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Influence of cold storage prior to and after ripening on quality factors and sensory attributes of 'Hass' avocados☆

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ABSTRACT

Partially-ripened avocados are often held in cold storage in an attempt to enable the consistent delivery of ripe fruit to food service or retail outlets. It is also common to hold fruit without any prior ripening for a few days to several weeks prior to ethylene treatment and final ripening. The purpose of this study was to determine the impact of these practices on ripening time and subsequent fruit quality. 'Hass' avocados were harvested from three different orchards on six harvest dates and placed at either 5 °C or 12 °C immediately after harvest or after ripening to average firmness values of either 80 N (partially ripe) or 19 N (near ripe). After storage the fruit were then ripened to eating firmness (4.4–6.7 N) and evaluated for quality parameters. A portion of the fruit at harvest firmness were stored for 0 d, 7 d, 14 d or 28 d and then treated with ethylene to ripen the fruit. Continual softening during storage resulted in fruit that had been ripened prior to storage being nearly at eating firmness at the end of 14 d, especially those stored at 12 °C. Stem end rot, body rot and pink staining of the vascular tissue occurred more frequently in the previously-ripened fruit after 14 d, the severity being greatest at 12 °C where the incidence exceeded 50% for both stem end rot and pink staining. Both harvest date and orchard influenced the amount of pink staining observed. Storage of firm fruit prior to ethylene treatment resulted in higher levels of both stem end rot and pink staining only when the storage time exceeded 14 d. In an additional study to assess the impact of the storage of partially-ripened fruit on both quality and sensory characteristics, 'Hass' avocados were harvested six times at monthly intervals, ripened to approximately 16 N firmness at 20 °C and then placed at either 1 °C or 5 °C for 7 d or 14 d. Following storage the fruit were ripened to eating firmness and evaluated. The occurrence of fruit quality defects was not affected by the timing of ripening. Sensory panelists liked fruit ripened prior to or after cold storage equally well and there were no differences among the ripening treatments, within a storage time, in the level of rich, nutty or grassy attributes that composed the fruit flavor. There were statistically-significant differences in the degree of textural creaminess among the ripening treatments, although these differences were inconsistent and slight. Storing partially ripe 'Hass' avocados for up to 4 d at either 1 °C, 5 °C or 12 °C does not greatly alter avocado quality, while storage for longer periods, such as 14 d, may be problematic due to enhanced development of decay and pink staining, especially at higher temperatures. Holding unripe avocados for longer than 14 d prior to ethylene treatment may predispose the fruit to the development of pink staining.

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1. Introduction

Consumers in the United States are generally supplied with avocados in the marketplace that have been ripened to a stage where they could be either readily consumed or eaten after a brief period of ripening. This is to a large degree a consequence of prior research demonstrating greatly enhanced consumer preference for pre-ripened fruit (Lee and Coggins, 1982) and the development of means to commercially implement the ripening process (Tokar, 2007). Not only is the consumer able to easily buy avocados that

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can be readily consumed but also the fruit-to-fruit variability in ripening time is reduced. Food service organizations also prefer the convenience of using ripened avocados as it greatly reduces the effort needed to ready the fruit for consumption.

Avocados do not ripen on the tree and so an extensive period of ripening must occur before consumption. Commercial ripening of avocados in California is most commonly performed in distribution centers or other dedicated facilities where the fruit is ripened to different levels of firmness, depending on the needs of the customer, prior to shipping to retail or food service outlets. Ethylene, at a concentration of 10–100 $\mu L\,L^{-1}$, is used to facilitate the ripening, which requires from 8 h to 36 h, depending on temperature and maturity of the fruit (Eaks, 1966; Anon., 2013). Although ripening immediately prior to marketing is preferred, it may be that the fruit are ripened and then placed into cold storage for a prolonged time awaiting demand and/or transportation. Although this is a common practice, there is insufficient information available regarding its influence on avocado fruit quality.

Holding of unripe avocados for extended amounts of time in cold storage is also common as packers and distributors respond to market demand. In addition, a large proportion of the fruit marketed in the United States is foreign in origin, sometimes necessitating lengthy transit times (21–28 d) while the fruit are in an unripe state. Fruit during long distance shipment may either be pretreated with 1-MCP (SmartFresh^{SM}) or shipped in controlled atmosphere equipped containers to prevent in-transit softening. These fruit will also benefit from ethylene ripening. Softening is known to occur in unripe avocados during cold storage (Zauberman et al., 1973; Eaks, 1976) and acts to reduce the time needed to achieve full ripeness after the fruit are warmed. Ethylene is often used during the time of final ripening to facilitate the ripening process. The impact of ethylene on fruit that have undergone such storage is, however, incompletely understood as are the effects on quality.

There have been a limited number of studies that have examined the effect of cold storage of ripened or partially-ripened fruit on subsequent fruit quality, but previous work has focused on susceptibility to chilling injury, principally discoloration of the skin or flesh, and thus has been limited in the type of quality evaluations that were performed. Zauberman et al. (1973) demonstrated that soft or semi-soft fruit can be stored for 4 weeks at temperatures ranging from 0 °C to 6 °C without the danger of chilling injury. Subsequently, Kosiyachina and Young (1976) established that avocados are most sensitive to chilling injury when the fruit are at the climacteric rise or peak stage of ripening, but are much less sensitive following the climacteric. In a more recent study, Marques et al. (2011) again addressed the issue of the effects on avocado fruit quality of storing fruit that had been partially ripened. This and prior research have not provided any information regarding its potential impact on sensory quality and have not investigated the interaction of this practice with fruit maturity. The main objective of our research was to provide a more detailed view of the impact of cold storage of partially-ripened avocados on fruit quality across the harvest season, including influences of the practice on sensory characteristics. Another objective was to examine the effect of ethylene on the time needed to achieve ripeness and quality of unripe fruit that have previously undergone various durations of cold storage.

