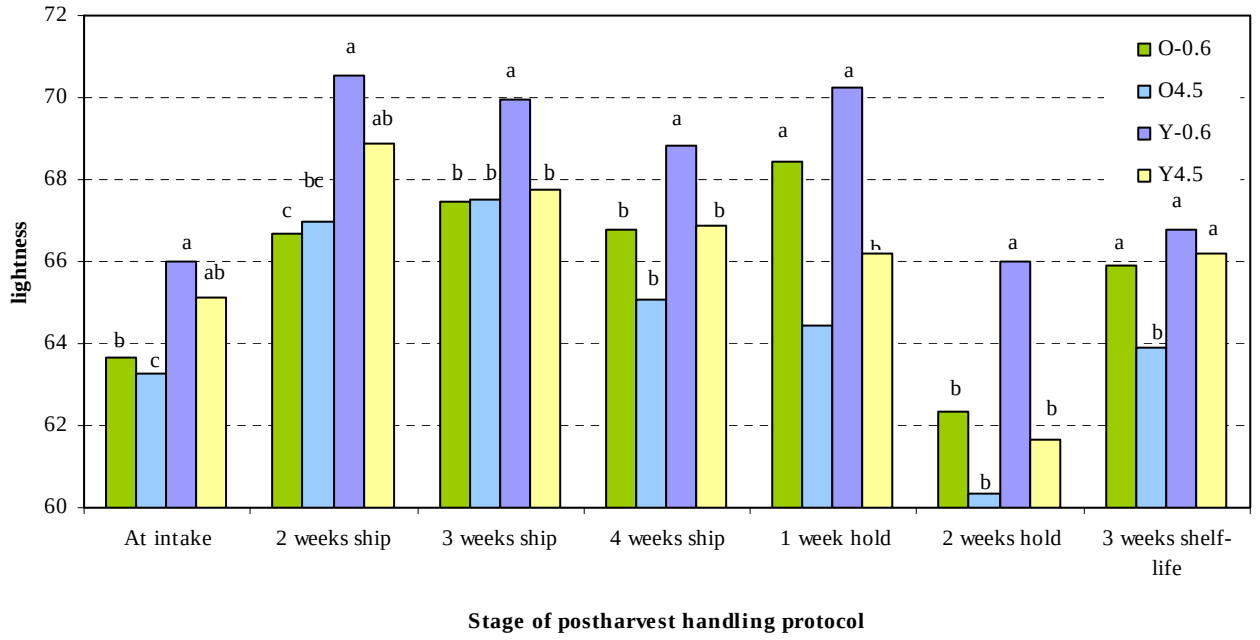

Fig. 4.3. Change in lightness of 'Palmer Navel' orange over time in orange (O) and yellow (Y) fruit during shipping at -0.6 °C or 4.5 °C during the 2002 season.

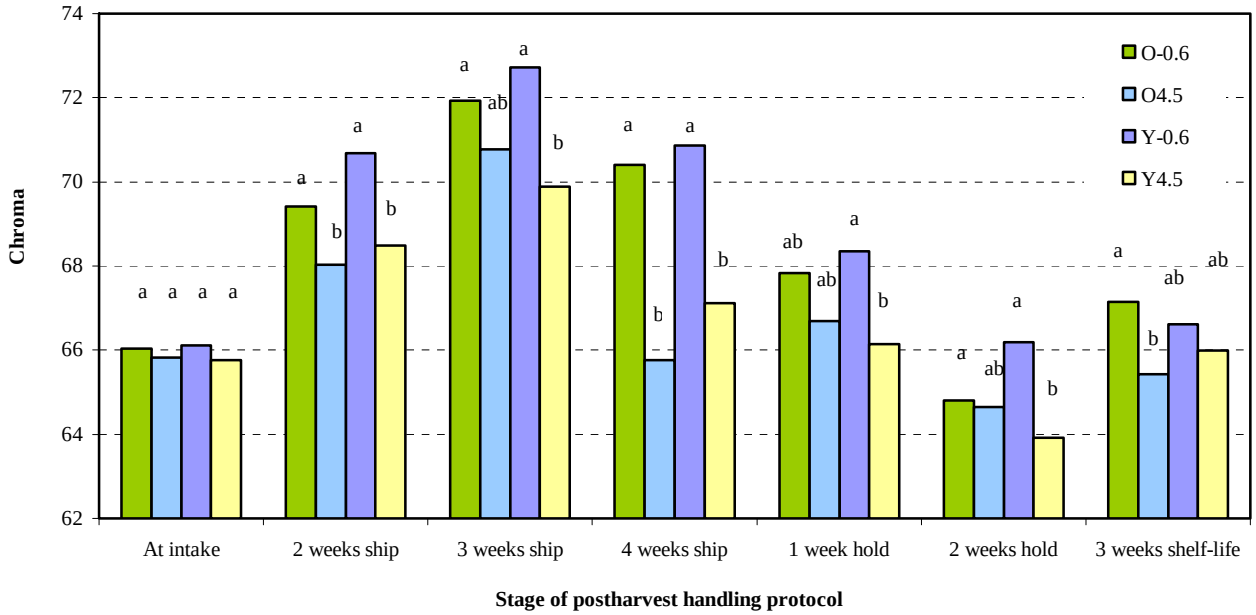

Fig. 4.4. Change in chroma of 'Palmer Navel' orange over time in orange (O) and yellow (Y) fruit during shipping at -0.6 °C or 4.5 °C during the 2002 season.

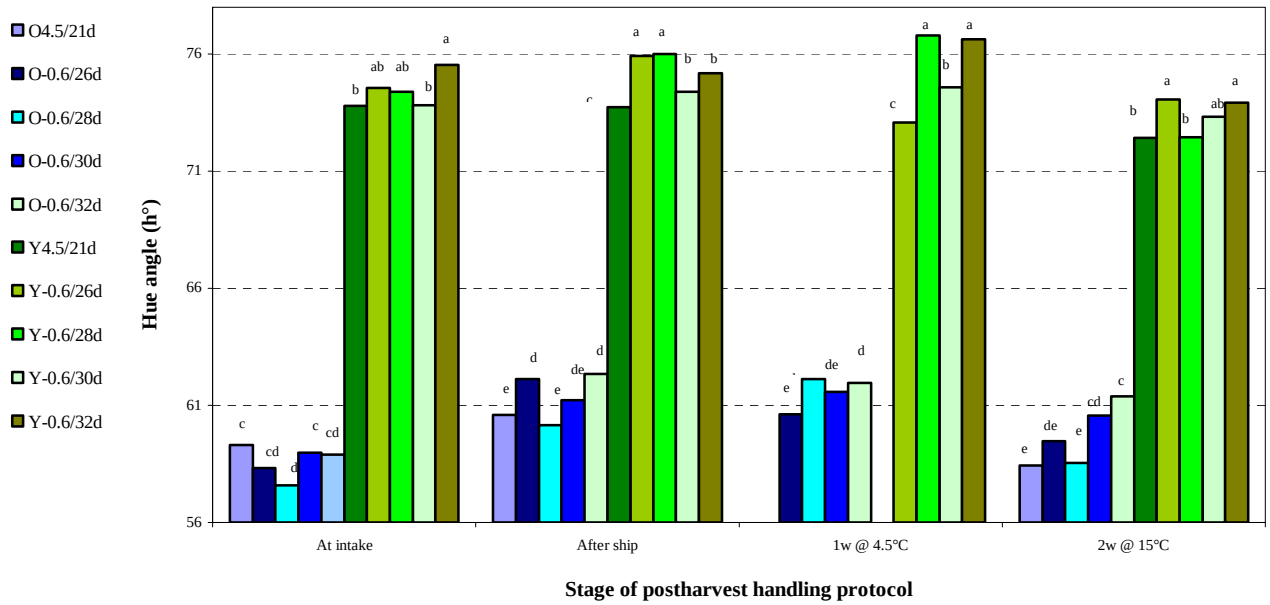

Fig. 4.5. Change in hue angle of 'Palmer Navel' orange in initially orange (O) and yellow (Y) fruit during shipping at -0.6 °C or 4.5 °C for varying durations followed by holding and shelf-life periods during the 2003 season.

