Cold Storage Duration Influences Ethylene Biosynthesis and Ripening of ‘Bartlett’ Pears

I. Tayfun Agar

Department of Horticulture, Faculty of Agriculture, University of Çukurova, 01330 Adana, Turkey

William V. Biasi and Elizabeth J. Mitcham¹

Department of Pomology, University of California, Davis, CA 95616

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Abstract. Ripening behavior of ‘Bartlett’ pears (*Pyrus communis* L.), with or without ethylene (C_2H_4) treatment, was assessed at harvest, and after 2, 4, 6 and 12 weeks of cold storage at $-1\text{ }^{\circ}\text{C}$. Fruit exhibited increasing rates of C_2H_4 production and consequently faster ripening rates with increased length of cold storage. Ripening characteristics were influenced by storage duration, but to different degrees. The data indicate that the threshold C_2H_4 concentration for softening may be lower than that for color change from green to yellow. Ethylene treatment for 24 h at harvest resulted in a rate of ripening equivalent to that following cold storage for 2 to 4 weeks, depending on the orchard location. Storage for 12 weeks significantly increased C_2H_4 production upon transfer to ambient temperature, indicating that fruit were reaching the end of their storage life. ‘Bartlett’ pears may ripen to a firmness of 14 N (ready to eat) at $20\text{ }^{\circ}\text{C}$ within 2.5 to 7 days depending upon the duration of prior cold storage.

Ripening of pears is induced by a threshold concentration of internal ethylene (C_2H_4) (Chen and Mellenthin, 1981). Less mature, freshly harvested ‘Bartlett’ pears lack the capacity to start autocatalytic C_2H_4 production and to produce significant levels of C_2H_4 (Puig et al., 1996). When ripened at $20\text{ }^{\circ}\text{C}$ immediately after harvest, ‘Bartlett’ pears ripen nonuniformly and typically fail to achieve good color, texture, and flavor (Agar et al., 1999a; Allen, 1930); conditioning at harvest with 10 Pa ($100\text{ }\mu\text{L}\cdot\text{L}^{-1}$) C_2H_4 at $20\text{ }^{\circ}\text{C}$ for 24 to 48 h starts endogenous C_2H_4 production and uniform ripening upon transfer of the fruit to air at $20\text{ }^{\circ}\text{C}$ (Agar et al., 1999 a, 1999 b).

Exposure of pears to low temperatures before (Wang et al., 1971) and after harvest (Looney, 1972; Sfakiotakis and Dille, 1974) stimulates C_2H_4 synthesis. While winter pear cultivars require 6 to 12 weeks of cold storage

to induce C_2H_4 synthesis and ripen (Chen and Mellenthin, 1981; Elgar et al., 1997; Lelièvre et al., 1997a), summer cultivars, such as ‘Bartlett’, require less chilling (Looney, 1972; Mitchell, 1990). Mitchell (1990) demonstrated that for California ‘Bartlett’ pears, the greatest effect of cold storage was with early season, less mature fruit, and that these pears required 2 weeks of chilling at $-1\text{ }^{\circ}\text{C}$, although some added benefit occurred after 5 weeks. However, Puig et al. (1996) concluded that 4 weeks of chilling at $-1\text{ }^{\circ}\text{C}$ induced adequate ripening of Oregon ‘Bartlett’ pears while 2 weeks of chilling did not.

The concentration of 1-aminocyclopropane-1-carboxylic acid (ACC) in many climacteric fruits, such as pear, is relatively low during the preclimacteric stage, but increases greatly during the climacteric, and then decreases in the postclimacteric stage (Yang and Hoffman, 1984). Ripening resistance in winter pears held at ambient temperatures immediately after harvest is generally related to the delayed synthesis of ACC and C_2H_4 in the tissue (van Eeden et al., 1991); exposure to chilling temperatures stimulates synthesis of both compounds (Blankenship and Richardson, 1985; Wang et al., 1985). For example, Lelièvre et al. (1997 a) demonstrated that exposure of ‘Passe-Crassane’ pears to low temperatures induced the expression of ethylene biosynthetic genes, including those responsible for ACC synthase (ACS) and ACC oxidase (ACO), and an accumulation of ACO protein. All of these are required for the starting of autocatalytic C_2H_4 production and fruit ripening upon warming. Exogenous C_2H_4 was unable to induce ACS gene expression

and C_2H_4 biosynthesis in nonchilled ‘Passe-Crassane’ pears. Storage at chilling temperatures stimulates both ACS and ACO activity, resulting in endogenous C_2H_4 production and uniform ripening when pears are placed at $20\text{ }^{\circ}\text{C}$ (Blankenship and Richardson, 1985; Chen et al., 1997; Wang et al., 1985; Yang and Hoffman, 1984).

