

# Ethylene Production by 'd' Anjou' Pears during Storage at Chilling and Nonchilling Temperature

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**Abstract.** To determine the ability of 'd' Anjou' pear fruit to produce climacteric ethylene postharvest, fruit were harvested at a mature green stage, chilled at  $-1^{\circ}\text{C}$  for various times, then transferred to  $20^{\circ}\text{C}$  to ripen. In addition, fruit were first held at  $20^{\circ}\text{C}$  for various times, then at  $-1^{\circ}\text{C}$  for various durations, followed by transfer to  $20^{\circ}\text{C}$  for 11 days. As storage time at  $-1^{\circ}\text{C}$  increased from 0 to 70 days, the time required to induce climacteric ethylene when transferred to  $20^{\circ}\text{C}$  progressively decreased from 90 to 0 days. The total storage time (sum of d at chilling and nonchilling temperature) needed to induce climacteric ethylene remained nearly constant (70 to 90 days). However, this was not the case with fruit held initially at  $20^{\circ}\text{C}$ , then transferred to  $-1^{\circ}\text{C}$ . The total storage time needed before the pears produced climacteric ethylene ranged from 70 to 110 days and increased with time of storage at  $20^{\circ}\text{C}$ . These fruit required more time at  $-1^{\circ}\text{C}$  than those first stored at  $-1^{\circ}\text{C}$ . The chilling requirement mechanism of 'd' Anjou' pears remains intact even during storage at nonchilling temperature and diminishes with senescence.

Summer pears respond promptly to chilling by producing climacteric ethylene (Looney, 1972), while winter pears require a considerable period of chilling to produce climacteric ethylene and to ripen. The amount of chilling required depends upon storage temperature (Gerasopoulos and Richardson 1995; Sfakiotakis and Dilley, 1974), variety (Knee, 1973; Wang et al., 1985), and fruit Ca concentration (Richardson and Al-Ani, 1982). 'd' Anjou' pears normally require 50 to 60 d storage at  $-1^{\circ}\text{C}$  (Chen et al., 1981; Chen and Mellenthin, 1981), but depending on the growing season may require only 30 d (Chen et al., 1982) or even no chilling (Wang et al., 1972) to begin to ripen at  $20^{\circ}\text{C}$ . However, 'd' Anjou' pears may eventually ripen at  $20^{\circ}\text{C}$  without prior exposure to chilling temperatures. Loss in firmness occurs first and climacteric ethylene production follows (Gerasopoulos and Richardson, 1995). The two events are completely separated in time when 'd' Anjou' pears are held continuously at nonchilling temperatures for a time equal to the chilling requirement (Gerasopoulos and Richardson, 1995).

The purpose of this study was to determine the time of storage at nonchilling temperature required by 'd' Anjou' pears to acquire the ability to produce climacteric ethylene as indicated by internal concentrations  $>1 \mu\text{L}\cdot\text{L}^{-1}$ .

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## Materials and Methods

'd' Anjou' pear fruits were harvested from the Mid-Columbia Experiment Station, Hood River, Ore., 147 d after full bloom. Flesh firmness, measured (two measurements per fruit) by a Hunter force model LKG-1 12-kg tester (Western Industrial Supply, San Jose, California) equipped with an 8-mm tip, was 70 N. After harvest, the fruits were transferred to perforated polyethylene film-lined 20-kg cardboard cartons and placed in storage at chilling ( $-1^{\circ}\text{C}$ ) and nonchilling ( $20^{\circ}\text{C}$ ) temperatures. All fruits with cork spot were removed and only sound fruits were used.

**Storage at  $-1^{\circ}\text{C}$ , then  $20^{\circ}\text{C}$ .** The pears were held first at  $-1^{\circ}\text{C}$  for 0 (at harvest-control), 25, 40, 55, 70, or 85 d, followed by a period at  $20^{\circ}\text{C}$ . To measure ethylene a 1-mL internal atmosphere sample was drawn by syringe from the water-immersed pears and injected into a flame ionization gas chromatograph (model 311; Carle Instruments, Fullerton, Calif.) equipped with a  $2.0 \times 4\text{-mm}$ , 80/100 mesh, activated alumina column. Internal fruit ethylene and flesh firmness were measured in five replicate pears three times per week during storage. The time at  $20^{\circ}\text{C}$  required by the fruits to produce climacteric ethylene was recorded.