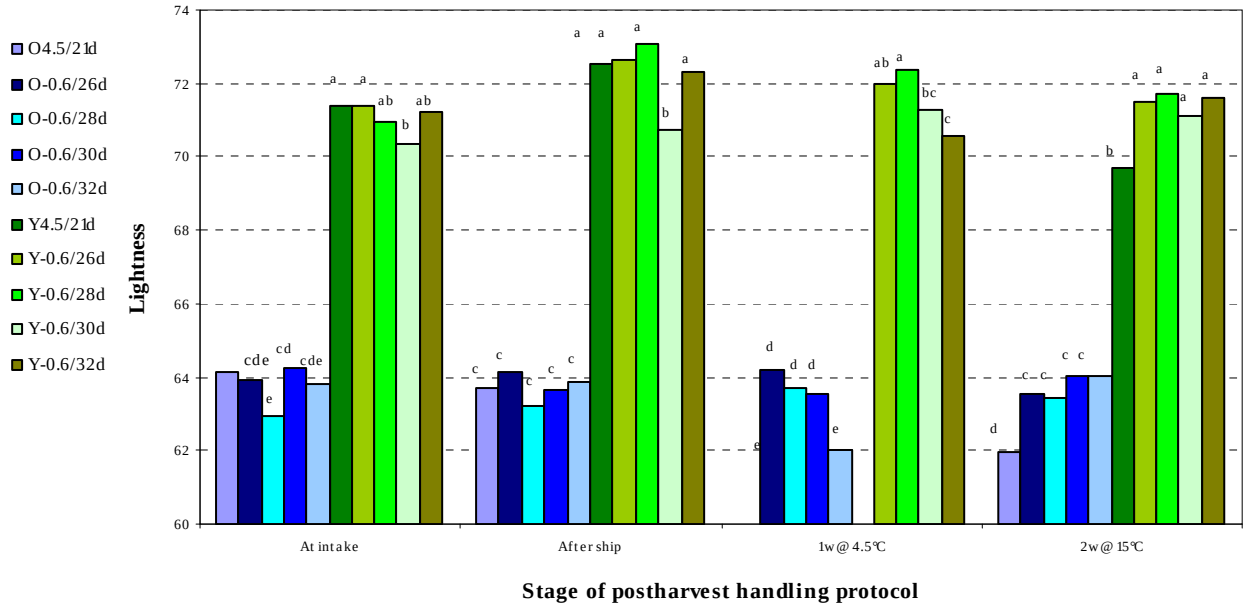

Fig. 4.6. Change in lightness of 'Palmer Navel' orange in initially orange (O) and yellow (Y) fruit during shipping at -0.6 °C or 4.5 °C for varying durations followed by holding and shelf-life periods during the 2003 season.

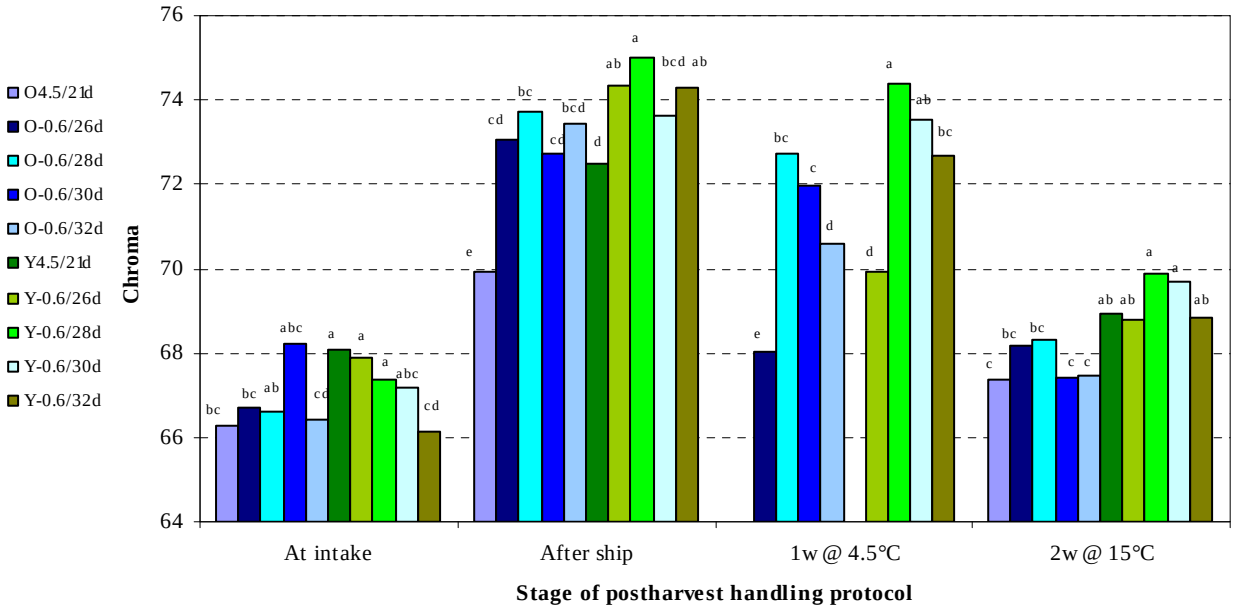

Fig. 4.7. Change in chroma of 'Palmer Navel' orange in initially orange (O) and yellow (Y) fruit during shipping at -0.6 °C or 4.5 °C for varying durations followed by holding and shelf-life periods during the 2003 season.

CHAPTER 5

OPTIMISATION OF POST-SHIPPING HOLDING TEMPERATURE TO ENHANCE RIND COLOUR OF *CITRUS* FRUIT

Abstract

Carotenoid biosynthesis is highly temperature sensitive and low shipping temperatures often lead to poor rind colour development. To comply with the USA's phytosanitary requirements for imported citrus, fruit is held at -0.6 ˚C for a minimum of 22 days. Shipping at -0.6 ˚C has an adverse effect on rind colour, but is essential to ensure exports. Various holding temperatures (low, intermediate and high) were evaluated to determine if an increased post-shipping holding temperature could improve rind colour. A high holding temperature (20 ˚C) caused colour degradation, whereas a low holding temperature (4.5 ˚C) resulted in a constant rind colour. Intermediate holding temperatures of between 11 ˚C and 15 ˚C were most effective in limiting the negative effects of sub-zero shipping temperatures on rind colour and re-initiated rind colour development.

Introduction

Temperature plays a pivotal role in rind colour development of *Citrus* fruit and can lead to either colour degradation or colour enhancement. Different carotenoids have different specific temperature requirements which result in the highest levels of carotenoid biosynthesis. For example, lycopene synthesis in tomato (*Lycopersicon esculentum* Mill.) is inhibited above 30 °C, although the synthesis of β-carotene in *Citrus* continues at this temperature (Wheaton and Stewart, 1973). β-citraurin biosynthesis is highly temperature sensitive and occurs at an optimum level at lower temperatures (Stewart and Wheaton, 1971).