2. Materials and methods

2.1. Avocado harvest (Tests 1A and 1B, 2006 season)

For Test 1 'Hass' avocados were harvested from three separate orchards in Ventura County, CA on six different harvest dates (January 23 (H1), March 20 (H2), April 24 (H3), June 5 (H4), July 17 (H5) and August 24 (H6)). Size 48 fruit (212–268 g) were utilized, with 500 fruit per orchard being harvested. Harvests H1, H3, and H5 were used for the experiments to determine the influence of partial ripening prior to storage on ripening time and fruit quality (part A). The remaining three harvests (H2, H4, and H6) were used to investigate the impact of cold storage prior to ethylene treatment and ripening on the same quality parameters (part B). Fruit for all of the tests were harvested in the morning and transported by air-conditioned vehicle to the Kearney Agricultural Center (KAC) in Parlier, CA. The fruit were held overnight at 12 °C, then sorted and assigned to the appropriate treatments on the following day. Care was taken to exclude blemished fruit.

2.2. Avocado harvest (Test 2, 2011 season)

For Test 2, 'Hass' avocados were obtained monthly during the avocado season in California beginning in March and ending in August from a commercial grove near Fillmore, CA (Table 1). At each harvest 450 avocados (size 48) were picked from 8 to 10 trees, the same trees being utilized each time. Care was taken to obtain fruit free of external blemishes. After harvest the fruit were transported in an air-conditioned vehicle to the Kearney Agricultural Center (KAC) in Parlier, CA. Upon arrival the fruit were randomized and placed into separate bins corresponding to the various treatments and the fruit placed at 5 °C and 90% RH. Average fruit weights and dry weights, a measurement of fruit maturity, were determined from 10 individual fruit from each of the harvests.

2.3. Ripening and storage treatments (Tests 1A and 1B)

To examine the effect of ripening prior to cold storage on subsequent fruit quality (part A) avocados were placed into cold storage either immediately after harvest (average firmness = 200 N) or following treatment with 25 $\mu L\,L^{-1}$ ethylene and ripening at 20 °C until the fruit were either partially ripe (average firmness = 80 N) or near ripe (average firmness = 19 N). In each case firmness was determined by use of a penetrometer (U.C. firmness tester, 8 mm tip). Fruit were stored at either 5 °C or 12 °C at 95% RH for either 4 d or 14 d. The storage times were selected to represent common avocado commercial handling practices in the United States, with 4 d or 14 d being typical short- or long-term storage durations, respectively. A temperature of 5 °C is used by the

Table 1
Average 'Hass' avocado fruit weight, dry weight and minimum and maximum dry weights for the five harvests for Test 2.

Harvest date	Average fruit weight (g)[x]	Average dry weight (%)[x]	Minimum dry weight (%)	Maximum dry weight (%)
15-Mar-11	219.3b	19.8c	18.0	21.4
27-Apr-11	242.3ab	23.5b	18.9	27.2
8-Jun-11	249.6ab	27.9a	23.1	31.8
13-Jul-11	281.1a	29.1a	26.6	31.3
17-Aug-11	278.6 a	29.2a	26.0	34.4

[x] Average fruit weights and dry weights determined on 10 individual fruit per harvest. Mean separation based on the 0.05% level using Tukey's test.

industry to slow ripening and maintain quality for as long as possible while 12 °C might be used to accelerate softening for the purpose of delivering ripened fruit at a specific time. After cold storage the fruit were held at 20 °C and 90–95% RH until the average flesh firmness was ≤6.7 N, at which time the fruit were considered to be fully ripe and at optimal eating firmness. In a separate experiment to assess the impact of prior cold storage on ripening time and quality (part B), avocados were placed in storage at 5 °C for 0 d, 7 d, 14 d, and 28 d the day after harvest and then ripened with 50 μL L^{-1} ethylene at 20 °C for 0 h, 24 h or 48 h following storage. The fruit were then ripened to eating firmness and assessed for fruit quality parameters. The impact of the various treatments on the timing of ripening was assessed by measurement of firmness and recording the number of days from when the treatments left cold storage until full ripeness was reached.

2.4. Ripening treatments (Test 2)

Additional testing was performed in 2011 to more fully determine the impact on avocado quality of cold storage on partially-ripened fruit. Avocados were stored for 4 d at 5 °C in 90% RH followed by ripening at 20 °C and 95% RH in the presence of 50–60 μL L^{-1} ethylene until the fruit were 13.3–17.8 N (average 15.6 N) firmness as determined by an Imada penetrometer (Imada, Northbrook, IL, USA, 8 mm tip). This ripened fruit will be referred to as near ripe, the same designation as used in Test 1, although it should be recognized that the level of firmness was slightly softer than that used in the prior test. Fruit at this firmness would be desirable for retail or foodservice operations needing fruit nearing but not at the fully ripe stage where the fruit are at optimal eating firmness. The purpose of the initial 4 d at 5 °C was to help synchronize the ripening times of the fruit. Near ripe fruit were placed at 5 °C for either 7 d or 14 d at either 1 °C or 5 °C and then ripened to full ripeness at 20 °C and 95% RH in the presence of 50–60 μL L^{-1} ethylene. Comparison fruit were kept in continuous 5 °C storage after harvest until ripening, under the same conditions as the near ripe fruit, the initiation of ripening being timed so that the fruit would be ripe at the same time as the near ripe treatments and then act as controls to help discern the effect of cold storage of partially ripe fruit.