**Storage at  $20^{\circ}\text{C}$ , then  $-1^{\circ}\text{C}$ .** Fruits were stored first at  $20^{\circ}\text{C}$  for 25, 40, 55, 70, or 85 d, then transferred to  $-1^{\circ}\text{C}$  for 10, 20, 30, 40, 50, 60, or 70 d, followed by transfer back to  $20^{\circ}\text{C}$  for 11 d. Internal ethylene and flesh firmness were measured every other day in five replicate fruits during ripening at  $20^{\circ}\text{C}$ . While the threshold for 'd' Anjou' pears may be in the range of 0.05 to  $0.5 \mu\text{L}\cdot\text{L}^{-1}$  ethylene (Wang et al., 1985), for comparative purposes an internal ethylene level of  $1 \mu\text{L}\cdot\text{L}^{-1}$  was arbitrarily chosen as having physiological activity. Sustained ethylene production above this level within 11 d of ripening at  $20^{\circ}\text{C}$ , leading to the climacteric rise in ethylene, is considered a clear indication of the satisfaction of the chilling requirement (Gerasopoulos and Richardson, 1996).

**Statistical analysis.** LSDs for comparisons between treatments at a particular time during ripening were calculated with the NCSS "Number Cruncher" (J. Hintze, Kaysville, Utah) statistical computer software.

## Results and Discussion

Firmness of 'd' Anjou' pears held only at  $-1^{\circ}\text{C}$  decreased slightly from 70 to 65 N, and the internal ethylene increased progressively to  $\approx 2 \mu\text{L}\cdot\text{L}^{-1}$ , by 98 d (Fig. 1). Firmness of fruit held only at  $20^{\circ}\text{C}$  decreased very slowly from 70 to 65 N during the first 21 d at  $20^{\circ}\text{C}$ . Thereafter, softening rapidly progressed up to 42 ( $\approx 35$  N), followed by a slower loss

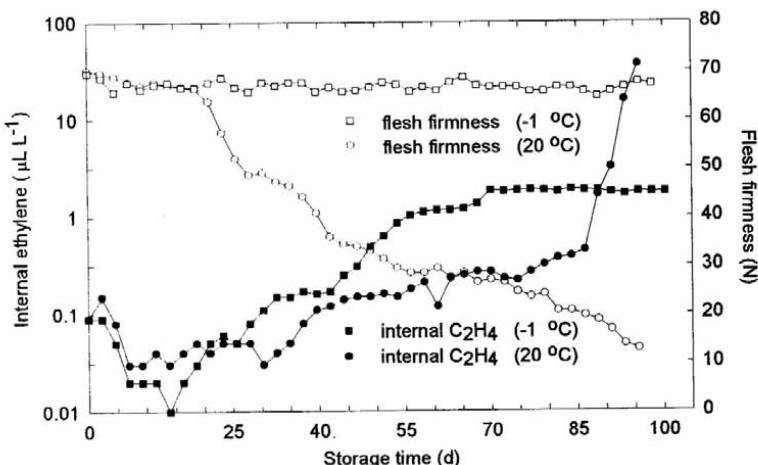


Fig. 1. Effect of storage at  $-1^{\circ}\text{C}$  or  $20^{\circ}\text{C}$  on level of internal ethylene and flesh firmness of 'd' Anjou' pears.

in firmness to 10 N at 98 d (Fig. 1). Ethylene concentrations in freshly harvested fruits subjected to  $-1^{\circ}\text{C}$  for 0 d or to storage at  $20^{\circ}\text{C}$  were no greater than  $0.3\ \mu\text{L}\cdot\text{L}^{-1}$ . The climacteric rise in ethylene appeared only after 90 d, the concentration reaching  $30\ \mu\text{L}\cdot\text{L}^{-1}$  within the next 6 d (Figs. 1 and 2). Similarly, the fruit color changed from green to yellow by day 21 (data not shown). Ripening and ethylene production of 'd' Anjou' pears within 70 to 90 d at nonchilling temperature, previously reported by Gerasopoulos and Richardson (1995) was attributed to fruit senescence.

