Low-temperature Storage Elicits Ethylene Production in Nonclimacteric Lychee (*Litchi chinensis* Sonn.) Fruit

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Abstract. Lychee (*Litchi chinensis* Sonn.) fruit were harvested at green, pink, and red developmental stages. The fruit of each group were used to determine developmentally and environmentally (chilling) regulated ethylene production. The rate of ethylene production in the fruit was green > pink > red. Storage at either 4 or 10 °C increased C₂H₄ production as much as 8.6-fold compared with control fruit stored at 25 °C. The green fruit were most responsive to chilling in terms of ethylene production.

Lychee, a member of the family Sapindaceae, is a subtropical evergreen plant indigenous to the southern part of China. The fruit is considered to be nonclimacteric (Biale, 1960), and has an edible translucent aril surrounded by a reddish, prickly, and leather-like shell. Discoloration or browning of the pericarp dramatically reduces its commercial value. The loss of red pigments and development of browning, which occur in <48 h under ambient conditions, are largely dependent upon the rate of desiccation, the increase in pH, and the length of storage time (Akamine, 1960; Campbell, 1959; Huang and Scott, 1985; Underhill and Critchley, 1994). The change in color is believed to be caused by degradation of anthocyanins, such as cyanidin-3-rutinoside, cyanidin-3-glucoside, malvidin-3-acetylglucoside, pelargonidin-3-glycoside, and pelargonidin-3,5-diglucoside (Peckett, 1980; Prasad, 1978). The activity of polyphenol oxidase (PPO; electrical conductivity 1.10.3.1) is responsible for the enzymatic oxidation of anthocyanins (Huang et al., 1990; Joslyn and Ponting, 1951).

Ethylene has been implicated in the activation of PPO (Elstner et al., 1976; Sharon and Kahn, 1979), and exogenous application of ethylene to lychee fruit promotes enzyme activity in pericarp tissues and subsequent browning (Jiang et al., 1986). Ethylene production is negligible prior to harvesting (Tongdee et al., 1982) but rises significantly after harvest, possibly because of wounding, then de-

creases to a basal level (Jiang et al., 1986).

Although heat treatment (60 °C water for 2 min) can induce the development of brown pigmentation in lychee fruit epicarp (Underhill and Critchley, 1993), chilling at 2 to 8 °C and 65% to 95% humidity can maintain the red color for as long as 5 weeks (Huang and Scott, 1985; Tongdee et al., 1982). Moreover, dipping fruit in ascorbic acid and citric acid, followed by deep-freezing, can maintain the red color for years (Nip and Liang, 1988). However, low or freezing temperatures induce browning; this is accelerated upon warming or thawing (Nip and Liang, 1988).

Hansen (1945) discovered that exposure of apple (*Malus domestica* Borkh.) fruit to temperatures <10 °C could enhance ethylene production upon return to a higher temperature. This temperature-dependent ethylene production was subsequently investigated by Burg and Thimann (1959). Cooper et al. (1969) studied the enhancement of fruit ripening by chilling-induced ethylene in citrus (*Citrus* sp.), pear (*Pyrus communis* L.), and avocado (*Persea americana* Mill.) fruits. The effect of chilling temperatures on ethylene biosynthesis was further examined in apple and tomato (*Lycopersicon esculentum* Mill.) fruit tissues (Mattoo and Lieberman, 1977), dwarf bean (*Phaseolus vulgaris* L.) leaf discs (Field, 1981), and cucumber (*Cucumis sativus* L.) fruit (Wang and Adams, 1980). These researchers all demonstrated that exposure to a temperature between 0 and 12 °C stimulated ethylene production on transfer to room temperature (Field, 1990). However, to our knowledge, chilling-inducible ethylene production in lychee fruit has not been reported before. In this work, therefore, we studied the production of ethylene during lychee fruit development, as well as following chilling, in order to determine the relationship between ethylene production and chilling sensitivity at different maturities.

Materials and Methods

Plant materials. Lychee fruit of different developmental stages were harvested from

Tai Tong Lychee Valley in Hong Kong, and transported to the laboratory within 2 h of harvesting. The fruit were classified into three groups of different developmental stages, i.e., green, pink, and red. About 1 kg of fruit in each group were used for the experiment.

Chilling treatment. Fruit were stored in 8-L airtight plastic chambers at 4, 10, or 18 °C. After 74 h of storage, fruit of all three developmental stages stored at 4 or 18 °C were transferred to 25 °C for 4 (4 °C) or 5 (18 °C) d, while the fruit treated at 10 °C were kept at the same temperature for 8 d before transfer to 25 °C for 2 d. Control fruit were held continuously at 25 °C. Ethylene production rates of the fruit were determined periodically during storage and after transfer to 25 °C.

Ethylene measurement. The containers were flushed with fresh air. After 2 h of equilibration and at intervals thereafter, 2 mL of gas were removed from each container with a plastic hypodermic syringe, and analyzed by gas chromatography (model HP5890; Hewlett-Packard, Wilmington, Del.) using a flame ion detector (FID) and a Porapak Q alumina column. Nitrogen was used as the carrier gas. The ethylene standard was purchased from Hong Kong Oxygen Ltd. Ethylene measurements on each group of fruit were performed in triplicate. The means and standard deviations were calculated from these replicate measurements.

