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Low-temperature cold shock may induce rind colour development of 'Nules Clementine' mandarin (*Citrus reticulata* Blanco) fruit

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Abstract

To simulate a rapid drop in temperature resulting from a cold front, 'Nules Clementine' mandarin (*Citrus reticulata* Blanco) fruit were hydrocooled to ~2 °C for 30 min and then transferred to a cold room set at 4 °C for 6 h to complete the cold shock treatment. Thereafter, fruit were incubated at 20 °C for ~72 h. In the 2002 season, low temperature treatment, or "cold shock", of 'Nules Clementine' mandarin improved rind colour to a level comparable with that of commercial ethylene degreening. Carotenoid concentration of cold-shocked fruit was similar to that of degreened fruit and nearly double that of untreated fruit. Chlorophyll concentration of cold-shocked and degreened fruit was nine times lower than that of untreated fruit. In subsequent experiments, however, where pre-harvest growing conditions were more conducive to natural rind colour development, this response could not be repeated.

Keywords: Carotenoids; Chlorophyll; *Citrus*; 'Clementine' mandarin; Cold shock; Ethylene degreening; Rind colour

1. Introduction

Rind colour of citrus fruit is largely determined by prevailing weather conditions during fruit maturation. Two temperature effects on rind colour of citrus fruit are important, namely, the requirement for optimum temperature favouring biochemical processes affecting chlorophyll degradation 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). By contrast, 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).

Fruit exposed to a day/night temperature of 20/5 °C developed a uniform orange colour, whereas a day/night temperature of 30/10 °C produced less well-coloured fruit (Erickson, 1960). 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. Citrus trees grown under more extreme conditions with large day–night fluctuations produce bright, orange-coloured fruit (Stearns and Young, 1942; Agusti, 1999). Air temperature below 13 °C causes chlorophyll degradation, revealing the underlying carotenoids and giving fruit a bright orange colour (Young and Erickson, 1961; Reitz and Embleton, 1986). As air and soil temperatures fall below 13 °C, chlorophyll degradation takes place as chloroplasts are converted to carotenoid-containing chromoplasts.

A rapid drop in temperature resulting from a "cold front", at or near fruit maturation, is often associated with the onset of so-called "colour break". To simulate this weather phenomenon in the laboratory, Oberholster (2001) exposed 'Valencia' sweet orange (*Citrus sinensis* [L.] Osbeck) flavedo discs to 4 °C for various periods, ranging from 2 to 10 h,

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and then incubated the discs at 22 °C for up to 96 h. Rind colour was improved after an incubation period of 24 h and the improvement in colour was more pronounced after 96 h. Apparently, carotenoid accumulation was enhanced in flavedo discs following the cold shock. Duration of the cold shock appears to be of secondary importance to the intensity of cold, but further investigation is required to determine the effects of cold shock on whole fruit.

Hydrocooling is an effective technology to rapidly cool fruit (Thompson et al., 2002), whereas forced-air cooling results in a more gradual decrease in temperature (Brosnan and Sun, 2001). For example, hydrocooling took <1 h to decrease the temperature of oranges by 88%, whereas forced-air cooling took between 1.5 and 3 h 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.

In an attempt to simulate a rapid drop in temperature resulting from a cold front, whole citrus fruit were hydrocooled. The objective of this study was to determine whether this postharvest "cold shock" treatment could trigger carotenoid biosynthesis and thereby enhance rind colour of fruit of early-maturing *Citrus* spp.

2. Materials and methods

2.1. Sites and plant material

In May 2002, 'Nules Clementine' mandarin (*C. reticulata* Blanco) fruit were selected from a commercial citrus packhouse in Gouda, Western Cape province, South Africa (33°19′S, 19°03′E). This experiment was repeated during November 2002 in Bakersfield, Kern county, CA, USA, using 'Nules Clementine' mandarin fruit grown in Maricopa, CA (35°04′N, 119°24′W), and selected from a commercial citrus packhouse in Bakersfield, CA. In May 2003, 'Nules Clementine' mandarin fruit were selected from a commercial citrus packhouse in Gouda, South Africa.

2.2. Treatments and experimental design

Two hundred 'Nules Clementine' mandarin fruit were sorted into two colour categories by visually selecting fruit according to colour categories T4 and T5. These colour categories are based on a series of photographs depicting various stages of rind colour development for mandarins (CRI, 2004), where T4 = yellow/orange strongly dominant with patches of green and T5 = yellow/orange becoming dominant over green. Within each colour category, 30 fruit were randomly allocated to treatments in a completely randomized block design with six replicates. Treatments included an untreated control, commercial ethylene degreening, cold shock, and a combination of cold shock and degreening.

Ethylene degreening was applied for 72 h using standard commercial practices (Krajewski and Pittaway, 2002) of 95% relative humidity, a temperature of 23 °C, an ethylene concentration of 2 μL L^{-1} and a carbon dioxide concentration <0.3%. The fruit were then subjected to commercial packhouse treatments, including fungicide application, waxing and packing. The fruit were drenched with 125 mg L^{-1} 2,4-dichlorophenoxyacetic acid, 500 mg L^{-1} Tecto® (thiabendazole) and 120 mg L^{-1} Sporekill® (dimethyldidecyl ammonium chloride). Fruit were stored under simulated shipping conditions of −0.6 °C for 28 days to comply 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 period at 15 °C for 21 days.

In the 2002 season, the cold shock treatment was applied by hydrocooling fruit using a commercial hydrocooler (MBB Engineers & York Refrigeration, Cape Town, South Africa). The thermostat of the hydrocooler was set to cool water to 2 °C. Fruit were hydrocooled for 30 min, until the fruit rind temperature was <4 °C. Directly after hydrocooling, fruit were transferred to a cold room set at 4 °C for 6 h to complete the cold shock treatment. After the cold shock period, fruit were incubated at 20 °C for ∼72 h or for a period equal to that of degreening to synchronise the treatments. In the 2003 season, 'Nules Clementine' mandarin fruit were exposed to various cold shock durations, viz. 2, 4 or 6 h, in an attempt to determine whether the duration of cold shock played a role in rind colour development.

2.3. Data collection

2.3.1. Rind and pulp temperature

Fruit from the cold shock treatment were selected randomly to monitor changes in rind and pulp temperature. 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 (1200 series Squirrel logger, Grant Instruments, Cambridge, England) to log temperature. Thermocouples were also attached to the water inlet and outlet.

2.3.2. Rind colour

Ten fruit were randomly selected from each of the replicates. A circle was drawn at the equatorial position of each 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). Visual observations relating to colour intensity and general appearance were also noted. Rind colour was measured directly after treatment, after 28 days at −0.6 °C, after 7 days at 4.5 °C and after 21 days at 15 °C.