Carotenoid biosynthesis in *Citrus* fruit is highly temperature sensitive and postharvest storage temperature is critical in rind colour development. The optimum postharvest storage temperature range for carotenoid biosynthesis and accumulation appears to be between 15 and 25 °C, whereas fruit stored at 30 °C showed less colour development (Wheaton and Stewart, 1973). Fruit held at 20 °C showed maximal colour development that continued over a longer period than fruit kept at 30 °C, whereas Agusti (1999) found that maximum rind colouration occurred at 15 °C, which was also associated with the highest increase in carotenoids and the highest rate chlorophyll degradation. Alternating temperatures of either 20/15 °C or 25/15 °C were found to have a favourable effect on colour development, while constant temperatures of 20 °C or 25 °C had less favourable results (Wheaton and Stewart, 1973). Le Roux (1997) found that fruit held at 20 °C, post-shipping, showed increased colour development compared with fruit held at 4.5 °C or 11 °C. Carotenoids are highly temperature sensitive and small variations (1 °C) from the optimum temperature may affect colour development (Young and Erickson, 1961).

The optimal temperature and duration thereof to re-initiate carotenoid biosynthesis after low-temperature shipping is not known. The objective of this research was to quantify the effects of post-shipping holding temperature on rind colour development and to identify the holding temperature which has the greatest effect on enhancing colour development.

Materials and Methods

Sites and plant material. In the 2002 and 2003 seasons, 'Palmer Navel' sweet oranges (*C. sinensis* [L.] Osbeck) (hereafter referred to as 'Palmer') were selected from the ALG Packhouse in Citrusdal, South Africa (32°40′S, 19°03′E). The fruit were drenched with 2,4-D (2,4-dichlorophenoxyacetic acid) (125 $mg \cdot L^{-1}$), Tecto® (thiabendazole) (500 $mg \cdot L^{-1}$) and

Sporekill® (dimethyldidecyl ammonium chloride) (120 $mg \cdot L^{-1}$) and then transported to Stellenbosch where fruit were subjected to various cold-storage regimes to simulate commercial shipping and holding conditions.

Treatments and experimental design. In the 2002 season, 3600 fruit were visually sorted into two colour classes, "yellow" and "orange", and all fruit were fully coloured with no green patches (Fig. 5.1). Fruit were stored at either 4.5 °C for 21 days or at -0.6 °C for 28 days to simulate commercial shipping conditions for Navel oranges exported to Europe or the USA, respectively. Fruit were then stored at 4.5 °C, 12.5 °C or 20 °C for 6 weeks (for fruit shipped at -0.6 °C) or 7 weeks (for fruit shipped at 4.5 °C) to compare the effect of post-shipping holding temperature on rind colour, i.e. there were 12 treatments: initial rind colour (2), shipping temperature (2), holding temperature (3). Each treatment had six replicates, and there were 50 fruit per replicate.

In the 2003 season, the same treatments were maintained, except that the holding temperatures were changed to 4.5 °C, 11 °C and 15 °C as data from the previous season showed that a holding temperature of 20 °C had a negative effect on rind colour.

Rind colour measurement. A circle was drawn at the equatorial position of 10 fruit using a permanent marker to ensure that consecutive colour measurements were made at the same position thereby minimising variation of rind colour from one position on the rind to another. Ten fruit per replicate were used in 2002, and eight fruit per replicate were used in 2003. Rind colour was quantified objectively using a colorimeter (Model NR-3000, Nippon Denshoku Kogyo, Tokyo) by measuring hue angle, lightness and chroma. Visual observations related to colour intensity and general appearance were also noted. In the 2002 season, rind colour was measured on four evaluation dates: after shipping, after 2 or 3 weeks

holding, after 3 or 4 weeks holding and after 5 or 6 weeks holding (all holding periods depended on shipping periods). In the 2003 season, rind colour was measured on six evaluation dates: after shipping as in 2002, then weekly for 6 weeks.

Statistical analysis. Data were subjected to analysis of variance using the general linear model (GLM) procedure of Statistical Analysis Systems (SAS Inc., Cary, N.C.), and means were separated using Fisher's LSD.

Results

2002 season. Rind colour was significantly better in fruit held at 12.5 °C than fruit held at 20 °C or 4.5 °C (Figs. 5.2 and 5.3; Tables 5.1 to 5.3); hue angle decreased over time and was significantly lower than for fruit held at 4.5 °C or 20 °C within 2 to 3 weeks of storage at 12.5 °C. Initially "orange" and "yellow" fruit had the lowest hue angles after post-shipping holding at 12.5 °C (~65° and ~69°, respectively) (Figs. 5.2 and 5.3). On the other hand, rind colour degradation occurred in fruit held at 20 °C as evidenced by increased hue angle (Figs. 5.2 and 5.3). Rind colour remained relatively stable in fruit held at 4.5 °C. The level of decay was lowest in fruit held at 4.5 °C (data not shown).

2003 season. Fruit held at 4.5 °C after shipping had the poorest rind colour (Fig. 5.4). Rind colour decreased for "orange" fruit, irrespective of shipping temperature, during the holding period, but was stable for "yellow" fruit at both shipping temperatures (Fig. 5.4). The largest improvement in rind colour was for "yellow" fruit held at 11 °C. "Orange" fruit shipped at 4.5 °C and held at either 11 °C or 15 °C also had good colour development. However, there were no significant differences in hue angle between these treatments. There were significant differences in lightness and chroma between "orange" and "yellow" fruit in both seasons, but

the effect of holding temperature on these components of colour was less marked (Tables 5.2 and 5.3).

Discussion and Conclusions

In the 2002 season, it appears that post-shipping, holding temperature played a greater role in rind colour development than shipping temperature. As holding temperature increased, the rate of decay increased and peaked at 20 °C. The high rate of decay observed during holding in the 2002 season may have influenced results, especially at the later evaluation dates. At each evaluation, decayed fruit were removed from the carton and the sound, adjacent fruit were surface cleaned with diluted NaCl solution. Holding temperature had a substantial effect on eventual fruit rind colour. Well-coloured fruit remained well-coloured throughout shipping and holding although the difference in hue angle between the two colour classes became narrower.

In the 2003 season, the holding temperatures were chosen on the basis of what was observed in the 2002 season. In an attempt to identify the ideal holding temperature, the ranges between the three holding temperatures were decreased and the 20 °C holding temperature was replaced with a lower temperature as the 2002 data indicated that 20 °C was too high to favour good colour development. These results contrast those of Le Roux (1997) who found that rind colour development was greatest when fruit were held at 20 °C. However, Le Roux (1997) did not hold fruit for longer than 1 week.

Holding temperature had a large effect on rind colour development and could be seen after 2 weeks at holding temperature. The effect of shipping temperature on rind colour development became negligible during holding. It appears that the most colour development takes place in

the intermediate temperature range, i.e. 11 to 15 °C. At 4.5 °C rind colour remains constant throughout the holding period. "Orange" fruit generally remained more well-coloured throughout the holding period, except at the last evaluation date where there were no significant differences among the O -0.6/4.5, Y -0.6/11 and Y 4.5/11 treatments. A trend can be seen by looking at the grouping of rind colour, while all treatments differed significantly at the last evaluation date, except Y -0.6/11, Y 4.5/11 and O -0.6/4.5, which were statistically similar and O 4.5/15 and O 4.5/11 which were statistically similar. Fruit held at 4.5 °C stood out as having the poorest eventual colour in both "yellow" and "orange" colour classes. Fruit held at 11 °C and 15 °C, within colour classes, were similar in colour throughout the holding period. "Orange" fruit remained the most well-coloured, in spite of shipping temperature, while a holding temperature of between 11 °C and 15 °C appeared to accelerate colour development. Holding temperature narrowed the difference in hue angle between "orange" and "yellow" fruit. By selecting the correct holding temperature, even after shipping at sub-zero temperatures, final colour can be significantly improved.