Fruits held at  $-1^{\circ}\text{C}$  for 25 d also showed a considerable delay (63 d) before  $0.03\ \mu\text{L}\cdot\text{L}^{-1}$  ethylene was exceeded at  $20^{\circ}\text{C}$  (Fig. 2). The climacteric began within the next few d, about the same time as in control fruit, i.e., by the 90th d (Table 1) and continued to rise to  $30\ \mu\text{L}\cdot\text{L}^{-1}$  (Fig. 2). Climacteric ethylene production was also delayed up to 34 and 23 d, in pears held for 40 or 50 d at  $-1^{\circ}\text{C}$  (Fig. 2) or a cumulative storage time of 74 and 73 d, respectively (Table 1). Once the pears had been held at  $-1^{\circ}\text{C}$  for at least 70 d, there was essentially no delay in their ability to produce climacteric ethylene, and a peak of  $40\ \mu\text{L}\cdot\text{L}^{-1}$  was attained to about 84 d (Fig. 2). Thus the chilling requirement of these pears was 70 d (Fig. 2), within the normal range reported for 'd' Anjou' (Chen et al., 1981; Chen and Mellenthin, 1981).

Pears initially held at  $20^{\circ}\text{C}$  for 25 d required an additional 70 d at  $-1^{\circ}\text{C}$ , or a cumulative 95 d at both temperatures, to produce climacteric ethylene when placed at  $20^{\circ}\text{C}$  temperature for 11 d; this was similar to freshly harvested pears (Fig. 3). Storage at  $20^{\circ}\text{C}$  for 25 d apparently does not affect the chilling requirement of pears. However, fruits held 40, 55, or 70 d at  $20^{\circ}\text{C}$  required an additional 40 to 50 d at  $-1^{\circ}\text{C}$  for a cumulative 90, 95, and 110 d, respectively, to produce climacteric ethylene during ripening at  $20^{\circ}\text{C}$  for 11 d (Fig. 3). Storage for 40 to 70 d at  $20^{\circ}\text{C}$  reduces the chilling requirement by 20 to 30 d compared to freshly harvested pears. 'd' Anjou' fruits held for 85 d at  $20^{\circ}\text{C}$  required the minimum amount of time (10 d) at  $-1^{\circ}\text{C}$  to produce climacteric ethylene at  $20^{\circ}\text{C}$  (Fig. 3).

Although softening and ethylene production are slowed during storage of pears at chilling temperatures ( $0^{\circ}\text{C}$ ), once chilling is satisfied ethylene production and softening develop concurrently during ripening (Blankenship and Richardson, 1985; Gerasopoulos and Richardson, 1995). Low storage temperature dramatically retards softening as ethylene synthesis capability continues to develop. Storage at chilling temperatures stimulates both ACC synthase and oxidase activity (Blankenship and Richardson 1985; Larrigaudiere and Vendrel, 1993) in addition to changing the mRNA population (Wilson et al., 1990), including ACC oxidase and ACC synthase mRNA transcripts (Lelievre et al., 1996).

Even though pears stored at  $20^{\circ}\text{C}$  for 25, 40, 55, or 70 d, then at  $-1^{\circ}\text{C}$  for 50 to 60, 50 to 70, 40 to 50, or 20 to 50 d, contained 1.5 to  $10\ \mu\text{L}\cdot\text{L}^{-1}$  internal ethylene, upon transfer to

Table 1. Effects of storage temperature and time on total time to climacteric rise of ethylene of 'd' Anjou' pears.<sup>a</sup>

Temp ( $^{\circ}\text{C}$ )		Time at $T_1$	Time at $T_2$	Total storage time
$T_1$	$T_2$	(d)	(d)	(d)
-1	20	0	90	90
		25	60	85
		40	35	75
		55	25	80
		70	0	70
		85	0	85
20	-1 <sup>b</sup>	0	70	70
		25	70	70
		40	50	90
		55	50	95
		70	40	110
		85	10	90

<sup>a</sup>Data obtained from Figs. 2 and 3.

<sup>b</sup>Fruits held an additional 11 d at  $20^{\circ}\text{C}$  after removal from  $-1^{\circ}\text{C}$ .