The effect of cold storage duration on C_2H_4 biosynthetic enzymes, C_2H_4 biosynthesis, and ripening has been characterized for winter pear cultivars (Blankenship and Richardson, 1985; Chen et al., 1997; Elgar et al., 1997; Lelièvre et al., 1997a), but not for ‘Bartlett’. The objective of this study was to investigate the ripening response, as well as associated changes in the activities of ACS and ACO, of ‘Bartlett’ pears from the three major pear-growing regions in California to cold storage durations ranging from 2 to 12 weeks.

Materials and Methods

Plant material. ‘Bartlett’ pears were harvested 1 week after the start of commercial harvest in 1997 and 1998 from 15 trees (five trees/rep) in the same commercial orchards in Sacramento (20 July 1997 and 27 July 1998), Mendocino (27 July 1997 and 21 Aug. 1998), and Lake (10 Aug. 1997 and 28 Aug. 1998) Counties, Calif. Fruit were transported in an air-conditioned vehicle on the day of harvest to the postharvest laboratory of the Univ. of California, Davis, and sorted to eliminate damaged fruit and to obtain fruit of uniform size and color within each pear-growing region. Unless otherwise stated, materials and methods, treatments, and results for the 1997 and 1998 seasons were similar, and thus this paper will focus on the 1998 experiment.

Treatments. Eighteen pears per growing location were selected for quality evaluation immediately after harvest. The remaining fruit from each growing location were randomly assigned to six treatments, three replicates, and four evaluation times and marked as such. In addition, 18 pears (six fruits/rep) for each treatment were allocated for C_2H_4 and CO_2 production measurements. Marked pears were placed in a telescoping pear box (16-kg) (one box/rep) and the box was filled with extra pears to provide an environment similar to a commercial box for the cold storage trials and subsequent ripening. Four groups of pears were placed immediately at $-1\text{ }^{\circ}\text{C}$ after harvest where they remained for 2, 4, 6, or 12 weeks. Two remaining groups were ripened immediately after harvest (0 weeks); one was treated with exogenous C_2H_4 , the other was not. For these two treatments, the boxes were placed in a tank and ventilated with a humidified flow of air or air + 10 Pa C_2H_4 ($100\text{ }\mu\text{L}\cdot\text{L}^{-1}$) at $3000\text{ mL}\cdot\text{min}^{-1}$ for 24 h at $20\text{ }^{\circ}\text{C}$. Thereafter, the C_2H_4 -treated boxes were kept in a separate room from the nontreated boxes, both at $20\text{ }^{\circ}\text{C}$, to avoid cross-contamination with C_2H_4 . For all six treatments, ripening behavior was assessed during the subsequent 7 d at $20\text{ }^{\circ}\text{C}$. The 7-d evaluation of pears that were stored for 12 weeks was not possible because of excessive softening.

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¹To whom reprint requests should be addressed. E-mail address: ejmitcham@ucdavis.edu

Flesh firmness. Flesh firmness was determined with an Ametek firmness tester (Ametek, Matfield, Pa.) mounted in a drill-press stand and fitted with an 8-mm probe. Skin was removed on two sides of the equatorial region of each pear and firmness was measured on each side.

Color. External skin color on opposite sides of each fruit was measured with a Minolta Chroma Meter (model CR-300; Minolta, Ramsey, N.J.) in CIEL*a*b* mode under CIE Standard Illuminant C. Changes in hue angle (h°), calculated as $h^\circ = \arctan b^*/a^*$ (deg) (McGuire, 1992), were used to indicate the color change from green to yellow during ripening.