2.5. Quality attribute evaluation

After final ripening the fruit were subjectively evaluated for peel color, overall external appearance, shrivel, external flesh to seed adhesion, seed germination, seed cavity browning, flesh bruising, diffuse flesh discoloration, stem end rot, vascular browning, vascular streaking, pink stain, uneven ripening, ease

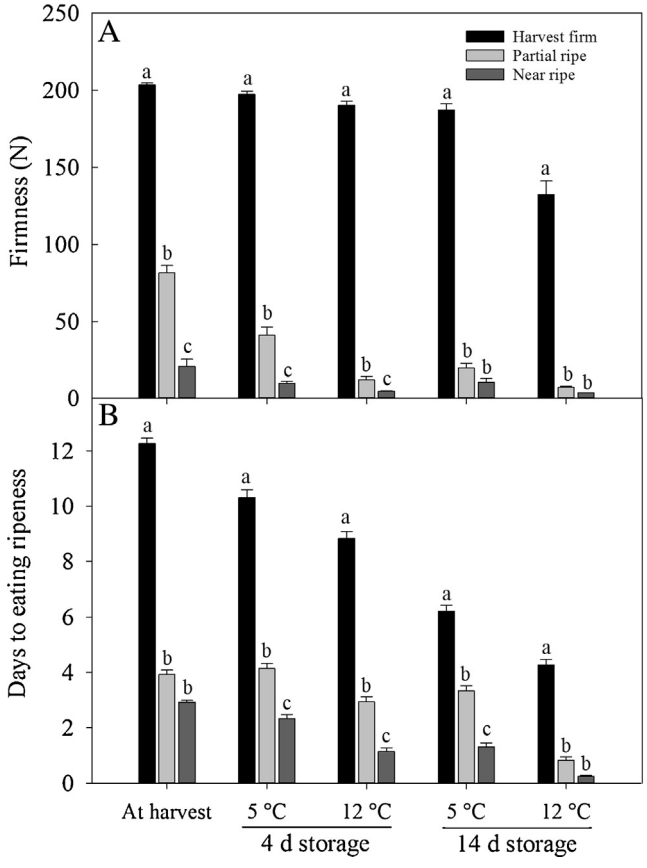

Fig. 1. Influence of limited ripening of 'Hass' avocados prior to cold storage on firmness (A) and days to eating ripeness (B) after cold storage (Test 1A). Fruit were either not ripened (harvest firm) or ripened to partial (partial ripe, 80 N) or near eating ripeness (near ripe, 19 N) with no subsequent storage or prior to either 4 d or 14 d storage at either 5 °C or 12 °C. Fruit were then ripened to eating firmness (less than 6.7 N) and the number of days needed to do so determined. Different letters within a storage time or storage time and temperature combination indicate statistical significance ($P \leq 0.05$).

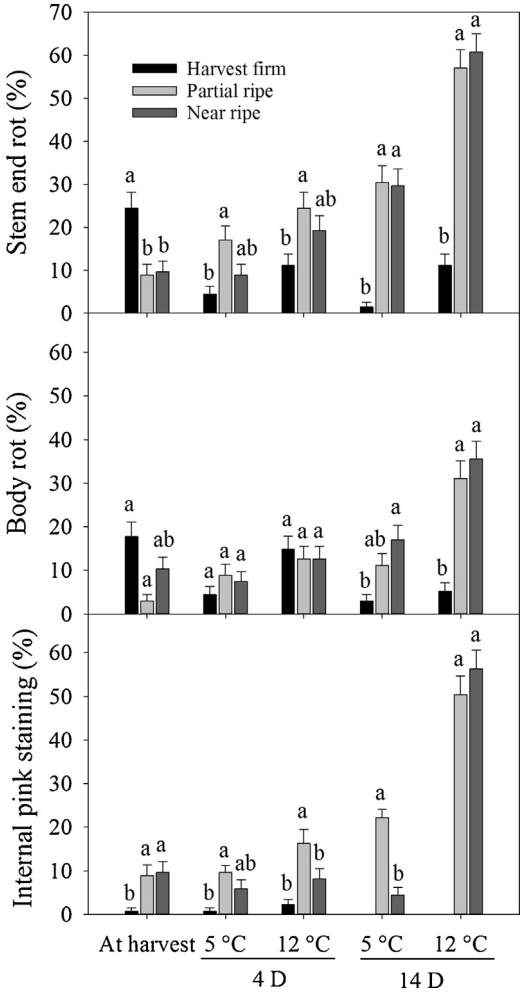

Fig. 2. Effect on the occurrence of fruit quality defects of storing 'Hass' avocados either unripened (harvest firm) or following ripening to either partial (partial ripe, 80 N) or near eating ripeness (near ripe, 19 N) prior to subsequent storage for either 4 d or 14 d at either 5 °C or 12 °C (Test 1A). Fruit were ripened to optimal eating firmness (less than 6.7 N) following each of the treatments. Different letters within a storage time or storage time and temperature combination indicate statistical significance ($P \leq 0.05$).

of peeling, body rot, tissue breakdown and vascular leaching as previously defined (White et al., 2009). Fifteen (Tests 1A and 1B) or thirty fruit (Test 2) were evaluated for each treatment. Of those attributes, many had little or no incidence and only the ratings of those that were of consequence will be further described. Peel color referred to external color where: 1 = emerald green; 2 = forest green; 3 = 20% colored black/purple on green; 4 = 60% colored black/purple on green; 5 = purple over 100% of peel surface; 6 = black over 100% of surface area. Appearance referred to external appearance where damage in the form of external discoloration and lenticel or nodule damage was assessed. Ratings were given where: 0 = no damage; 1 = slight; 2 = approximately 25% of the fruit affected; 3 = 50% or more of the fruit affected. Pink staining was evaluated with each rating referring to the following percentages of the interior of the fruit affected: 0 = 0%; 1 = 10%; 2 = 25%; 3 = 50%. Signs of seed germination were noted in terms of incidence only, while uneven ripening and body and stem end rot were evaluated both for incidence and severity. Severity was rated using a similar 0–3 rating scale.