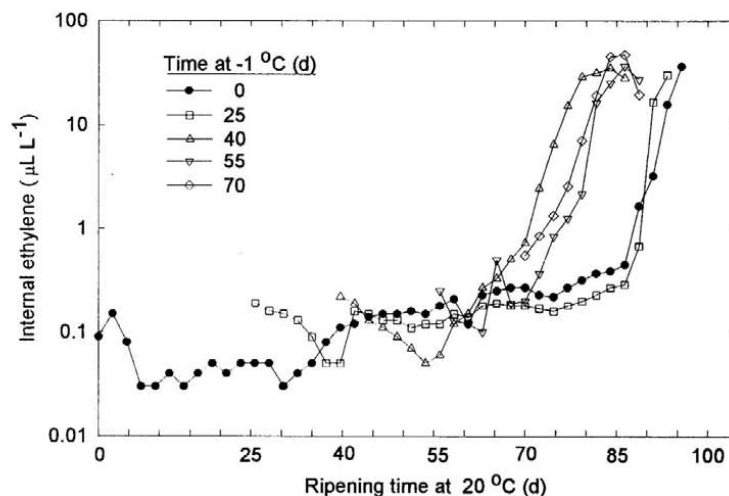


Fig. 2. Internal ethylene level in 'd' Anjou' pears stored at  $-1^{\circ}\text{C}$  for 0, 25, 40, 55, and 70 d, followed by ripening at  $20^{\circ}\text{C}$ .  $\text{LSD}_{0.05} = 4.6$ .

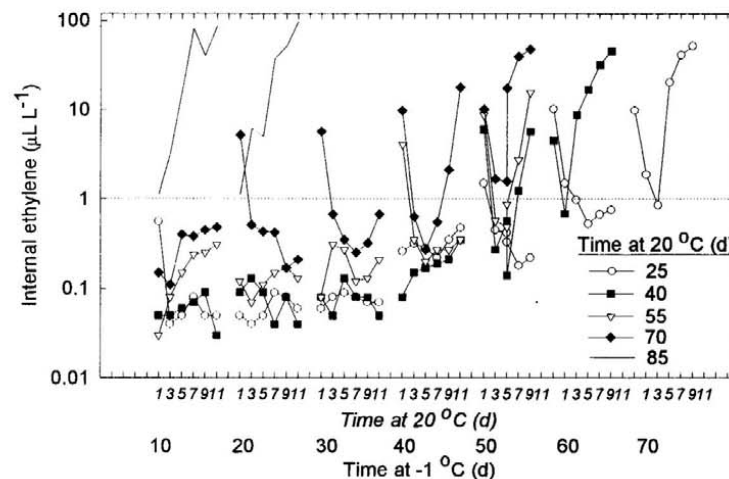


Fig. 3. Internal ethylene level in 'd' Anjou' pears stored at  $20^{\circ}\text{C}$  for 25, 40, 55, 70, and 85 d, then at  $-1^{\circ}\text{C}$  for 10 to 70 d, followed by ripening at  $20^{\circ}\text{C}$  for 11 d.  $\text{LSD}_{0.05} = 6.2$ .

$20^{\circ}\text{C}$ , this was not sustainable, falling to  $0.3\ \mu\text{L}\cdot\text{L}^{-1}$  by the 7th d. This suggests that the initial high ethylene production was due to conversion of accumulated ACC to ethylene (Blankenship and Richardson, 1985).

The time required by 'd' Anjou' pears held at  $-1^{\circ}\text{C}$  to produce climacteric ethylene upon rewarming appears to decrease linearly with

increasing time at  $-1^{\circ}\text{C}$  (Table 1). Alternatively, the total time that the fruit requires at  $-1^{\circ}\text{C}$  followed by  $20^{\circ}\text{C}$  to produce sustainable ethylene is relatively constant (Table 1). However, this was not the case for fruits stored first at  $20^{\circ}\text{C}$  and then at  $-1^{\circ}\text{C}$  (Fig. 3, Table 1). The loss in firmness and changes in other characteristics occurring during storage at the

20 °C (Gerasopoulos and Richardson, 1995) do not appear to reduce the time at -1 °C needed to produce climacteric internal ethylene at 20 °C. The chilling requirement of 'd' Anjou' pears first held at 20 °C dramatically decreases when firmness falls below 30 N (after the 70th day). As a result, the sum of time at 20 °C followed by -1 °C is not constant (Table 2) as it is for fruits stored at -1 °C followed by 20 °C (Table 1). This value appears to increase with the loss in firmness and to decline only when firmness is about 30 N (Fig. 1, Table 2).

These data suggest that in 'd' Anjou' pear, a winter variety that requires chilling to ripen, postharvest storage determines which of two distinct routes is followed to reach ripening and production of climacteric ethylene. Storage at chilling temperatures appears to stimulate the ripening mechanism, as evidenced by climacteric ethylene production. Holding pears at nonchilling temperatures, however, alters the sequence of the different components in ripening. The loss in firmness precedes the rise in ethylene. This loss in firmness, and possibly other factors, reduce the chilling requirement. Climacteric ethylene production without chilling may be attributed to senescence; the chilling requirement may be satisfied or reduced to nil by the same mechanism.

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