Table 5.1. The effect of shipping and holding temperature on hue angle of 'Palmer Navel' orange fruit during the 2002 and 2003 seasons. In the 2002 season, fruit were held at 4.5, 12.5 or 20 °C, while in the 2003 season, fruit were held at 4.5, 11 or 15 °C.

Treatment	After Shipping		2w after ship		3w after ship		5w after ship		6w after ship	7w after ship
	2002	2003	2002	2003	2002	2003	2002	2003	2003	2003
Initial colour (I)										
Yellow	73.35[a z]	73.45[a]	73.68[a]	72.17[a]	72.71[a]	70.22[a]	72.69[a]	69.09[a]	69.78[a]	69.51[a]
Orange	68.52[b]	58.47	69.09[b]	58.01[b]	69.76[b]	56.71[b]	69.09[b]	56.41[b]	60.26[b]	57.03[b]
Shipping temp (S)										
-0.6 °C	70.80[a]	65.75[a]	71.42[a]	65.99[a]	71.18[a]	64.30[a]	70.96[a]	63.42[a]	63.83[b]	64.13[a]
4.5 °C	71.08[a]	66.17[a]	71.35[a]	64.82[b]	71.30[a]	62.63[b]	70.82[a]	62.08[b]	66.21[a]	62.68[b]
LSD	0.75	0.47	1.02	0.45	0.92	0.46	1.09	0.84	0.66	0.46
Holding temp(H)										
4.5 °C	70.38[b]	66.13[a]	70.90[b]	66.68[a]	70.13[b]	66.72[a]	71.04[b]	65.96[a]	68.95[a]	69.40[a]
12.5 / 11°C	70.88[ab]	65.71[a]	68.91[c]	64.93[b]	68.97[c]	62.12[b]	67.11[c]	63.68[b]	61.14[c]	59.45[c]
20 / 15 °C	71.56[a]	66.04[a]	74.35[a]	64.60[b]	74.62[a]	61.54[c]	74.53[a]	61.54[c]	64.89[b]	61.36[b]
LSD	0.92	0.58	1.24	0.55	1.12	0.56	1.34	0.56	0.80	0.56
P-values										
I x S x H	0.0032	0.7730	0.9251	0.8279	0.9755	0.0097	0.3554	0.0650	<0.0001	0.0572
I x S	0.0005	0.0010	0.4327	0.0009	0.1539	<0.0001	0.1909	<0.0001	<0.0001	<0.0001
I x H	0.0285	0.1142	0.0168	<0.0001	0.0056	0.6912	0.8429	0.0204	<0.0001	0.0016
S x H	0.0367	0.2498	0.3621	0.2015	0.4697	0.4687	0.7331	0.1292	<0.0001	0.0440
I	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
S	0.4693	0.0783	0.9015	<0.0001	0.7959	<0.0001	0.7890	<0.0001	<0.0001	<0.0001
H	0.0434	0.3201	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[z] Means with the same letter are not significantly different (P>0.05).

Table 5.2. The effect of shipping and holding temperature on lightness of 'Palmer Navel' orange fruit during the 2002 and 2003 seasons. In the 2002 season, fruit were held at 4.5, 12.5 or 20 °C, while in the 2003 season, fruit were held at 4.5, 11 or 15 °C.

Treatment	After Shipping		2w after ship		3w after ship		5w after ship		6w after ship	7w after ship
	2002	2003	2002	2003	2002	2003	2002	2003	2003	2003
Initial colour (I)										
Yellow	70.28[a z]	71.25[a]	70.52[a]	70.15[a]	69.57[a]	68.49[a]	69.80[a]	68.29[a]	68.20[a]	66.56[a]
Orange	66.99[b]	62.07[b]	68.08[b]	61.77[b]	67.43[b]	60.62[b]	67.89[b]	60.74[b]	62.08[b]	59.46[b]
Shipping temp (S)										
-0.6 °C	68.62[a]	66.30[b]	69.15[a]	66.28[a]	68.77[a]	64.93[a]	68.79[a]	64.81[a]	64.50[b]	63.19[a]
4.5 °C	68.65[a]	67.02[a]	69.46[a]	65.65[b]	68.22[b]	64.18[b]	68.89[a]	64.22[b]	65.78[a]	62.83[a]
LSD	0.48	0.45	0.57	0.54	0.49	0.46	0.57	0.34	0.46	0.45
Holding temp(H)										
4.5 °C	68.24[b]	66.77[a]	68.42[b]	66.11[a]	68.55[a]	66.81[a]	69.14[b]	65.96[a]	66.29[a]	64.93[a]
12.5 / 11°C	68.76[ab]	66.50[a]	68.78[b]	65.84[a]	67.89[b]	64.88[b]	66.93[c]	63.89[b]	63.37[b]	62.11[b]
20 / 15 °C	68.90[a]	66.71[a]	70.71[a]	65.95[a]	69.05[a]	63.98[b]	70.46[a]	63.68[b]	65.75[a]	61.99[b]
LSD	0.58	0.55	0.69	0.66	0.61	0.57	0.70	0.42	0.56	0.55
P values										
I x S x H	0.0186	0.7195	0.2682	0.7461	0.2019	0.4296	0.0024	0.8860	<0.0001	0.4458
I x S	0.0113	0.2679	0.6538	0.2141	0.1284	0.0640	0.8345	0.0087	<0.0001	0.0795
I x H	0.0770	0.1989	0.1307	0.0225	0.0002	0.7509	0.0879	0.1177	<0.0001	0.0085
S x H	0.0126	0.1247	0.5251	0.8522	<0.0001	0.7539	0.4304	0.9971	<0.0001	0.6803
I	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
S	0.9215	0.0022	0.2668	0.0228	0.0301	0.0019	0.7139	0.0010	<0.0001	0.1171
H	0.0667	0.5887	<0.0001	0.7156	0.0014	0.0035	<0.0001	<0.0001	<0.0001	<0.0001

[z] Means with the same letter are not significantly different (P>0.05).

Table 5.3. The effect of shipping and holding temperature on chroma of 'Palmer Navel' orange fruit during the 2002 and 2003 seasons. In the 2002 season, fruit were held at 4.5, 12.5 or 20 °C, while in the 2003 season, fruit were held at 4.5, 11 or 15 °C.