Gas analysis. Carbon dioxide and C_2H_4 production rates were measured daily at 20 °C and standard pressure for each replicate and treatment. Six pears (≈ 1 kg of fruit) were sealed in a 3.7-L jar for 5 to 30 min, depending on the stage of ripeness, and the headspace was sampled with a 10-mL syringe. An infrared CO_2 analyzer (model PIR-2000R; Horiba Instruments, Irvine, Calif.) was used for CO_2 measurements. A gas chromatograph (model 211; Carle Instruments, Anaheim, Calif.) with FID detector and alumina column was used to analyze for C_2H_4 .

ACC-synthase and ACC-oxidase activity. ACS activity was assayed as described by Gorny and Kader (1997) with three replications per treatment. Activity of ACO was also assayed as described by Gorny and Kader (1997) with three replications per treatment. Peel tissue including ≈ 1 mm cortical tissue was assayed immediately upon removal from air, C_2H_4 , or storage treatments.

Statistical analysis. Data were analyzed by analysis of variance using the general linear model (GLM) procedure (SAS Inst., Cary, N.C.), with mean separation by least significant difference (LSD) with a significance level of $P \leq 0.05$.

Results

'Bartlett' pears grown in Sacramento County. Increasing the duration of cold storage up to 12 weeks increased the rate of softening (Fig. 1A), color change (Fig. 1D), respiration (data not shown), and C_2H_4 production (Fig. 1G), and the activities of ACS (Fig. 2A) and ACO (Fig. 2D). Ripening-associated changes were negligible in pears held in air at 20 °C for 7 d immediately after harvest (0 weeks). These pears retained their initial firmness (Fig. 1A) and color (Fig. 1D) and exhibited extremely low ACS and ACO activity (Fig. 2A, 2D), resulting in <1 $pmol \cdot kg^{-1} \cdot s^{-1}$ C_2H_4 production (Fig. 1G) during the 7 d.

A 24-h C_2H_4 treatment at harvest (0 weeks + C_2H_4) resulted in similar fruit softening rates (Fig. 1A) and color change (Fig. 1D) as in fruit stored for 4 weeks at -1 °C. However, C_2H_4 production (Fig. 1G) and ACS activity (Fig. 2A) were similar to those of fruit stored for 2 weeks at -1 °C, and ACO activity (Fig. 2D) was intermediate between that of fruit stored for 2 and 4 weeks. During ripening at 20 °C, ACS and ACO activity were 2.5 and

1.5 times as high in pears previously stored for 4 weeks at -1 °C (Fig. 2A and D), and C_2H_4 production was 2.5 times as high as in that of fruit treated with C_2H_4 for 24 h at harvest (Fig. 1G). Flesh firmness of pears decreased 4 N during storage for 12 weeks at -1 °C. Immediately upon removal from 12 weeks of cold storage (0 d), the ACS (Fig. 2A) and ACO activities (Fig. 2D) were 14 times as high, and C_2H_4 production (Fig. 1G) was 290 times as high as fruit cold-stored for 6 weeks or less, although they ripened to a similar firmness after 5 d at 20 °C as did fruit stored at -1 °C for 6 weeks. Fruit held in cold storage for 12 weeks exhibited the highest softening rate among all the treatments, reaching 26 N and 13 N firmness after 3 and 5 d of ripening at 20 °C (Fig. 1A). All cold-stored or C_2H_4 -treated pears softened to 24 N or less after 7 d at 20 °C.

'Bartlett' pears grown in Mendocino County. The rate of ripening-associated changes increased incrementally with increasing length of cold storage. Fruit held at 20 °C immediately after harvest (0 weeks), without added C_2H_4 , did not soften appreciably (Fig. 1B) or change color (Fig. 1E), and exhibited extremely low ACS (Fig. 2B) and ACO (Fig. 2E) activities,

resulting in production of <50 $pmol \cdot kg^{-1} \cdot s^{-1}$ C_2H_4 (Fig. 1H) after 7 d of ripening.