2.6. Sensory evaluation

Sensory panelists were employees of the Kearney Agricultural Center in Parlier, CA, with most having performed sensory evaluation of avocados in the past. Panelists were provided a training session instructing them in the use of hedonic and line scales, the meaning of the sensory characteristics being evaluated, and on how to properly cleanse the palate by the use of small pieces of carrot and a sip of water. Generally 12–14 panelists attended each tasting. Sensory evaluations were held in a dedicated sensory evaluation laboratory at the Kearney Agricultural Center. Panelists were seated in individual three-sided evaluation booths that were designed to standardize the testing environment. Samples were received through a small door in the booth. On the day of sensory testing fruit were removed from the storage rooms and allowed to adjust to ambient temperature conditions in the laboratory. Four fruit from each treatment were selected for sensory evaluation based upon size and firmness as determined by penetrometer measurement of firmness, the main goal being to provide fully ripe fruit to the panelists. Preparation consisted of cutting the fruit in half, top to bottom, and removing the seed. Only the mid-section of the fruit was used for sensory evaluation, with the portions above and below the seed cavity and within 13 mm of the penetrometer puncture being discarded. The remainder of the fruit was cut into pieces approximately 13 mm square and placed into 30-mL paper soufflé cups that were labeled with 3-digit random numbers for presentation to the panelists. Six samples were presented in random order to each panelist in a tasting session. Panelists rated each sample for overall liking using a 9-point hedonic scale where 1 = dislike extremely and 9 = like extremely. Then the sample was rated for the degree of creaminess, richness, nuttiness and grassiness by placing marks on 150-mm line scales corresponding to the intensity of the sensory attribute present. Twelve fruit were tasted for each treatment at each harvest date, with each fruit being tasted by 10 panelists.

2.7. Statistics

Analysis of the data from the storage of partially ripened fruit in Test 1A was accomplished by one-way ANOVA within storage treatment and duration across the three harvest dates and three orchards using SPSS (SPSS, Chicago, IL, USA). An alternative one-way ANOVA (SPSS) was performed within ripening treatment and across storage temperatures and times to assess the relative effects of these treatments. One-way ANOVA (SPSS) was also used to examine the effect of storage duration prior to ripening in that

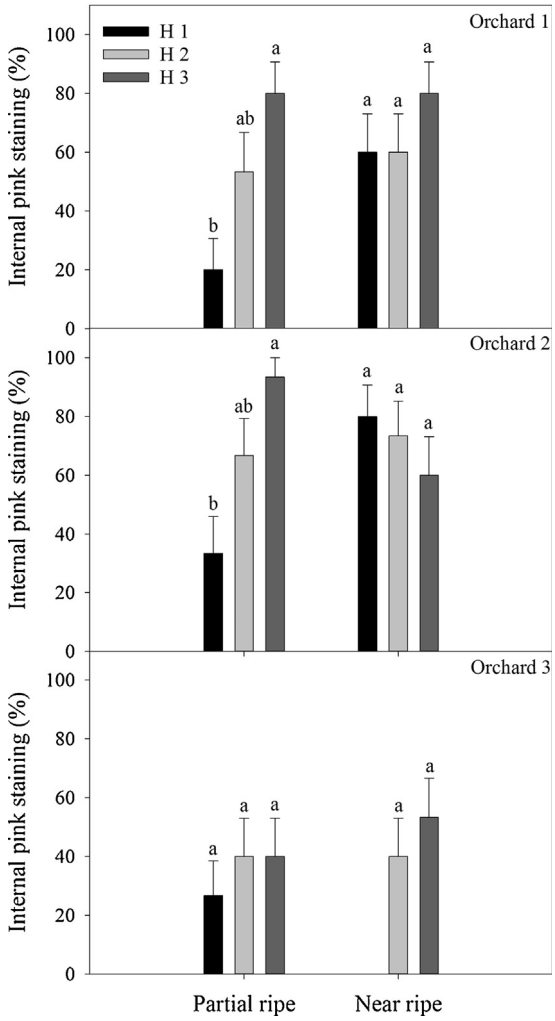

Fig. 3. Influence of orchard and harvest date (H) on the incidence of pink staining in 'Hass' avocados stored following ripening to either partial (partial ripe, 80 N) or near eating (near ripe, 19N) ripeness prior to subsequent storage for 14 d at 12 °C (Test 1A). Different letters within a ripeness stage indicate statistical significance ($P \leq 0.05$).

portion of Test 1B by performing the analysis within each of the ripening times across the three harvest dates and three orchards. An additional analysis was conducted for the attribute pink staining to examine harvest date and orchard effects using a general linear model in SPSS. A separate one-way ANOVA examined the effect of ripening time within each of the storage duration treatments prior to ripening. Tukey's test was used to perform all mean separations at $P \leq 0.05$. Means were based upon 135 fruit for the partial ripening portion of the testing and upon 120 fruit for the post-storage ripening. For the data from Test 2 an analysis using a general linear model with harvest date, ripening treatment and storage time as fixed effects was conducted using SPSS for both quality and sensory attributes. The mean for each quality factor within a treatment and harvest date was based upon 35 fruit for each harvest. For the sensory evaluation, means were based upon 12 fruit per treatment for each harvest date, with each fruit being tasted by 10 panelists. Since an analysis indicated that storage time had a statistically-significant effect on both quality and sensory attributes, separate analyses were conducted for each of the two storage times using harvest date and ripening treatment as the fixed effects. Mean comparisons among harvest dates or ripening treatments within a storage time were performed using Tukey's test. The significant interaction of harvest date and