Treatment	After Shipping		2w after ship		3w after ship		5w after ship		6w after ship	7w after ship
	2002	2003	2002	2003	2002	2003	2002	2003	2003	2003
Initial colour (I)										
Yellow	72.45[a z]	72.90[a]	69.60[a]	71.41[a]	69.92[a]	70.47[a]	67.67[a]	71.59[a]	71.99[a]	69.96[a]
Orange	71.14[b]	72.16[a]	69.31[a]	69.45[b]	69.84[a]	68.53[b]	66.75[b]	69.54[b]	69.70[b]	67.42[b]
Shipping temp (S)										
-0.6 °C	71.74[a]	71.27[b]	69.23[a]	70.52[a]	69.79[a]	69.65[a]	67.09[a]	70.83[a]	70.49[b]	68.98[a]
4.5 °C	71.86[a]	73.79[a]	69.67[a]	70.33[a]	69.97[a]	69.35[a]	67.33[a]	70.31[a]	71.21[a]	68.40[b]
LSD	0.59	0.84	0.63	0.73	0.58	0.40	0.71	0.55	0.43	0.53
Holding temp(H)										
4.5 °C	72.00[a]	72.38[ab]	72.41[a]	72.19[a]	73.03[a]	71.45[a]	69.26[a]	73.18[a]	73.70[a]	70.68[a]
12.5 / 11°C	71.73[a]	72.08[b]	68.81[b]	69.95[b]	70.72[b]	68.86[b]	67.09[b]	69.66[b]	68.68[c]	67.65[b]
20 / 15 °C	71.67[a]	73.13[a]	67.13[c]	68.42[c]	65.88[c]	68.20[c]	65.29[c]	68.87[c]	70.15[b]	67.74[b]
LSD	0.72	1.02	0.77	0.90	0.71	0.49	0.87	0.68	0.53	0.65
P values										
I x S x H	0.8230	0.3701	0.3470	0.8186	0.0646	0.0149	0.0442	0.0368	<0.0001	0.0063
I x S	0.9260	0.0013	0.2686	0.1857	0.0476	0.0008	0.9044	0.7086	0.5158	0.0266
I x H	0.5238	0.6979	0.8399	0.0442	0.0021	0.8954	0.1189	0.0939	0.3382	0.1881
S x H	0.9172	0.5444	0.4439	0.2134	<0.0001	0.2946	0.1976	0.2958	0.0312	0.0202
I	<0.0001	0.0796	0.3583	<0.0001	0.7907	<0.0001	0.0117	<0.0001	<0.0001	<0.0001
S	0.7000	<0.0001	0.1632	0.6070	0.5526	0.1297	0.5040	0.0628	0.0015	0.0311
H	0.6092	0.1181	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[z] Means with the same letter are not significantly different (P>0.05).

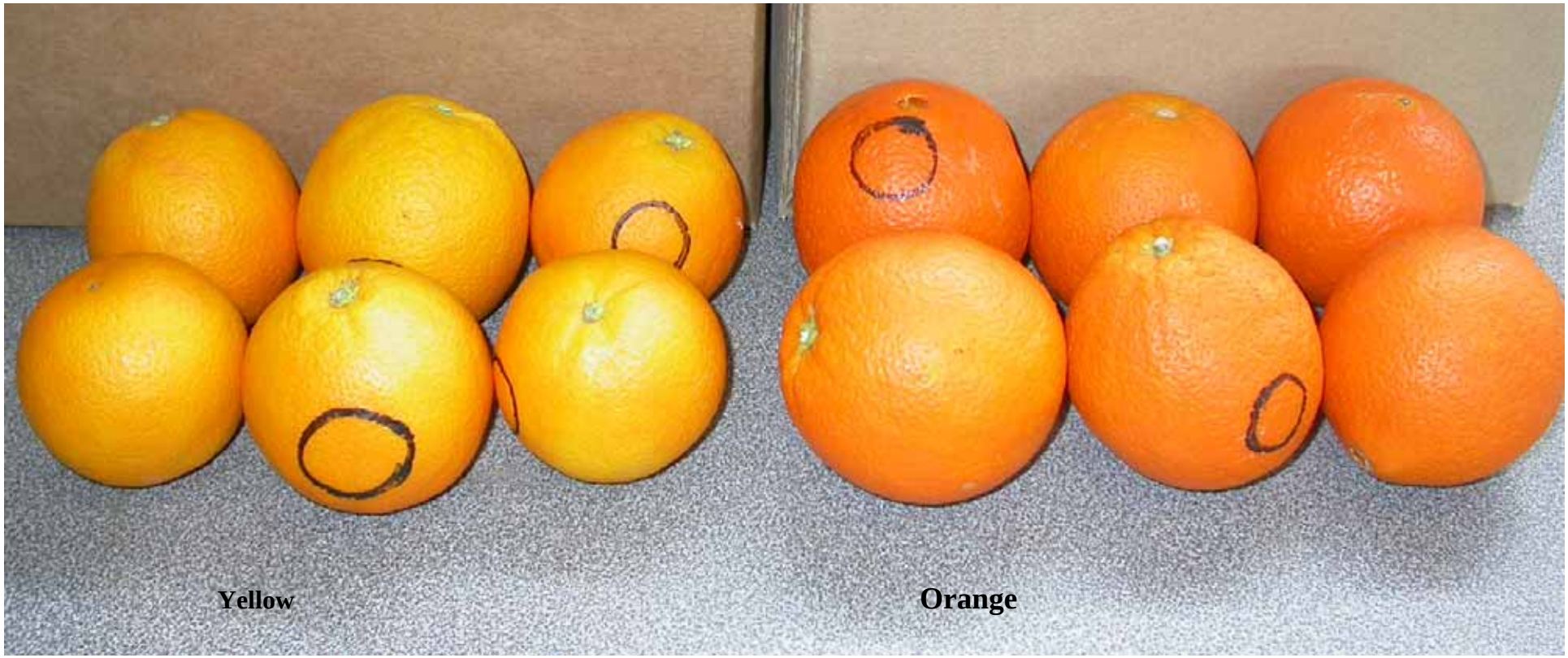

Fig. 5.1. ‘Palmer Navel’ sweet orange fruit sorted into “yellow” and “orange” colour classes prior to shipping and post-shipping holding treatments.