A 24-h C_2H_4 treatment at harvest (0 weeks + C_2H_4) resulted in a softening rate between that of fruit stored for 2 and 4 weeks at -1 °C (Fig. 1B). The rate of change in color of fruit treated with C_2H_4 for 24 h at harvest was similar to that of fruit stored for 2 weeks at -1 °C (Fig. 1E); however, their C_2H_4 production (Fig. 1H), and ACS (Fig. 2B) and ACO (Fig. 2E) activities were slightly lower. After 7 d of ripening at 20 °C, fruit previously stored for 4 weeks at -1 °C were twice as soft and yellow, had twice as much ACS activity, similar ACO activity, and twice as much C_2H_4 production as did fruit stored for 2 weeks. Pears stored for 12 weeks exhibited 140 times as high ACS (Fig. 2B) and six times as high ACO (Fig. 2E) activities and 13 times as high C_2H_4 production (Fig. 1H) as did pears stored for 6 weeks, but the rate of softening was not affected. During 5 d of ripening at 20 °C, pears stored for 12 weeks exhibited a 2-fold decrease in ACS activity and a 4-fold increase in ACO activity; however, C_2H_4 production remained unchanged at above 1000 $pmol \cdot kg^{-1} \cdot s^{-1}$. All stored or C_2H_4 -treated pears softened to between 12 and 26 N after 7 d ripening at 20 °C.

Fig. 1. Effects of storage duration and C_2H_4 treatment on changes in firmness (N) (A, B, C), color (h°) (D, E, F), and C_2H_4 production ($pmol \cdot kg^{-1} \cdot s^{-1}$) (G, H, I) of 'Bartlett' pears harvested in Sacramento (A, D, G), Mendocino (B, E, H), or Lake (C, F, I) Counties during 7 d at 20 °C. Mature-green pears from each location were ripened at 20 °C, without (0 weeks) or with 10 Pa (100 $\mu L \cdot L^{-1}$) ethylene (0 weeks + C_2H_4) for 24 h at harvest, and after storage for 2, 4, 6, and 12 weeks at -1 °C. Data points represent means of three replicates $\pm$ SE. Hue angle is attributed to colors as yellow (90°) and green (180°) or an intermediate between any adjacent pair of colors.

'Bartlett' pears grown in Lake County. The rate of ripening-associated changes at 20 °C increased with duration of storage. Pears held at 20 °C after harvest (0 weeks) without exogenous C_2H_4 softened slightly (77 to 71 N after 5 d and 49 N after 7 d of ripening) (Fig. 1C). In addition, these pears exhibited a slight color change from 112 to 105 h° (Fig. 1F), and some increase in ACS and ACO activities (Fig. 4C and F), resulting in production of more than 200 pmol·kg⁻¹·s⁻¹ C_2H_4 (Fig. 1I) after 7 d at 20 °C.

Fruit treated with ethylene at harvest (0 weeks + C_2H_4) softened more slowly than did fruit stored for 2 weeks at -1 °C (Fig. 1C); however, firmness of pears from the two treatments was similar after 7 d at 20 °C. Skin color (Fig. 1F), C_2H_4 production (Fig. 1I), and ACS (Fig. 2C) and ACO (Fig. 2F) activities of fruit treated with C_2H_4 were also between those of nontreated fruit held continuously at 20 °C and those of pears stored for 2 weeks at -1 °C before ripening. Firmness of pears stored at -1 °C decreased 12 N in firmness during 12 weeks (Fig. 1I); upon removal from storage, they exhibited 25 times higher ACS activity (Fig. 2C), 3.5 times higher ACO activity (Fig. 2F), and 40 times higher C_2H_4 production (Fig. 1I) than did fruit stored for 6 weeks. Action of ACO and C_2H_4 production increased during 5 d of ripening at 20 °C (Fig. 1I and 2F), while ACS activity decreased (Fig. 2C). The rate of softening of fruit stored for 6 weeks was similar

to that of fruit stored for 12 weeks, although in the 12-week-stored pears ACS activity was twice as high, ACO activity was 1.5 times as high, and C_2H_4 production was 1.3 times as high after 5 d of ripening as in pears stored for 6 weeks. All cold-stored or C_2H_4 -treated pears softened to between 11 and 15 N after 7 d at 20 °C.