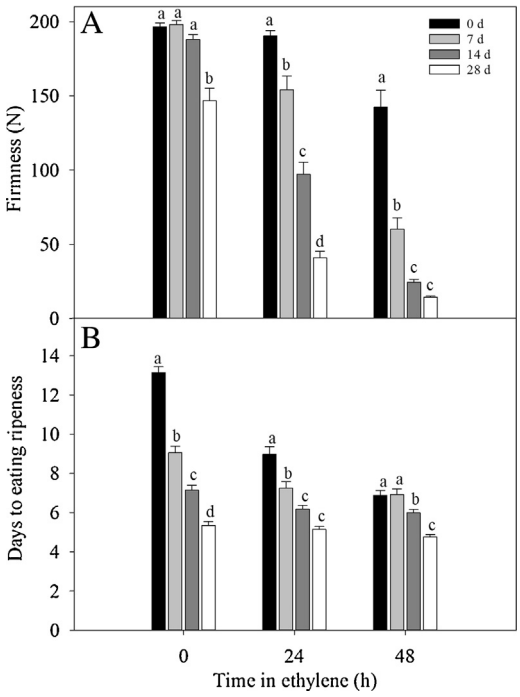

Fig. 4. Firmness (A) and days to eating ripeness (B) of 'Hass' avocados following storage at 5 °C for 0 d, 7 d, 14 d, or 21 d and ripening with ethylene for 0 h, 24 h, or 48 h (Test 1B). Different letters within an ethylene exposure time indicate that the differences are statistically significant ($P \leq 0.05$).

ripening treatment for the sensory attribute 'creamy' was further examined by performing a one-way analysis using SPSS for the effect of ripening treatment for each of the harvest dates for each storage at each of the two storage times. The effect of harvest date on average fruit weight and dry weight were analyzed with a one-way analysis of variance, using Tukey's test to perform the mean separations, the means being the average of 10 individual fruit.

3. Results and discussion

3.1. Influence of partial ripening prior to storage on time to full ripeness and quality (Test 1A)

In Test 1A, as would be expected, partially ripening avocados with ethylene prior to cold storage initiated the softening process and lessened the amount of time needed to become fully ripe either following harvest or upon removal from cold storage (Fig. 1A and B). With increasing storage time both fruit that had received ripening (partial ripe and near ripe) became significantly softer ($P \leq 0.05$), as did fruit that had not received any ripening prior to storage (harvest firm), although these unripe fruit showed very little loss in firmness during storage with the exception of 14 d at 12 °C (Fig. 1A). The softening of unripe fruit in cold storage is in agreement with prior research (Lizana et al., 1992), although Zauberman et al. (1977) found softening to only occur during storage at temperatures of 10 °C and above. Within either 4 d or 14 d of storage fruit held at 12 °C softened at a significantly more rapid rate than those at 5 °C ($P \leq 0.05$) as reflected both in firmness following the storage treatments and days to ripeness. Differences between ripening treatments in terms of the number of days needed to achieve ripeness became less pronounced with increasing storage. After 14 d at 12 °C, less than a day of additional ripening after removal from cold storage was needed to achieve full ripeness for either of the treatments that had been received some degree of ripening prior to storage. Given the continued softening that occurs in avocados during storage, care would need to be taken by packers and distributors with partially-ripened fruit that they do not become over-ripe prior to reaching the consumer. Avocados become too soft in texture as the fruit becomes overripe, potentially rendering it less acceptable to consumers.

The incidence of stem end rot, body rot, and pink staining of the vascular tissue were all significantly affected by the ripening treatments (Fig. 2) whereas other fruit quality parameters were not influenced (data not shown). For fruit not stored in the cold both partial ripe and near ripe fruit had a lesser incidence of stem end rots than did fruit that did not undergo a partial ripening treatment. This was most likely due to the longer amount of time needed to ripen this fruit (8–9 d longer) that allowed a greater amount of time for the development of decay. The fruit that were not partially ripened prior to storage showed a declining amount of decay with storage. This observation is in line with the observations of Hopkirk et al. (1994) and Smilanick et al. (2002). In avocados receiving cold storage, however, stem end rots occurred more frequently in the partially ripened fruit, particularly in fruit stored for 14 d. In this fruit decay rates exceeded 30% and 50% for fruit stored at 5 °C and 12 °C, respectively. Similarly, body rot was also increased at both storage temperatures in the partially ripened fruit stored for 14 d, the incidence of decay being greater than 30% in the ripened fruit

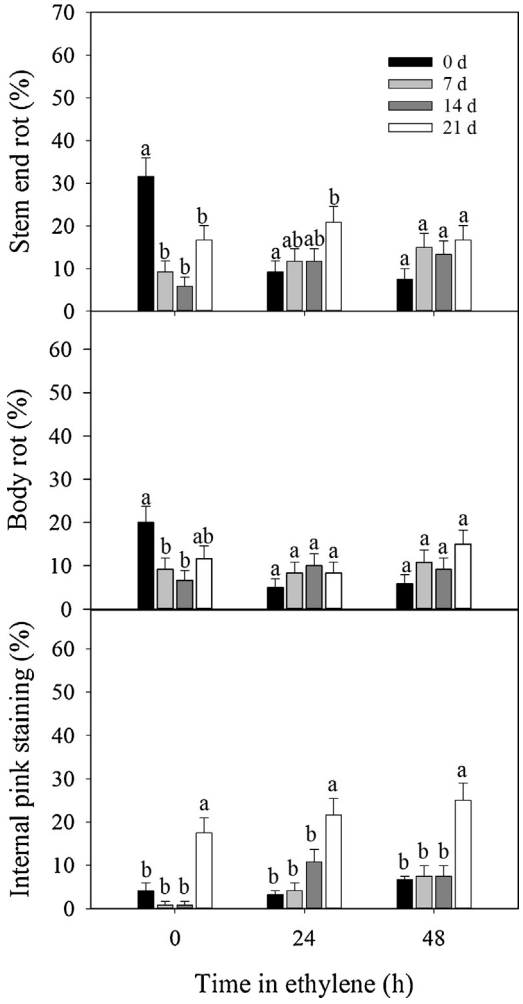

Fig. 5. Influence of storage at 5 °C for 0 d, 7 d, 14 d, or 21 d prior to exposure to ethylene for 0 h, 24 h or 48 h and final ripening to optimal eating firmness (less than 6.7 N) on the occurrence of fruit quality defects in 'Hass' avocados (Test 1B). Different letters within an ethylene exposure time indicate statistical significance ($P \leq 0.05$).