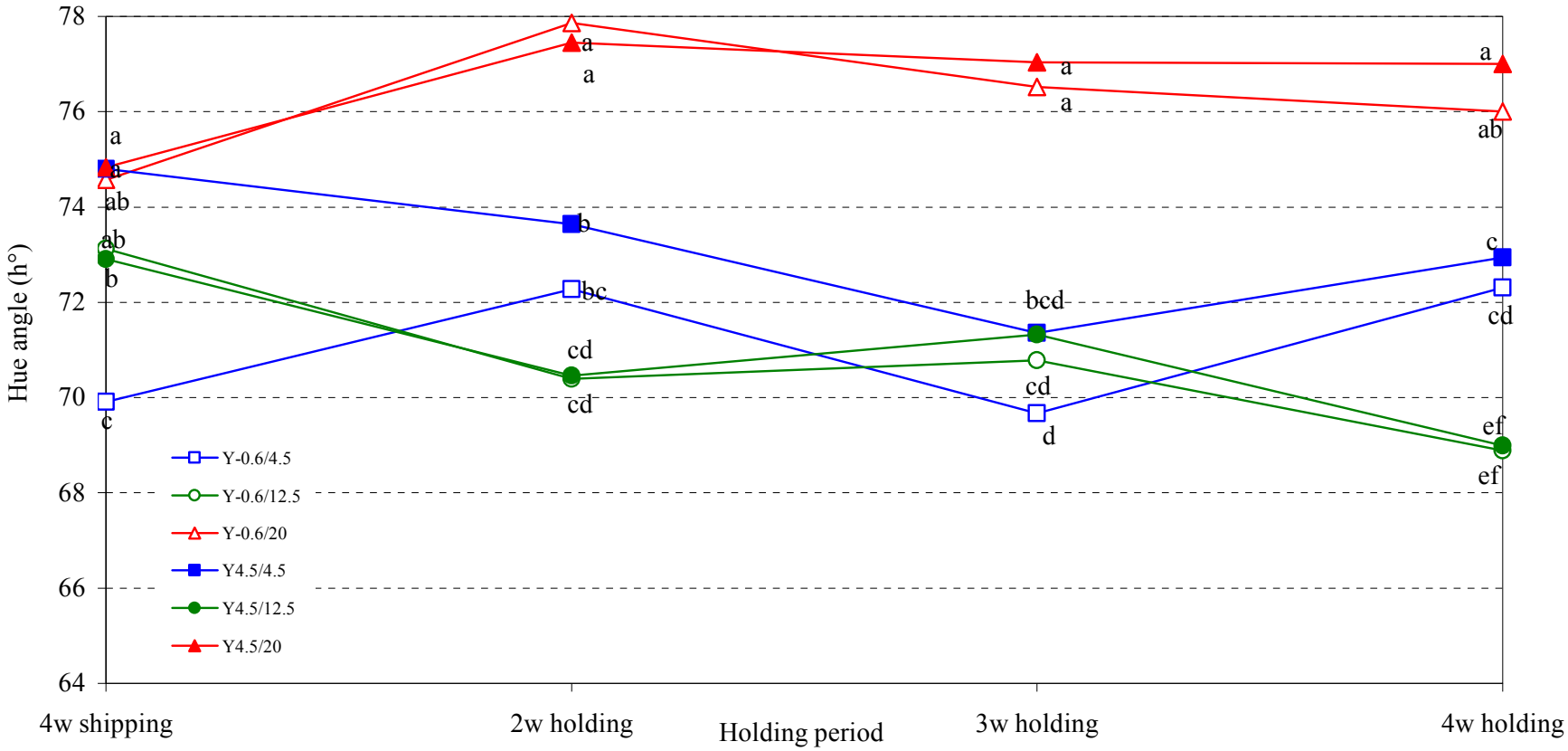

Fig. 5.2. Change in hue angle of "yellow" (Y) fruit during holding, at 4.5, 12.5 and 20 °C after being shipped at -0.6 °C or 4.5 °C in the 2002 season. (Letters indicating statistical differences apply to both Figs. 5.2 and 5.3).

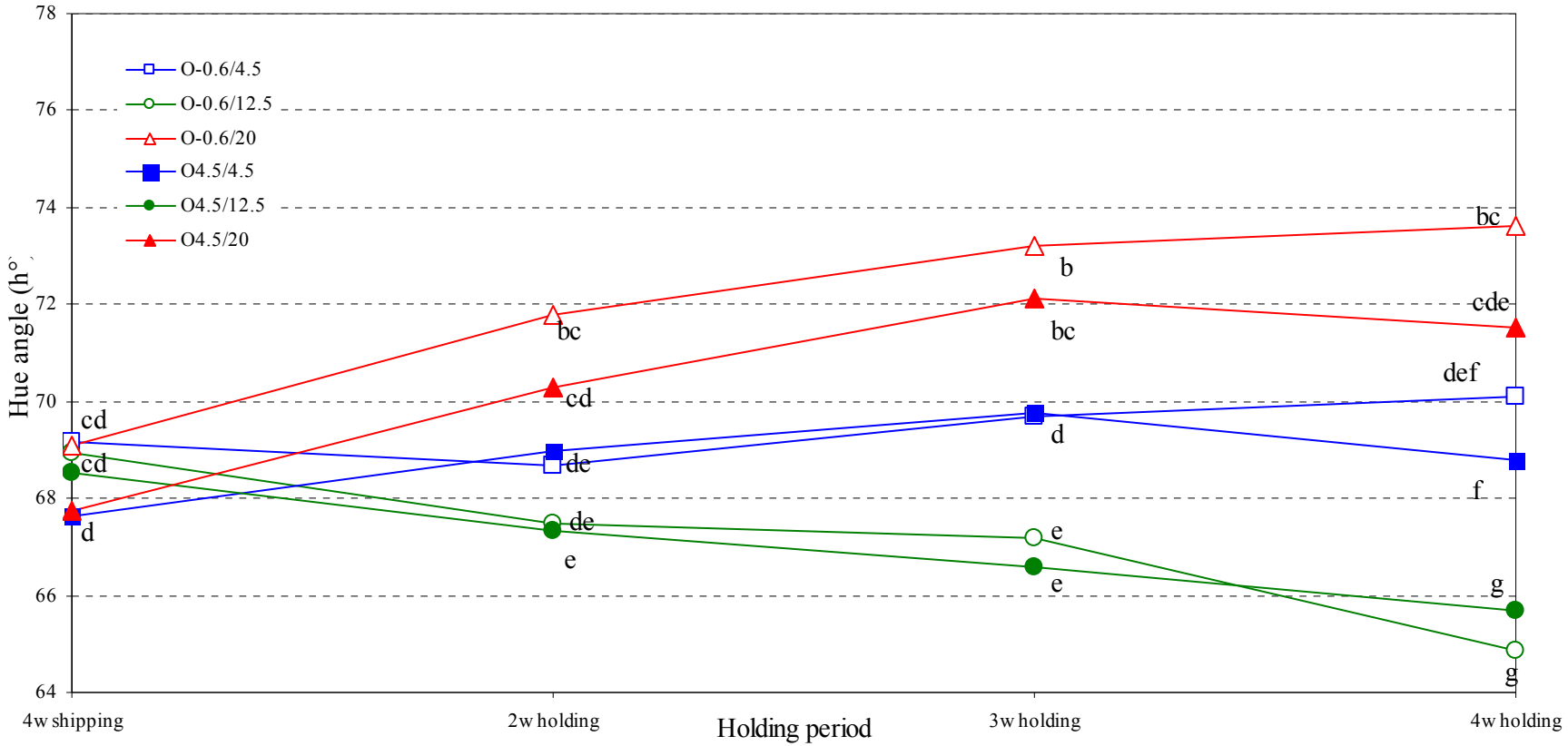

Fig. 5.3. Change in hue angle of "orange" (O) fruit during holding, at 4.5, 15 and 20 °C after being shipped at -0.6 °C or 4.5 °C in the 2002 season. (Letters indicating statistical differences apply to both Figs. 5.2 and 5.3).

