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Endogenous ethylene regulates accumulation of α- and β-carotene in the pulp of harvested durian fruit

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ABSTRACT

Durian (*Durio zibethinus* Murr.) cv. Chanee fruits were harvested at commercial maturity and stored at 25 °C for 9 days. Respiration rates and ethylene production increased during storage, while pulp firmness decreased. The concentrations of the two main pulp carotenoids (α- carotene and β-carotene) increased by more than 30% during storage. The minor carotenoids zeaxanthin also increased, while an increase in lutein was not statistically different from controls. Treatment at 25 °C with 500 μL L^{-1} 1-methylcyclopropene (1-MCP) for 12 h delayed the increase in respiration rate and in ethylene production. It also resulted in a later decrease in pulp firmness. 1-MCP largely prevented the increase in pulp carotenoid concentrations. Ethephon treatment (brushing the stem cut surface) increased the measured ethylene and slightly hastened the decrease in pulp firmness, but did not measurably affect the respiration and carotenoid concentrations. It is concluded that the postharvest increase in pulp α-carotene and β-carotene concentrations is an integral part of pulp ripening in this cultivar.

1. Introduction

Durian is a tropical fruit with a typical smell and taste. Durian fruit is chilling-sensitive, and is therefore often stored at about 20–25 °C, i.e. only slightly below ambient temperatures in the tropics (with maxima about 30–33 °C). Fruit ripening is regulated by endogenous ethylene, as 1-MCP (1-methylcyclopropene), an inhibitor of ethylene perception (Blankenship and Dole, 2003; Watkins, 2006) delayed softening (Maninang et al., 2011; Amornputti et al., 2014).

Fruit pulp colour depends on the durian cultivar, varying between pale to deep yellow. Yellow fruit colour is often due to carotenoids (Wei et al., 2014). We previously reported that the pale yellow pulp in ripe (ready to eat) cv. Monthong and the deep yellow colour in ripe cv. Chanee were correlated with an 11-fold higher concentration of β-carotene and about a 60-fold higher concentration of α-carotene in ripe cv. Chanee. These two carotenoids accounted for about the total carotenoid concentration in the pulp, as the levels of lutein and zeaxanthin were very low (Wisutiamonkul et al., 2015).

Carotenoid synthesis in fruit was inhibited by 1-MCP, depending on the carotenoid and whether pulp ripening in the species is regulated by endogenous ethylene (climacteric fruit) or not (non-climacteric fruit). In non-climacteric fruit no effect is to be expected, but in climacteric fruit the results were not uniform. For example, in mature green apricot (*Prunus armeniaca*) fruit, which is climacteric, 1-MCP had no effect on the increase of the concentrations of phytoene, phytofluene and β-carotene (Marty et al., 2005) while 1-MCP prevented the increase in lycopene and β-carotene concentrations in the climacteric papaya fruit (*Carica papaya*; Fabi et al., 2007) and total carotenoids in the climacteric tomato (Moretti et al., 2002) and dragon fruit (*Selenicereus megalanthus*; Deaquiz et al., 2014). 1-MCP applied before ripening also inhibited the increased expression of genes involved in carotenoid synthesis, for example in nectarine (Ziliotto et al., 2008).

Ethylene or ethephon (2-chloroethylphosphonic acid), a compound that slowly releases ethylene, has been used to hasten fruit ripening, e.g. in banana (Jiang et al., 1999). Durian can also ripen with ethylene (Ketsa and Pongkool, 1995) or ethephon (Cheyglinted, 1993). Ethylene induced peel yellowing or even browning. Consumers do not prefer this as it seems that the fruit is not fresh. For this reason many growers, wholesalers, and retailers in Thailand induce durian ripening by quickly dipping the fruit stalk (peduncle) into an ethephon solution, or brush the stalk with