stored at 12 °C (Fig. 2). Although both stem end and body rots occurred frequently in the partially ripened fruit that had been stored, most were graded as slight as the incidence of moderate to severe decays was 10% or less in all of the treatments (data not shown). The percentage of fruit with pink staining of the vascular tissue was significantly enhanced by partial ripening of the fruit that were ripened at harvest and did not undergo cold storage in comparison to fruit that did not undergo partial ripening treatments (Fig. 2). Storage acted to exacerbate the negative effect of partial ripening on this disorder, particularly in the avocados stored for 14 d at 12 °C where it was observed in more than half of the fruit. In contrast, fruit that had not undergone any ripening prior to cold storage for this duration had no incidence of the disorder. In order to better understand the occurrence of pink staining, data from the treatment with the highest incidence (14 d at 12 °C) was graphed to show the impact of harvest date and orchard (Fig. 3). There was clearly a large orchard effect on the incidence of pink staining with orchard 3 having a much lower incidence than orchard 1 or orchard 2 ($P \leq 0.05$) at both stages of ripeness prior to storage. Also, the disorder was significantly more common as the season progressed in the fruit from orchards 1 and 2. Pink staining of the vascular tissue is described in the International Avocado Quality Manual (White et al., 2009) but is not commonly observed in California (Arpaia, personal communication). This disorder, although a significant issue in our evaluations in this test, was not noted by either Kosiyachina and Young (1976) or Marques et al. (2011) in their studies of the cold storage of partially-ripened fruit. Field temperature records were examined for the 2006 season in comparison to other harvest seasons and nothing unusual was observed (data not shown), nor were there any differences between the three orchards that were believed to be conducive to this disorder. The prevalence of decay in the avocados stored for 14 d at 12 °C was also examined as a potential link to the occurrence of pink staining. The overall incidence of body rot across ripening stage and harvest was 31%, 40%, and 29% for orchards 1, 2, and 3, respectively (data not shown). Stem end rot incidence averaged across ripening stage and harvest was 60%, 64%, and 52% for orchards 1, 2, and 3, respectively (data not shown). Correlations between either form of decay and pink staining were not statistically-significant (data not shown).

3.2. Influence of prior cold storage of unripe fruit on time to full ripeness and quality (Test 1B)

The second objective of Test 1 was to examine the impact of cold storage on the time required to ripen avocados both with and without ethylene and also on the resulting fruit quality of the ripe fruit. Avocados softened very slowly in cold storage in the absence of any ethylene treatment and there was a significant decline in firmness only after 28 d of storage (Fig. 4A, time 0). Nonetheless, the prior storage time resulted in a lessening of the time needed to reach eating firmness (Fig. 4B, time 0). Fruit in cold storage for 28 d required nearly 8 d less to reach full ripeness than did fruit ripened without ethylene immediately after harvest. Application of ethylene (24 h and 48 h exposures) acted to greatly accelerate the loss in firmness and progressively minimize the storage effect (Fig. 4A). Following 48 h of ethylene exposure fruit that had not undergone prior cold storage required only 2 d longer to reach full

Table 2
Effect of partial ripening to an average firmness of 15.6 N and subsequent cold storage at either 1 °C or 5 °C for 1 or 2 weeks on quality attributes of 'Hass' avocados over five separate harvests (Test 2). All fruit were ripened to eating firmness prior to evaluation.

	Color Rating[x]	Appearance	Seed germ. (%)[y]	Uneven ripen.	Body rot
1 week cold storage after ripening					
Harvest (H)					
March 15	1.5c	0.5a	0b	15a	4b
April 27	3.2b	0.7a	0b	0b	28a
June 8	4.2a	0.8a	5b	0b	10b
July 13	3.7ab	0.1a	0b	0b	0b
August 17	4.3a	0.1a	39a	0b	0b
Ripening treatment (R)					
After 5 °C storage	3.7ab	0.9a	10a	0b	14a
Partial ripening prior to 1 °C storage[z]	3.4b	0.2b	5a	5a	5b
Partial ripening prior to 5 °C storage[z]	3.9a	0.2b	7a	5a	8ab
Effect					
H	*	NS	*	*	*
R	NS	*	NS	NS	*
H × R	NS	*	*	*	*
2 weeks cold storage after ripening					
Harvest (H)					
March 15	3.5c	0.9a	0b	10a	23a
April 27	3.8b	0.2c	0b	0b	20a
June 8	3.9b	0.3c	1b	0b	1b
July 13	4.0b	0.5b	0b	0b	0b
August 17	4.4a	0.1c	18a	0b	0b
Ripening treatment (R)					
After 5 °C storage	4.3c	0.1b	5a	4a	3b
Partial ripening prior to 1 °C storage[z]	3.3a	0.9a	5a	4a	9ab
Partial ripening prior to 5 °C storage[z]	4.0b	0.2b	4a	3a	13a
Effect					
H	*	*	*	*	*
R	*	*	NS	NS	*
H × R	*	*	NS	NS	*

