Involvement of Stress-induced Ethylene Biosynthesis in Fruit Softening of ‘Saijo’ Persimmon

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Summary

The possible involvement of stress-induced ethylene in softening of ‘Saijo’ persimmon fruit with or without >95 % CO₂ treatment for 16 hr at 25 °C, a technique widely used for the removal of astringency (Constant Temperature Short Duration (CTSD) method) was investigated. To confirm the role of ethylene in fruit softening, fruit were treated with 1-methylcyclopropene (MCP), a strong inhibitor of ethylene perception. The softening of treated fruit was inhibited markedly compared with non-treated fruit. Fruits, with or without CO₂ treatment, were held in high (>95 % RH) or low humidity (40 – 60 % RH) at 20 °C to determine the effects of water and CO₂ stresses on postharvest ethylene production and fruit softening. Without CO₂ treatment, ethylene production and fruit softening were retarded significantly by high humidity. Whereas, with CO₂ treatment, a considerable amount of ethylene was produced and fruit softened, irrespective of humidity. These results indicate that ethylene is involved in fruit softening and that its evolution is stimulated not only by water stress but also by CO₂ stress in ‘Saijo’ persimmon fruit.

Key Words: CO₂ stress, Diospyros kaki, ethylene, MCP, water stress.

Introduction

Storage- and shelf-life of persimmon fruit vary widely among cultivars. The astringent type Japanese persimmon fruit ‘Saijo’ softens rapidly after removal of astringency so that its market is restricted near the production area, in spite of its high quality. In persimmon fruit including ‘Saijo’ fruit, ethylene production concurrent with fruit softening and enhancement of fruit softening by exogenous ethylene has been reported, indicating a close relationship between ethylene and fruit softening (Itamura et al., 1991; Itamura et al. 1997; Takata, 1982). However, there is no direct evidence to demonstrate the role of endogenous ethylene in postharvest fruit softening.

1-Methylcyclopropene (MCP) is an inhibitor of ethylene action that blocks ethylene receptors and prevents ethylene effects in plant tissues (Sisler and Serek, 1997). Therefore, MCP provides a useful tool to study the role of ethylene in various physiological processes (Fan et al., 1999; Golding et al., 1998; De Wild et al., 1999). Furthermore, since MCP is nontoxic and odorless, in future it may be applied as a commercial postharvest treatment in many horticultural crops (Sisler and Serek, 1997; Watkins et al., 2000).

Apart from internal factors which regulate ethylene biosynthesis developmentally in plant tissues, such as ripening fruit, senescing flower and germinating seed, a variety of biotic and abiotic external factors induce ethylene production (Yang and Hoffman, 1984; Morgan and Drew, 1997). These external factors include mechanical wounding, heavy metals, waterlogging, drought (water stress), chilling and elevated CO₂ (CO₂ stress). The astringent type persimmon fruit are often subjected to applications that remove astringency, such as spraying with alcohol vapor, dipping in hot water or exposing to elevated carbon dioxide level. For example, the Constant Temperature Short Duration (CTSD) treatment which utilizes elevated CO₂ involves holding the fruit in >95 % CO₂ for a short duration (12 – 48 hr) at constant temperature (20 – 30 °C) (Matsuo et al., 1976). These applications for the removal of astringency may turn out to be stress factors, which can induce ethylene produc-
tion by the fruit. Beside the stress during destringency treatment, we have demonstrated that in forcing-cultured ‘Tonewase’ persimmon fruit, water stress stimulates ethylene production and advances postharvest fruit softening and that an alleviation of water loss using perforated polyethylene bag package delays fruit softening (Nakano et al., 2001). This water stress-induced ethylene may be involved in the softening of ‘Saijo’ fruit.

In this study, first, ‘Saijo’ fruit were treated with MCP to clarify the involvement of ethylene on the fruit softening and to estimate the potential of MCP for extending shelf-life. Second, fruit, with or without CO\textsubscript{2} treatment equivalent to CTSD method, were held in high or low humidity condition to test the effects of CO\textsubscript{2} treatment and relative humidity on ethylene production and fruit softening.

Materials and Methods

‘Saijo’ Japanese persimmon (Diospyros kaki Thunb.) fruit were harvested at commercial maturity from the research farm of Okayama University, Okayama, Japan.