Discussion

Ripening of pears is closely associated with climacteric C_2H_4 production and respiration, both factors being influenced by cold storage (Sfakiotakis and Dilley, 1974). 'Bartlett' produced more C_2H_4 and consequently ripened faster at 20 °C as storage at -1 °C was prolonged. As time at -1 °C increased from 2 to 12 weeks, the time required to induce climacteric C_2H_4 production upon transfer to 20 °C progressively decreased from 5 to 0 d. Changes in firmness and skin color during ripening were rapid after an initial delay, especially in fruit stored for shorter periods, in agreement with the results of Elgar et al. (1997) for New Zealand-grown 'Bosc' and 'Comice' pears. There was about a 3-d lag at 20 °C before ripening changes were detected in fruit that were stored for 2 and 4 weeks and in those that were C_2H_4 -treated at harvest (0 weeks) in our study, indicating that pears from these treatments needed more time to respond for the start of ripening. The variation in softening rates among individual pears

also decreased with time at -1 °C, indicating a more uniform ripening behavior.

'Bartlett' pears require less chilling to stimulate ripening than do winter pears (Looney, 1972; Mitchell, 1990). However, ripening rate was much slower after 2 than after 4 weeks of storage at -1 °C, especially in pears from Sacramento and Mendocino Counties. The effect of storage times between 2 and 4 weeks is not known, but pears stored for 2 weeks ripened to just under 26 N within 7 d. Puig et al. (1996) also found that 2 weeks of chilling at -1 °C was not sufficient to completely ripen Oregon 'Bartlett' pears during 7 d at 20 °C (fruit softened from 80 to 53 N); however, 4 weeks of chilling fully induced ripening and was approximately equivalent to treatment with 10 Pa C_2H_4 at harvest.

Although 2 weeks of cold storage greatly improved ripening in comparison with nonstored, non- C_2H_4 -treated fruit (0 weeks), between 2 and 4 weeks of cold storage appears to be necessary to fully induce ripening of 'Bartlett' pears. Sfakiotakis and Dilley (1974) found that storage of Michigan 'Bosc' pears at 5 °C for 1.5 or 3 d was sufficient to establish the full potential for C_2H_4 production, although 3 d was more effective than 1.5 d. These results contrast with our results with 'Bartlett' pears stored at -1 °C, where the full potential for C_2H_4 production was not reached even after 2 weeks of storage. We do not know whether the difference in response was due to cultivar, fruit maturity, or storage temperature.

Fig. 2. Effects of storage duration and C_2H_4 treatment on changes in ACC synthase activity (ACC in nmol·g⁻¹·h⁻¹) and ACC oxidase activity (C_2H_4 in nmol·g⁻¹·h⁻¹) of 'Bartlett' pears harvested in Sacramento (A and D), Mendocino (B and E), or Lake (C and F) Counties during 7 d at 20 °C. Mature-green pears from each growing location were ripened at 20 °C, without (0 weeks) or with 10 Pa (100 µL·L⁻¹) ethylene (0 weeks + C_2H_4) for 24 h at harvest, and after storage for 2, 4, 6, and 12 weeks at -1 °C. Data points represent means of three replicates ± SE.

Various ripening-related characteristics were influenced by cold storage to different degrees. Although 6 and 12 weeks of storage resulted in similar rates of firmness loss in pears grown in Lake and Mendocino Counties, the rates of skin yellowing and C_2H_4 production were very different. In agreement with our findings, Lelièvre et al. (1997b) stated that fruit softening is one of the ripening processes that is most sensitive to C_2H_4 , while color change can be either C_2H_4 -dependent or independent according to the type of pigment involved and the species. Therefore, cold storage or exogenous C_2H_4 treatment may selectively induce faster softening, but not other aspects of ripening to the same extent (Gerasopoulos and Richardson, 1997; Wang et al., 1972).

Similar rates of ripening achieved by 6 and 12 weeks of cold storage in pears grown in Mendocino and Lake Counties might be an indication that we fully induced ripening with the 6-week treatment, and therefore longer storage periods would have no further effect. Fruit from all locations that were cold-stored for 12 weeks had 1) higher ACS and ACO activity, and 2) C_2H_4 production upon removal from cold storage than did those stored for shorter periods, indicating that they were reaching the end of their storage life. Our results agree those of Blanpied (1977), who reported that the storage life of New York 'Bartlett' pears is usually limited to, at most, 10 weeks of cold storage in air and, if 'Bartlett' pears are held longer than 10 weeks, they usually ripen in storage.

