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Low temperature conditioning reduces chilling injury while maintaining quality and certain bioactive compounds of 'Star Ruby' grapefruit

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ABSTRACT

In the current study, influence of storage temperature (11 and 2 °C) and low temperature conditioning (7 days at 16 °C before cold storage at 2 °C) on the bioactive compounds in 'Star Ruby' grapefruit (*Citrus paradisi* Macf.) were examined. Fruits stored at 11 °C showed no CI; while fruits stored at 2 °C showed highest CI. Conditioning treatment (CD) reduced the incidence of CI. Carotenoids and flavonoids were significantly higher after 16 weeks in fruits stored at 11 °C. Low temperature storage (2 °C and CD) helped to retain ascorbic acid for a longer period (12 weeks). Higher furocoumarins and taste scores along with less decay development were observed in CD fruits. Conditioning treatment can be utilized to reduce CI and to maintain taste and certain bioactive compounds of grapefruits during prolonged storage at low temperature. However, for a short storage period, 11 °C temperature is more effective.

1. Introduction

Quality is an important attribute which influences marketability of fruits and vegetables. Storing fresh produce at low temperature is commonly practiced to enhance storage life, reduce storage losses and to retain the quality of fruits and vegetables by slowing their rate of metabolic activities. However, tropical and subtropical fruits are sensitive to low temperature storage and develop chilling injuries (CI) when stored at low temperature for prolonged period. Among the different citrus fruits, lemons, limes and grapefruits are highly susceptible to CI (Kader & Arpaia, 2002). The most common symptoms of CI manifested in citrus fruits are internal discolouration, browning of flavedo and albedo, pitting, surface lesions, and water soaked tissues (Grierson, 1986; Porat, Weiss, Cohen, Daus, & Aharoni, 2004). Grapefruit (*Citrus paradisi* Macf.) develops CI (pitting or brown staining of flavedo) when stored at temperatures below 10 °C. Early season and late season grapefruits are more sensitive to CI as compared to those harvested in the midseason (Grierson, 1974).

Various treatments used in our lab and by others, including the temperature conditioning treatment (Porat, Pavoncello, Peretz, Ben-Yehoshua, & Lurie, 2000), intermittent warming (Da Vis & Hofmann, 1973), use of different waxes and vegetable oils (Aljuburi & Huff, 1984) as well as modified atmosphere packaging (Porat, Weiss, Cohen, Daus, & Aharoni, 2004) are reported to reduce CI incidence. Pre-storage temperature conditioning is one of the most common treatments used to prevent the incidence of CI, by increasing the cold stress tolerance. Conditioning treatment of citrus fruits to enhance fruit quality has been extensively studied (Biolatto, Vazquez, Sancho, Carduza, & Pensel, 2005; Hatton & Cubbedge, 1982, 1983; Porat et al., 2000). In conditioning treatment fruits are cured at relatively higher temperature prior to cold storage. It is further categorized as high temperature conditioning and low temperature conditioning.

Low temperature conditioning is reported to reduce CI in several crops such as avocados, cucumbers, eggplants, grapefruits, lemons, limes, mangoes, papayas, sweet peppers, tomatoes and zucchini squash (Wang, 1994; Woolf, Cox, White, & Ferguson, 2003). In grapefruits, low temperature conditioning is carried out at either 21 °C for 3 days or at 16 °C for 7 days (Hatton & Cubbedge, 1982, 1983). Conditioning fruits at 16 °C for 7 days was reported to be better as compared to 21 °C for 7 days in minimizing incidence of CI in grapefruits stored at 1 °C (Hatton & Cubbedge, 1983).

Recent study showed that high temperature conditioning at 37 °C for 1–2 days had no negative effect on flavonoids, vitamin C and antioxidant capacity in the chilling sensitive 'Fortune' mandarin pulp (Lafuente, Ballester, Calejero, & González-Candelas, 2011). The treatment was conducted taking into consideration the single (16 days at 1.5 °C) and double (32 days at 1.5 °C) quarantine treatments required for eradication of the Mediterranean fruit

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fly, with maximum storage period of 32 days. However, influence of low temperature conditioning (7 days at 16 °C) on the bioactive compounds including limonoids, furocoumarins and carotenoids in 'Star Ruby' grapefruit juice vesicles during prolonged cold storage has not been investigated.

Grapefruit contains diverse class of bioactive compounds such as limonoids, flavonoids, furocoumarins and vitamins. These bioactive compounds help in reducing the risks from various chronic disorders such as cardiovascular diseases, cancer and inflammation, by protecting against the free radicals (Benavente-García & Castillo, 2008; Manners, 2007). Previous studies in our laboratory have demonstrated that limonoids and flavonoids can inhibit the growth of human neuroblastoma (Poulose, Harris, & Patil, 2006), colonic adenocarcinoma cells (Poulose et al., 2006) and oral carcinogenesis (Miller et al., 2008). In addition, limonoids and flavonoids also enhance the levels of phase – II detoxifying enzymes such as glutathione-S-transferase and NAD(P)H: quinone reductase (Perez et al., 2009, 2010). Therefore, it is essential to understand the influence of storage period, storage temperature and low-temperature conditioning treatment on the contents of bioactive compounds present in grapefruit juice vesicles. The present study reports changes in the levels of bioactive compounds such as ascorbic acid, carotenoids, limonoids, flavonoids and furocoumarins present in the juice vesicles of 'Star Ruby' grapefruit stored for 16 weeks at 11, 2 °C and in preconditioned fruits (7 days at 16 °C) stored at 2 °C. To the best of our knowledge, this is first report on the influence of low-temperature conditioning treatment and prolonged cold storage on limonoids and furocoumarins present in 'Star Ruby' grapefruit juice vesicles.