Results

Since lychee fruit is a nonclimacteric fruit, a rise in ethylene production during fruit ripening, i.e., at the pink and red stages, was not expected. Consistent with our expectation, the detached green fruit produced more ethylene, (3.3 to 3.5 nL·g⁻¹·h⁻¹) within the first 2 d of storage at 25 °C, than did the pink and red fruit (Fig. 1). Tongdee et al. (1982) reported that the production rate prior to harvesting was negligible; thus, the initial higher levels of ethylene production (1.3 to 3.7 nL·g⁻¹·h⁻¹) determined here might represent a wounding response (Underhill and Critchley, 1993). Our data suggest that the green fruit are more sensitive to wounding than are the red and pink fruit.

One day after harvest, the rate of ethylene production at 25 °C dropped significantly to 0.9 nL·g⁻¹·h⁻¹ in green fruit and to 0.6 nL·g⁻¹·h⁻¹ in pink and red fruit (Fig. 1). These rates are similar to those reported before by Jiang et al. (1986). After 2 d at 25 °C, the rate of ethylene production had diminished to 0.15 to 0.4 nL·g⁻¹·h⁻¹ (Fig. 1). This basal level was maintained at 0.1 to 0.55 nL·g⁻¹·h⁻¹ throughout 8 d of storage at 25 °C.

Ethylene production by fruit stored at 18 °C for 74 h did not increase significantly upon transfer to 25 °C (Fig. 1), whereas production by fruit stored at 4 °C for 74 h surged upon transfer (Fig. 1). The rates of production were 6.5-, 7.3-, and 7.7-fold as high in green, pink, and red fruit, respectively, as the rate in control fruit held continuously at 25 °C (Fig. 1). The ethylene production rates of these chilled fruits persisted for 4 d with some fluctuation (Fig. 1). The rates in green, pink, and red fruit exposed to 18 °C increased 1.9-, 2.0-, and 1.2-fold,

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respectively, 4 d after the temperature shift (Fig. 1).

Since ethylene is involved in flowering of lychee, and induction of flowering requires exposure to 10 or 13 °C treatment (Chen and Ku, 1988), we determined if a longer exposure to 10 °C could also increase the rate of ethylene production of lychee fruit. Fruit were treated at 10 °C for 8 d, then transferred to 25 °C. On transfer to the higher temperature, ethylene production rates of green, pink, and red fruit rapidly increased 8.6-, 3.6-, and 3-fold, respectively (Fig. 2), and the rate in green fruit continued to increase for 2 d after the temperature shift. However, the rates in pink and red fruit reached plateaus between 1 and 2 d after transfer to 25 °C (Fig. 2).

Discussion

Wang and Adams (1982) established that the controlling step in chilling-inducible ethylene biosynthesis is ACC (1-aminocyclopropane-1-carboxylic acid) formation, rather than conversion of ACC to ethylene (Field, 1984). The marked increase in ethylene production following low-temperature treatment of lychee fruit is probably also controlled by ACC formation. In our recent study of chilling-inducible ethylene production in citrus peel, we found that the steady-state level of an ACC synthase gene transcript was enhanced significantly during and after 3 to 6 d of treatment at 4 °C (data not shown). This finding suggests that the increase in ethylene production in lychee fruit following chilling may also be attributed in part to an increase in ACC synthase mRNA accumulation.

The chilling sensitivity of the plant cell is believed to be related to membrane function and a phase transition in membrane state (Lyons et al., 1979). Such transitions in the membrane could affect the membrane-bound enzymes, such as the ethylene receptor, ETR1, that signals ethylene responses in plant tissues (Ecker, 1995). Chilling might induce ACC synthase and ACC oxidase gene transcription by perturbation of the ethylene signal transduction pathway. In our work, Arrhenius plot analysis was not applied to ethylene production by lychee fruit to identify the critical chilling-sensitive temperature because of questions concerning the reliability of this method (Bagnall and Wolfe, 1978). Instead, we arbitrarily chose four different temperatures to study chilling-inducible ethylene biosynthesis. Based on our results, both 4 and 10 °C may be below the critical chilling-sensitive temperature for lychee fruit, since ethylene biosynthesis was greatly stimulated by both temperature treatments.

Some reports on the study of lychee fruit have linked ethylene synthesis to PPO (Jiang et al., 1986). Continued study of PPO and its inducer, ethylene, in fruit of different developmental stages might reveal the molecular and physiological basis for fruit pericarp browning, a very important postharvest problem associated with lychee fruit, which is commonly attributed to degradation of the anthocyanin pigments by PPO activity (Huang et al., 1990).

Fig. 1. Ethylene production rates of intact lychee fruit of three maturities (green, pink, and red) stored at (A) constant 25 °C; (B) 3 d at 18 °C; and (C) 3 d at 4 °C, then 25 °C.

Fig. 2. Ethylene production rates of intact lychee fruit of three maturities (green, pink, and red) during 8 d at 10 °C and subsequent transfer to 25 °C.

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