Effect of MCP treatment on ethylene production and fruit softening of ‘Saijo’ persimmon fruit

Soon after the harvest, some fruit were treated with MCP at the estimated concentration of 300 nl·liter\textsuperscript{-1} for 3 hr as described previously (Nakatsuka, et al., 1997; Nakano et al., 2001); the rest were untreated. Then, fruit, with or without MCP treatment, were subjected to CO\textsubscript{2} treatment by holding fruit in a desiccator filled up with >95 % CO\textsubscript{2} for 16 hr at 25 °C. After the CO\textsubscript{2} treatment, fruit were held at 20 °C and ambient laboratory humidity (40 - 60 % RH). The rates of ethylene production and percentage of softened fruit were determined immediately after harvest and during storage at 20 °C. Ethylene production was measured by enclosing individual fruit in an air tight chamber for 1 hr at 20 °C, withdrawing for each determination 1·ml of headspace gas from the chamber, and injecting into a gas chromatograph (model GC-4CMPF, Shimadzu, Kyoto, Japan) fitted with a flame ionization detector and an activated alumina column. The percentage of softened fruit was determined according to the softening index reported by Iwata et al. (1969), whereby the softened fruit in this experiment was defined as a fruit that reached stage III of the index (soft to be easily crushed with fingers, or part of fruit surface becomes water-soaked appearance) (Tamura, 1986).

Effects of CO\textsubscript{2} treatment and relative humidity on ethylene production and fruit softening in ‘Saijo’ persimmon fruit

After harvest, fruit with or without CO\textsubscript{2} treatment as described above were held in high or low humidity at 20 °C. High humidity was maintained in a container through which humidified air was flowed at 250 ml·min\textsuperscript{-1}, >95 % RH, whereas the low humidity was that of the ambient laboratory, 40 - 60 % RH. Weight loss, the rates of ethylene production and percentage of softened fruit were determined immediately after harvest and during storage at 20 °C as described above. Weight loss was expressed as percentage of loss from the initial weight.

Results and Discussion

Effect of MCP treatment on ethylene production and fruit softening of ‘Saijo’ persimmon fruit

Since we attempted to compare the response of ‘Saijo’ fruit to CO\textsubscript{2} treatment with that of ‘Tonewase’ fruit (Nakano et al., 2001), the duration of CO\textsubscript{2} treatment conducted in this study was 16 hr, which resulted in an incomplete removal of astringency in ‘Saijo’ fruit (data not shown).

In the control fruit, ethylene production increased on the 2nd day after harvest (i.e. on the 1st day after CO\textsubscript{2} treatment) (Fig. 1). It decreased on the 3rd day and then increased again on the 5th day. Concomitant with the second increase in ethylene production, the percentage of softened fruit increased, resulting in softening of 90 % of the fruit within a week (Fig. 2). When ethylene response in the fruit was prevented by MCP treatment, the fruit softening was inhibited markedly. No softened fruit was observed within a week in the MCP plot which indicates clearly that ethylene is involved in fruit softening of ‘Saijo’ persimmon. Although softened fruit appeared concurrent with the second ethylene increase, it seemed that the cumulative effect of ethylene from the 2nd to the 5th day caused the softening. The results presented in this section also suggest that MCP has a high potential for maintaining firmness and extending the storage- and self-life of ‘Saijo’ fruit. As mentioned above, MCP is nontoxic and odorless. In the future, MCP may be used as a valuable tool in prolonging the

![Fig. 1. Effect of postharvest MCP treatment on the rate of ethylene production in ‘Saijo’ persimmon fruit. Each point represents the mean of the 10 fruits. Vertical bars show ± SE (n=10).](image-url)
storage and/or shelf life of "Saijo" fruit.

The initial increase in ethylene production observed in control fruit on the 2nd day after harvest was enhanced by MCP treatment, while the second increase on the 5th day after harvest did not occur in MCP-treated fruit. These observations suggest that the initial ethylene biosynthesis is under negative-feedback regulation and that the second is under positive-feedback regulation. The second increase of ethylene would be attributed to the action of the initial ethylene. In general, ripening-related ethylene biosynthesis in climacteric fruits is considered to be under positive-feedback regulation; the reduction of ethylene evolution by MCP treatment has been reported in ripening fruit of tomato (Nakatsuwa et al., 1997), apple (Fan et al., 1999) and pear (De Wild et al., 1999). On the contrary, ethylene biosynthesis, especially expression of ACC synthase genes, stimulated by external factors, such as wounding in winter squash (Nakajima et al., 1990) and tomato (Tatsuki and Mori, 1999) and auxin in mungbean hypocotyl (Yoon et al., 1997), is under negative-feedback regulation or is regulated independent of ethylene. In "Tonewase" fruit, we also demonstrated that water stress-induced ethylene production is regulated independent of ethylene (Nakano et al., 2001). It seems that the initial increase in ethylene production might be induced by external factors, such as water and CO₂ stresses.