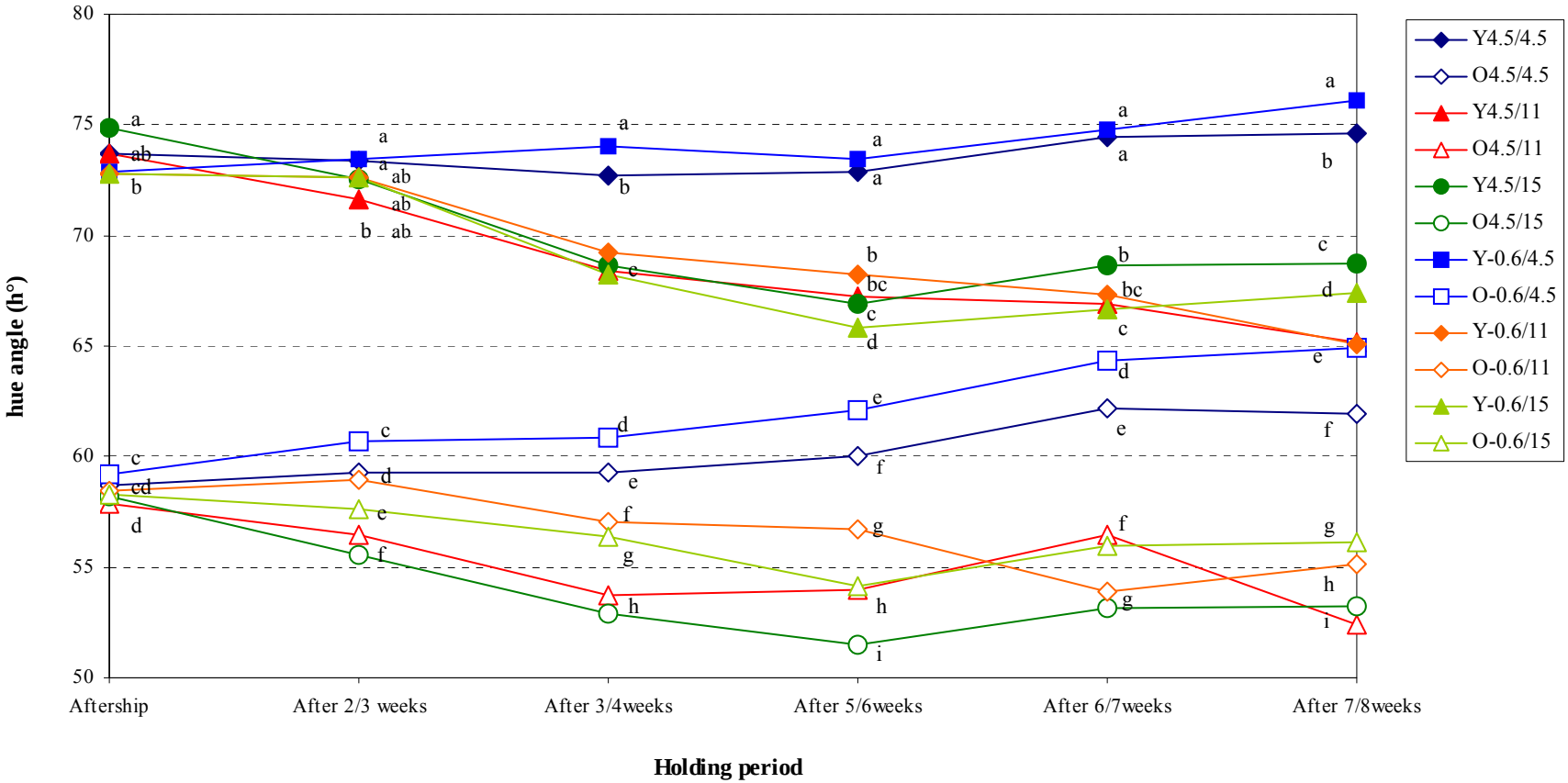

Fig. 5.4. Change in hue angle of "orange" (O) and "yellow" (Y) fruit during holding at 4.5, 11 and 15 °C in the 2003 season after being shipped at -0.6 °C or 4.5 °C.

CHAPTER 6

OVERALL DISCUSSION AND CONCLUSIONS

Cold shock was applied under postharvest conditions to simulate a rapid drop in temperature resulting from a "cold front". Rind colour of 'Nules Clementine' mandarin fruit was significantly improved by cold shock treatment in one experiment. If this response could be consistently achieved, cold shock could partially substitute ethylene degreening. However, cold shock did not consistently improve rind colour of 'Nules Clementine' mandarin and 'Navel' orange.

Cold shock was unsuccessful in most cases and appeared to have a negative effect on fruit colour as treated fruit had a slightly paler rind colour than degreened fruit. This response may have been due to chlorophyll breakdown rather than carotenoid biosynthesis, thereby allowing the underlying yellow-orange pigments to be exposed. There could be various reasons for the inconsistent performance of cold shocked fruit. Temperature data from California for the period before treatment showed that minimum temperatures were favourable for good natural colour development on the tree. These ideal temperature conditions may have initiated good colour development. Therefore, cold shock may be more effective during warm autumn conditions leading to poor rind colour.

The response to cold shock treatment may vary from one *Citrus* species to another, e.g. 'Navel' oranges may be less sensitive to cold shock than 'Nules Clementine' mandarin. Cold weather may have initiated natural colour development in the later-maturing 'Navel' orange fruit than in 'Nules Clementine' mandarin which usually mature before weather conditions become conducive for natural colour development. Therefore, the key to the success of cold

shock may lie in applying the treatment earlier in the season before a drop in temperature initiates natural colour development on the tree.

Furthermore, experimental cold shock temperatures may have been sub-optimal and may have negatively affected carotenoid biosynthesis, leading to pale-coloured fruit. Alternatively, the incubation temperature used may have been too low to trigger colour development. It may, therefore, be necessary to increase the temperature at which cold shock is applied as cold shocked fruit had a yellow rind, which may have been a result of the destruction of carotenoids under very low temperatures (4 °C), whereas in nature, temperatures below 13 °C are sufficient to cause a colouring response.

Following the positive results on 'Nules Clementine' mandarin in the 2002 season and the need to reduce the negative effects of degreening on fruit shelf-life, further research on refining the cold shock treatment is required.

Shipping temperature is known to affect rind colour (Gilfillan, 1988; Le Roux, 1997). However, the effect of sub-zero shipping temperature has not previously been quantified. In this study, there was a significant difference in rind colour between fruit shipped at 4.5 °C and fruit shipped at -0.6 °C. Hue angle increased at both shipping temperatures, i.e. colour degradation occurred. There were no significant differences in rind colour between fruit shipped at -0.6 °C for different durations, indicating that the USA phytosanitary requirement for cold sterilisation at -0.6 °C for a minimum of 22 days did not negatively affect the colour of initially well-coloured fruit more than any other treatment. However, shipping at sub-zero (-0.6 °C) or low (4.5 °C) temperatures does not favour colour development. Thus, fruit need to be well-coloured before shipping as no further colour development occurs in transit. Sub-zero shipping temperature does not improve rind colour of fruit. Therefore, initial rind colour

is the most important factor determining rind colour on arrival at the market, especially when low-temperature shipping conditions are used.

Low-temperature (<5 °C) shipping of citrus fruit has previously been shown to limit rind colour development (Le Roux, 1997). However, the current study highlights the role of low-temperature shipping on the loss of rind colour, possibly via carotenoid degradation. Individual fruit with deep orange initial colour (~60° hue, <65 lightness) prior to shipping at low temperatures are better able to withstand colour loss associated with low-temperature shipping, whereas fruit with pale orange initial colour (~75° hue, >70 lightness) prior to shipping at low temperature were pale after shipping.

The perceived loss of rind colour following shipping at sub-zero temperatures is probably due to carotenoid degradation. Therefore, initial rind colour plays a critical role in final product quality. Depending on market destination, and hence, shipping temperature, fully coloured yet pale fruit should not be packed for markets sensitive to rind colour.

Post-shipping holding temperature had a substantial effect on eventual rind colour. Well-coloured fruit remained well-coloured throughout shipping and holding although the range in hue angle between the two colour classes decreased. The most rind colour development took place in the intermediate temperature range, from 11 to 15 °C. A holding temperature of 20 °C was too high to favour good colour development, whereas at 4.5 °C rind colour remained constant throughout the holding period. “Orange” fruit generally remained more well-coloured throughout the holding period, regardless of shipping temperature. By selecting the optimal holding temperature, even after shipping at sub-zero temperatures, final rind colour can be significantly improved.