2. Materials and methods

2.1. Chemicals

The solvents used for the extractions were of ACS-grade, while HPLC-grade solvents were used for quantitative analysis (Fisher Scientific Research, Pittsburgh, PA, USA). Narirutin, naringin, didymin, poncirin, limonin, nomilin, lycopene, β-carotene, 6′,7′-dihydroxybergamottin (DHB), bergamottin and butylated hydroxytoluene (BHT) were obtained from Sigma Aldrich Co. (St. Louis, MO, USA). Deacetyl nomilinic acid glucoside (DNAG) was purified in the lab according to our previously published methods (Poulose, Jayaprakasha, Mayer, Girennavar, & Patil, 2007).

2.2. Plant material

'Star Ruby' grapefruits were purchased in April, 2009 from a commercial packinghouse at the Hachula valley in North Israel. The fruits were harvested at optimal maturity and were further washed, sorted, dipped in 1,000 μL L^{-1} Imazalil fungicide and coated with commercial 'Zivdar' polyethylene-based wax emulsion (Safe-Pack Ltd., Kfar Saba, Israel), in the packinghouse, as per the commercial practice.

2.3. Storage and conditioning treatment

Grapefruits were divided into three lots, one for each temperature treatment, 11, 2 °C, and conditioning treatment (CD) in which fruits were subjected to 16 °C for 7 days, followed by storage at 2 °C. The grapefruits in all three treatments were stored for period of 16 weeks and the samples were collected at an interval of 4 weeks, with subsequent 1 week storage at 20 °C to simulate shelf life conditions. Juice samples, prepared by homogenizing three peeled fruits, were used for quality analysis. Additionally, juice samples were lyophilized and sent to the Vegetable and Fruit Improvement Center, Texas A&M University, College Station for quantification of bioactive compounds.

2.4. Fruit quality analysis

A model PAL-1 digital refractometer (Atago, Tokyo, Japan) was used to determine the total soluble solids (TSS), while acidity percentages were measured by titration to pH 8.3 with 0.1 M NaOH by means of an automatic titrator model CH-9101 (Metrohm, Herisau, Switzerland). Each measurement comprised of five replications, where each replication was prepared from three fruits.

Colour measurements were taken using a Chromo Meter, model CR-200 (Minolta, Tokyo, Japan). Fruits (n = 15 per treatment) were circled with a black marker on their equatorial side, and the peel colour within these circles was determined by measuring their hue angle; a hue angle of ~90° represents yellow, ~60° orange, and ~30° red colour.

Fruit weight loss was evaluated at an interval of 4 weeks by weighing 10 fruits per treatment before and after the storage, and calculating their percentages of weight loss.

2.5. Evaluation of decay and chilling injury

Decay incidence was determined as the number of fruits manifesting decay symptoms (mainly green mold) in each treatment after each storage interval as compared to the total number of fruits, and expressed as decay percentage. All treatments included three replications, with each replication containing fifteen fruits.

CI was evaluated by sorting the fruits after each storage interval into four categories according to their peel damage severity: none (score 0; no pitting), slight (score 1; a few scattered pits), moderate (score 2; pitting covering up to 30% of the fruit surface), and severe (score 3, extensive pitting covering > 30% of the fruit surface). Overall CI incidence was determined as the total number of fruits manifesting CI symptoms in each treatment after each storage interval as compared to the total number of fruits, and expressed as CI percentage. All treatments included three replications, with each replication containing fifteen fruits.

2.6. Sensory analysis

Fruit sensory quality was evaluated at 4 weeks storage intervals with subsequent 1 week storage in shelf life conditions at 20 °C. Separated segments of peeled grapefruits were cut into halves and placed into covered glass cups. Each treatment included a mixture of cut segments prepared from five individual fruits. Fruit taste was evaluated by a sensory panel consisting of 10 members; five males and five females, aged between 25 and 62 years. Each panelist assessed the various attributes of three samples, based on an unstructured 100 mm scale, with anchor points 'very weak' and 'very strong' for each attribute. Sensory data were recorded as distances (mm) from the origin. The samples were identified by means of randomly assigned three-digit codes.

2.7. Ascorbic acid determination

Ascorbic acid content in fruits was determined by titrating the juice with 2,6-dichlorophenolindophenol (Hiromi, Kuwamoto, & Ohnishi, 1980) and comparing the titration volumes with 0.1% ascorbic acid (Sigma–Aldrich, St. Louis, MO). The results were expressed as mg of ascorbic acid per 100 ml of juice.

2.8. Carotenoids analysis

Sample preparation for carotenoid analysis was carried out according to our previously published method (Chaudhary, Jayap-