Effects of CO₂ treatment and relative humidity on ethylene production and fruit softening in "Saijo" persimmon fruit

Irrespective of CO₂ treatment, relative humidity during postharvest shelf life had a large effect on fruit weight loss (Fig. 3). Fruit held in low RH lost > 0.8% of their initial fresh weight per day, while fruit held in high RH lost < 0.16% per day. Without CO₂ treatment, fruit held in low RH produced considerable ethylene on the 2nd day after harvest and then softened rapidly (Fig. 4, 5), whereas those held in high RH produced no detect-
able level of ethylene until the 10th day after harvest; meanwhile softening was retarded significantly. These results show that water stress–induced ethylene production is involved in fruit softening of ‘Saijo’ fruit as was shown in forcing cultured ‘Tonewase’ fruit (Nakano et al., 2001). Therefore, as a first step to extend the storage – and/or shelf – life of ‘Saijo’ fruit, water loss should be alleviated by keeping the fruit under high RH.

Fruit held in low RH after CO₂ treatment produced larger amounts of ethylene and softened more rapidly than those held in low RH without CO₂ treatment (Fig. 4, 5). Even in the fruit held in high RH after CO₂ treatment, a considerable amount of ethylene was produced on the 2nd day after harvest; thereafter, fruit softening proceeded rapidly until 60% had softened within a week. These results indicate that in addition to water stress, CO₂ treatment equivalent to CTS method can stimulate ethylene production in ‘Saijo’ fruit. Induction of ethylene biosynthesis by CO₂ stress has been reported in various horticultural crops including banana, eggplant and cucumber fruit (Kubo et al., 1990; Mathooko et al., 1995; Mathooko, 1996). Moreover, in ‘Triumph’ persimmon fruit, an elevated level of CO₂ has been shown to cause stress symptoms such as accelerated fruit softening, increased pulp injury and enhanced fruit respiration (Guelfalt – Reich et al., 1975).

Among the treatments for the removal of astringency, CTS method was developed to alleviate the toxic effects of carbon dioxide by minimizing the duration of exposure and to give the least damage to fruit (Matsuo et al., 1976; Itamura et al., 1991). CTS method is an established commercial technique for removal of astringency in ‘Tonewase’ or ‘Hiratanenashi’ persimmon fruit. Maybe, the reason is that in these cultivars the induction of ethylene by CTS method was not observed (Nakano, 2001). Tolerance of fruit to elevated levels of CO₂ may differ among cultivars, and ‘Saijo’ fruit may be more sensitive to CO₂ than ‘Tonewase’ or ‘Hiratanenashi’ fruit (Itamura et al., 1993). Moreover, for the complete removal of astringency in ‘Saijo’ fruit, a longer duration of CO₂ treatment than that conducted in this study may be required as mentioned above, which may generate stronger stress in the fruit. As reviewed in Mathooko (1996), a commodity’s tolerance to an elevated level of CO₂ depends on its physiological condition and maturity, as well as internal CO₂ concentration, duration of exposure, internal O₂ concentration and temperature. We also found that young fruit is more sensitive to water stress than mature fruit in ‘Hiratanenashi’ persimmon (Nakano et al., 2001). Therefore, further studies on the effects of harvest maturity and growing conditions in combination with studies on the effects of O₂ and CO₂ concentrations, duration and temperature on the response of ‘Saijo’ fruit to CO₂ treatment would be helpful in establishing the optimal condition for removing the astringency.

In conclusion, the results presented in this study show that ethylene production is induced by not only water stress but also by CO₂ stress, which, in turn, causes fruit softening in ‘Saijo’ Japanese persimmon. To reduce these stress–induced ethylene, further study aimed at establishing the optimal CO₂ treatment coupled with high humidity condition will be required. Inhibition of the ethylene response in the fruit by MCP treatment may be another way of extending storage – and/or shelf – life of ‘Saijo’ fruit in the future.

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**Literature Cited**


カキ ‘西条’ におけるストレス誘導エチレン生成と果実軟化

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摘 要

カキ ‘西条’ 果実の軟化に対するエチレンの関与を明らかにするとともに、果実のエチレン生成に及ぼすCTSD押出に相当する CO₂処理と貯蔵中の温度条件の影響を調査した。

‘西条’ 果実を 95% CO₂ で 16 時間処理した後、温度 20℃ 湿度 40% の条件下で貯蔵すると、収穫後 2 日 (CO₂ 処理後 1 日) でエチレン生成が検出され、収穫後 7 日より軟化果実が多発した。1-methylcyclopropene (MCP) によりエチレンの作用を阻害すると、この急激な軟化は完全に抑えられた。CO₂ 処理を行わず、果実を

低湿度下 (40%–60%) および高温度下 (>95%) で貯蔵するとき、低温度で貯蔵した果実は収穫後 2 日よりエチレン生成が誘導され、急激な軟化が観察された。高温度で貯蔵した果実はエチレン生成・軟化発生ともに収穫後 10 日まで抑えられた。一方、CO₂ 処理果実は、高温度で貯蔵した場合でも収穫後 2 日よりエチレン生成とそれに伴う急激な軟化がみられた。

以上より、‘西条’ 果実の収穫後の軟化には、水ストレスおよび脱水処理に伴う CO₂ ストレスによって誘導されるエチレンが関与していることが示された。