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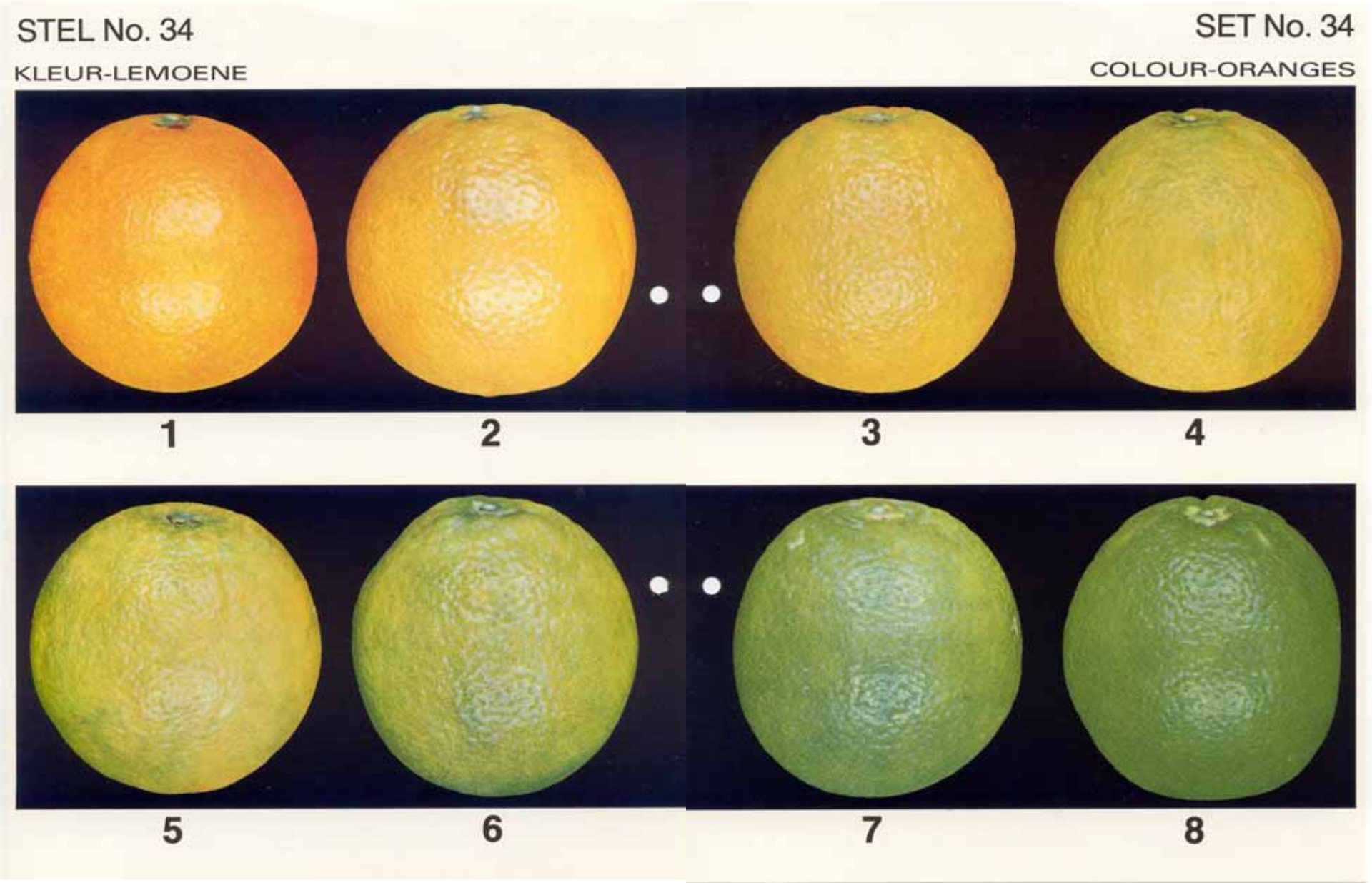
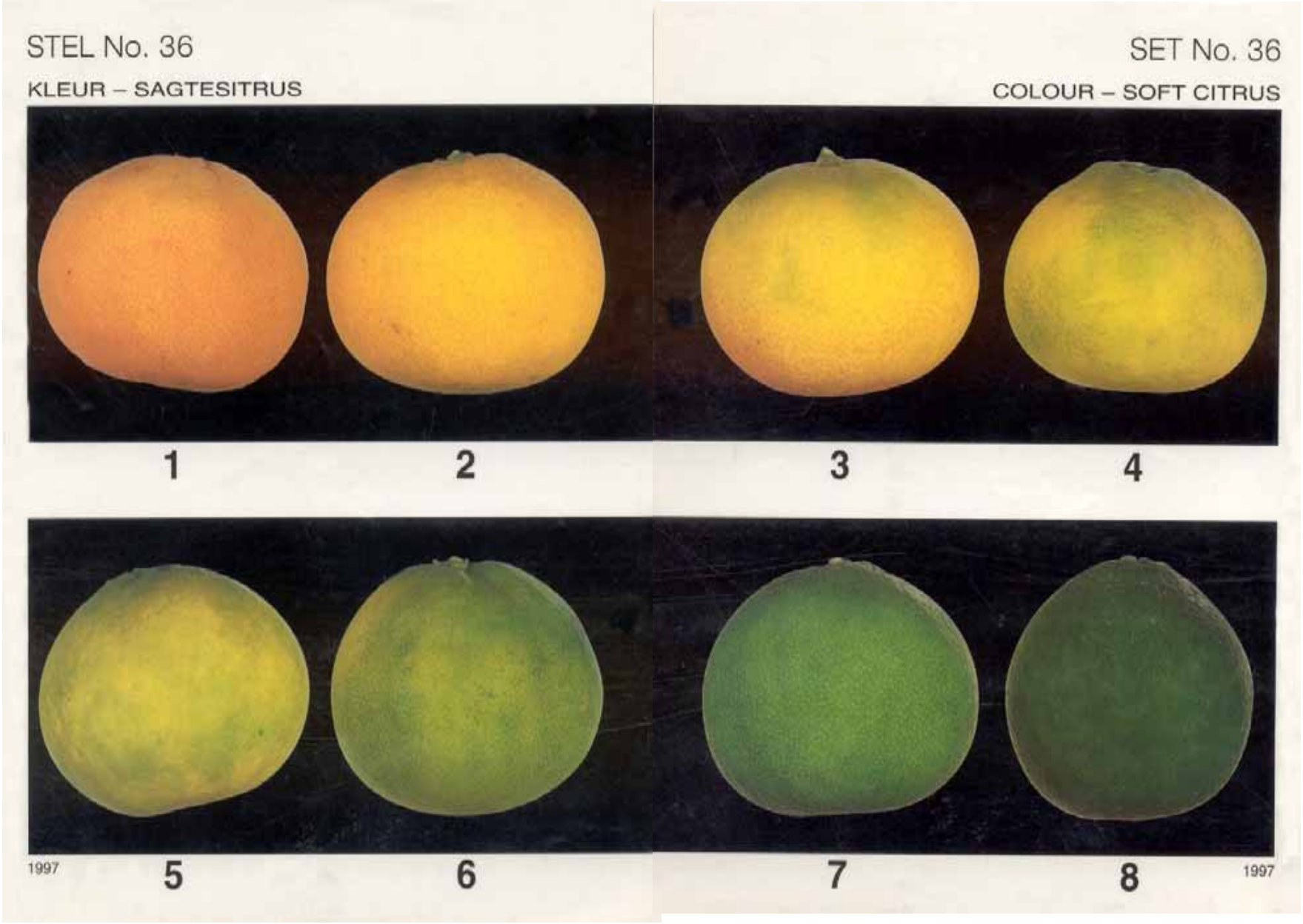


Appendix 1. Rind colour rating chart for oranges (CRI, 2004a).



Appendix 2. Rind colour rating chart for soft citrus (CRI, 2004b).