Holding fruit at 20 °C without C_2H_4 immediately after harvest resulted in little softening or change in skin color in fruit from all three growing locations. The resistance to ripening was probably a result of very low ACS and ACO activity and consequently low C_2H_4 production. Ethylene treatment at harvest induced ripening of fruit from all three locations within 7 d at 20 °C, and the rate of fruit ripening was equivalent to or faster than that of fruit cold-stored for 2 weeks (Lake and Mendocino) or 4 weeks (Sacramento). Of the three pear-growing locations studied, distinct differences in ripening characteristics were evident. In general, fruit from Lake and Mendocino Counties ripened faster in response to a given length of cold storage than did fruit from Sacramento County. The differences between locations are probably related to the more advanced maturity of the fruit from Mendocino and Lake Counties (data not shown), resulting in pears with a higher capacity to ripen (Agar et al., 1999a). Advanced maturity of the Mendocino and Lake fruit could be evidenced by slightly higher ACS and ACO activity at harvest than that of Sacramento fruit. Also, firmness of Mendocino and Lake fruit at harvest were 76 and 77 N; 9 and 8 N softer than that of Sacramento County fruit (85 N), respectively. Cooler preharvest temperatures in Mendocino and especially in Lake County may induce ACS and ACO enzyme activity, resulting in higher endogenous C_2H_4 production, which triggers ripening.

Activities of ACS and ACO in the pears were very low at harvest. Our results suggest that the activity of neither enzyme was signifi-

cantly induced by holding the fruit at 20 °C immediately after harvest (0 weeks). Although ACO activity increased significantly in C_2H_4 -treated pears after three and five d at 20 °C, the increase in ACS activity was small in Sacramento and Mendocino fruit, resulting in relatively low C_2H_4 production. These results suggest that there may be a differential response of the C_2H_4 -synthesizing enzymes to exogenous C_2H_4 , which may result in higher ACO, but not ACS, activity. Our results seem to agree with the findings of Blankenship and Richardson (1985), who stated that ACO activity is induced prior to ACS activity during cold storage and that ACS is the rate-limiting step in C_2H_4 biosynthesis in 'Anjou' pears.

The rate of C_2H_4 production in pears grown in Lake County and cold-stored for 6 weeks declined after 5 d at 20 °C, despite a significant increase in ACO and ACS activities. Also, C_2H_4 production of Lake County pears that were stored for 12 weeks increased slightly between 3 and 5 d of ripening despite decreases in ACS and ACO activities during the same period. These data may indicate that: 1) other factors besides ACS and ACO activities are influencing fruit C_2H_4 production, 2) enzyme activity in vitro can exceed that in vivo, or 3) both.

Lelièvre et al. (1997a) also reported that ACO gene expression and activity can be induced by either chilling or short-term exogenous C_2H_4 treatment in 'Passe-Crassane' pears. They also found that ACS and C_2H_4 biosynthesis may not be regulated by exogenous C_2H_4 alone in nonreceptive fruits, but that a chilling treatment may also be required prior to the C_2H_4 treatment. Contrary to these latter findings, we were able to induce ripening in 'Bartlett' pears, including induction of ACS and ACO activity, without cold storage, by treating with 10 Pa C_2H_4 at harvest.

Our findings with California 'Bartlett' pears suggest that 4 weeks of cold storage at -1 °C or treatment with 10 Pa C_2H_4 at harvest stimulates ACS and ACO activities upon transfer of the fruit to 20 °C and results in satisfactory ripening. If faster ripening is desired after short cold storage durations (<4 weeks), the fruit should be treated with exogenous C_2H_4 at the start of ripening. Our data indicate that the rate of ripening that can be expected at retail markets or at canning facilities depends greatly on the length of the cold storage period prior to ripening; 'Bartlett' pears may ripen to 14 N (ready to eat) within 2.5 to 7 d depending upon the length of prior cold storage.

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