[x] Subjective ratings of exterior fruit color and appearance, 35 fruit evaluated per replication. Color: 1 = emerald green; 2 = forest green; 3 = 20% colored black/purple on green; 4 = 60% colored black/purple on green; 5 = purple over 100% of peel surface; 6 = black over 100% of surface area; appearance: 0 = no damage; 1 = slight; 2 = approximately 25% of the fruit affected; 3 = 50% or more of the fruit affected.
[y] Percent incidence of seed germination, uneven ripening and body rot, 35 fruit evaluated per replication.
[z] Ripened at 20 °C prior to cold storage at either 1 °C or 5 °C.

eating ripeness than did non-stored fruit (Fig. 4B). This underscores the ability of ethylene to help synchronize required ripening times, regardless of the prior storage history, making it easier to provide more uniform quality to avocado consumers. Fruit that have been previously stored, however, react more quickly to ethylene exposure in terms of a loss of firmness as evidenced by the lack of change in firmness in the non-stored (0 d) fruit after 24 h of ethylene exposure.

There were statistically-significant effects of cold storage duration on the incidence of stem end rot, body rot and pink staining of the vascular tissue (Fig. 5) but no influence of time in ethylene on any of the other quality measures that were evaluated (data not shown). Stem end rot was less in fruit that had received cold storage prior to ripening without ethylene as compared to fruit that were had no cold storage. This effect on stem end rot was most likely due to the very long time (>13 d) at warm temperature for ripening to occur in fruit that did not receive ethylene or cold storage. This was also observed for body rot, although to a lesser degree. With the exception of fruit stored for 21 d and ripened for 24 h in ethylene, there was no enhanced loss of quality in any of the other ethylene-treated fruit due to prior cold storage. The occurrence of pink vascular staining was significantly increased by cold storage for 21 d prior to ripening either with or without ethylene.

3.3. Influence of partial ripening prior to storage on sensory and overall quality (Test 2)

A shortcoming of prior efforts by researchers to better understand the effects on of cold storage of partially-ripened avocados on subsequent fruit quality was a lack of information on the sensory impact of such treatments. A second test (Test 2) was conducted to provide additional information regarding this question as well as to provide data over a greater number of harvests than was given by Test 1. Details regarding the characteristics of the fruit at harvest for Test 2 are given in Table 1. Average fruit size for the initial harvest was significantly less than that of the final two harvests, while dry weights increased until the third harvest on July 8 when the dry weights plateaued in value. The increase in dry weight with maturation was typical of what has been previously reported for 'Hass' avocado in California (Obenland et al., 2012). Even though avocados from the initial harvest were below the minimum dry weight standard of 20.6% in the California Agricultural Code, they were legal to pick based upon the mandated release date.

There was little or no incidence of shrivel, flesh to seed adhesion, seed cavity browning, flesh bruising, flesh discoloration, vascular browning, vascular streaking, pink stain, difficulty in peeling, stem end rot, tissue breakdown or vascular leaching in any of the treatments (data not shown). Those avocado quality factors that did show statistically-significant change due to either harvest maturity or ripening treatment are shown in Table 2. In comparison to fruit that had not been partially ripened prior to storage, color development was unchanged following storage for 1 week but slightly slowed by partial ripening followed by storage for 2 weeks. External appearance never reached a mean damage rating of slight (value of 1) throughout all of the treatments and so was not an important factor, even though there were significant differences due to harvest and ripening treatment. Seed germination was highest in fruit from the final harvest and had little incidence in the earlier harvests, regardless of treatment. The increased occurrence of seed germination in late maturity fruit has

Table 3
Effect of partial ripening to an average firmness of 15.6 N and subsequent cold storage at either 1 °C or 5 °C for 1 or 2 weeks on likeability (hedonic score) and sensory attributes of 'Hass' avocados as evaluated by a sensory panel over five separate harvests (Test 2).

	Hedonic[x] (1–9 score)	Creamy[y] (mm from origin of 150 mm line scale)	Rich[y]	Nutty[y]	Grassy[y]
1 week cold storage after ripening					
Harvest (H)					
March	5.9c	68.1d	72.3c	61.5c	68.9a
May	6.5b	72.8cd	79.6bc	67.9bc	59.3b
June	7.1a	78.6c	99.3a	78.8ab	47.5c
July	7.1a	92.4b	101.7a	88.4a	46.0c
August	6.4b	102.0a	88.5b	73.7b	42.0c
Ripening treatment (R)					
After cold storage	6.6a	79.4b	88.9a	73.7a	54.7a
Partial ripening prior to 1 °C storage[z]	6.6a	79.5b	88.9a	74.9a	53.9a
Partial ripening prior to 5 °C storage[z]	6.6a	85.9a	87.9a	74.7a	51.4a
Effect					
H	*	*	*	*	*
R	NS	*	NS	NS	NS
H × R	NS	*	NS	NS	NS
2 weeks cold storage after ripening					
Harvest (H)					
March	6.1b	68.2c	80.5b	66.4b	60.4a
May	6.2b	71.7c	74.0b	58.7b	55.7ab
June	6.8a	78.7b	99.4a	77.3a	48.1bc
July	7.0a	89.2a	97.7a	82.1a	44.1c
August	6.8a	83.4ab	94.2a	78.2a	44.3c
Ripening treatment (R)					
After cold storage	6.6a	78.1a	89.4a	73.6a	50.0a
Partial ripening prior to 1 °C storage[z]	6.6a	76.2a	89.8a	72.1a	51.1a
Partial ripening prior to 5 °C storage[z]	6.5a	80.1a	88.5a	71.9a	51.0a
Effect					
H	*	*	*	*	*
R	NS	NS	NS	NS	NS
H × R	NS	*	NS	NS	NS

[x] Hedonic score where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely.
[y] Ratings that used a 150-mm scale where a higher number indicated a greater intensity of each of the attributes.
[z] Ripened at 20 °C prior to cold storage at either 1 °C or 5 °C.

been previously noted (White et al., 2009) and may be modulated by the concentration of ethylene within the fruit (Hershkovitz et al., 2009). The incidence of seed germination was unchanged as a result of ripening treatment. In contrast, uneven ripening was only found in fruit from the first harvest. The occurrence of this disorder was not influenced by ripening treatment. A small amount (less than a 1 rating, indicating a few small areas) of body rot was present in some of the fruit, the prevalence being greatest in the early harvests. There was no difference in the average amount of body rot present after one week among the treatments, although there was a slightly greater amount found in the partially-ripened fruit stored at 5 °C in comparison to the fruit that had not been ripened prior to storage. Taken as a whole, although there were statistically-significant differences among the treatments for a given storage time, there were no changes in any of the quality attributes as a result of ripening treatment that would have substantially altered the marketability of the avocados. These results contrast with those from Test 1 in that storage disorders were increased to a much greater degree in that test due to partial ripening, even at 5 °C, one of the two storage temperatures used in Test 2. This highlights the impact that fruit source can potentially have on the resulting fruit quality following such treatments.

Our results from both tests differ those of Marques et al. (2011), who also examined the effect of cold storage of fruit that had been previously ripened, in that there was not any effect of ripening treatment on the development of internal flesh browning observed. In fact there was no incidence of internal or external discoloration characteristic of chilling injury in any of our treatments, even in the early-season harvest that are thought to be the most sensitive to chilling injury (Toerien, 1986). These authors found diffuse discoloration to be the major quality defect associated with partial ripening of fruit prior to storage, although it was thought not to be a problem as long as storage temperatures were kept at 5 °C and above and storage limited to 10 d. Darkening of the avocado mesocarp is commonly described as one of the major symptoms of chilling injury (Chaplin et al., 1982; Cutting et al., 1990).

The hedonic score, a measure of likeability, increased with advancing harvest date (Table 3) as has been previously noted (Harding, 1954; Lee et al., 1983). Sensory panelists associated the increase in hedonic score with greater richness, nuttiness and creaminess and a decline in grassiness in agreement with prior research (Obenland et al., 2012). There was no effect of pre-ripening the fruit prior to storage on the hedonic score, nor on richness, nuttiness or grassiness. There was, however, a statistically-significant impact of pre-ripening on the perception of creaminess by the sensory panelists. In order to more fully present the effect of pre-ripening on this sensory attribute a separate table is presented, showing the interaction of treatment with harvest date (Table 4). There was no consistent pattern to the differences, with fruit that had been pre-ripened having significantly less creaminess in select treatments in harvests from May to July, but having a higher creaminess rating in fruit from the August harvest following one but not two weeks of storage following pre-ripening.

Sensory quality had not been previously evaluated as part of any previous study to determine the effect of pre-ripening on avocado quality, so it was important to determine if there was any negative effect on flavor of this practice. Although there may have been some effect of pre-ripening on avocado texture as described by the sensory attribute “creamy”, this appears to have had little impact on the sensory quality of the fruit given the lack of difference between hedonic scores of the various treatments (Table 3). The lack of impact on flavor is also suggested in an analysis on fruit from one of the harvest dates indicating no differences in aroma volatiles (data not shown), some of which have been shown to influence flavor in avocados (Obenland et al., 2012), among the ripening treatments.

Table 4
Effect of partial ripening and subsequent storage at either 1 °C or 5 °C for 1 or 2 weeks on the creamy rating of ‘Hass’ avocados as judged by a sensory panel over five separate harvests (Test 2).

Ripening treatment	Creamy rating[x]				
	Harvest month				
	March	May	June	July	August
	1 week storage[y]				
After cold storage	65.3a	69.2b	79.2a	95.6ab	91.6b
Partial ripening prior to 1 °C storage[z]	71.6a	76.9a	80.2a	96.8a	110.2a
Partial ripening prior to 5 °C storage[z]	67.2a	72.5ab	76.4a	84.8b	104.1a
	2 weeks storage[y]				
After cold storage	64.3a	70.6a	82.6a	89.3a	83.7a
Partial ripening prior to 1 °C storage[z]	68.3a	72.4a	78.7ab	93.8a	87.4a
Partial ripening prior to 5 °C storage[z]	71.9a	72.0a	75.0b	83.3a	79.0a

[x] Textural rating ranging from watery (0) to dry (150) using a 150 mm line scale. Numbers represent means of 12 individually tasted fruit, each fruit being tasted by 8–10 panelists. Values within a storage time and harvest month followed by a different letter are statistically-significant from each other in the column ($P \leq 0.05$).
[y] Amount of storage after partial ripening. Total storage times were equivalent for pre-ripened fruit and fruit that had not been pre-ripened prior to storage.
[z] Ripened at 20 °C prior to cold storage at either 1 °C or 5 °C.

4. Conclusions

This research has extensively examined in two separate tests, over multiple harvests and including sensory evaluations, the common commercial practice holding of partially-ripened fruit in cold storage and found that that it can be safely implemented if the storage time is limited to 4 d, but that there is a risk of the development of storage disorders if longer storage times are used, particularly at 12 °C. The degree of risk was found to be dependent on the initial nature of the fruit used as in Test 2 of this study the fruit were in general more resistant to the development of these disorders in comparison to Test 1. This was also indicated by the strong influence of harvest date and orchard on pink staining incidence in partially ripened fruit. Cold storage prior to the application of ethylene reduced the number of days after ethylene exposure for fruit to reach ripeness and did not negatively affect fruit quality if the length of storage time was limited to 14 d or less.

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