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such a solution. Concentrations used vary mostly between about 3000–5000 $\mu L\,L^{-1}$ or higher. This treatment prevents peel yellowing or browning (Paull and Ketsa, 2014). Cheyglinted (1993) showed the rationale of this ethephon treatment. Cv. Chanee fruit harvested at 75% maturity either failed to ripen or ripened abnormally, whilst fruit harvested at 85% maturity reached good eating quality. Normal ripening, i.e. yellowing of the pulp and development of full flavour and aroma of fruit harvested at 75% maturity was obtained if treated with 1000 or 2000 $\mu L\,L^{-1}$ ethephon at the fruit stalk. Ripening of 85% mature fruit was slightly advanced when treated with ethephon. Harvesting at 75% maturity combined with ethephon treatment extended shelf life, compared with later harvesting and no such treatment.

It is not known if durian pulp carotenoid production after harvest is regulated by endogenous ethylene. The objective of this study, therefore, was to investigate the effect of 1-MCP. Additionally we tested the effect of ethephon, brushed at the fruit stalk. We used cv. Chanee fruit. It was hypothesized that carotenoid synthesis would be part of the ripening process, hence be inhibited by 1-MCP and promoted by ethephon.

2. Materials and methods

2.1. Plant material

Flowers on durian (*Durio zibethinus* Murr. cv. Chanee) trees, growing in a commercial orchard in Chanthaburi province (Eastern Thailand), were tagged one week after anthesis. Fruit were harvested 15 weeks (about 105 days) after anthesis, which is the commercially mature harvest stage. Fruit were submerged in 0.5 $mL\,L^{-1}$ imazalil solution for 20 s to control fruit rot caused by *Phytophthora palmivora*. Fruit were transported to the laboratory in a temperature-controlled truck (25 °C), where they arrived within a day of harvest. Transport took about 6 h.

2.2. Ethephon and 1-MCP treatments

One group (100 fruit) served as controls. In another group (100 fruit) an aqueous 4800 $\mu L\,L^{-1}$ ethephon was brushed at the surface of the cut stalk, applying about 0.2 mL per stalk and dried in air at 25 °C. A third group was placed in a 71 L sealed container and treated with 500 $\mu L\,L^{-1}$ 1-MCP for 12 h at 25 °C. 1-MCP was generated by adding water to 1-MCP (EthylBloc®, Floralife Inc., Walterboro, SC, USA) powder, which was placed a glass vial. This resulted in a final concentration of 500 $\mu L\,L^{-1}$ of 1-MCP in the air. Fans were used in the chambers to maintain air circulation. A fourth group of fruit was first treated with 1-MCP, as described above, and then immediately brushed with ethephon and dried as described. Fruit were stored at 25 °C and 85-90% RH.

2.3. Ethylene production and respiration rate

Measurement of ethylene production and respiration rate followed the method described by Palapol et al. (2015). Individual durian fruits were placed into 13.5 L airtight jars for 30 min at 25 °C, after which a 5 mL gas sample was taken from the air space and injected into a gas chromatographs equipped with a flame ionization detector (GC-14, Shimadzu, Tokyo, Japan) for ethylene, and a thermal conductivity detector (GC-RIA, Shimadzu, Kyoto, Japan) for carbon dioxide.

2.4. Colour, total soluble solids (TSS), firmness, and carotenoids

Pulp colour was quantified using a Minolta Chromameter (Model CR-300, Minolta, Osaka, Japan), recording hue, L^* and b^* (Hunter scale) values. Colour readings were taken twice at the equatorial region of each fruit. Values were averaged per fruit, then over the batch tested. Pulp firmness was measured using a hand-held fruit firmness tester (Effegi, Alfonsine, Italy) equipped with a cylindrical plunger 0.5 cm in diameter. The plunger was inserted to a depth of 0.5 cm and recorded in Newtons per square cm. Pulp TSS was determined using 4 g FW, adding 12 mL of distilled water, followed by homogenization. The homogenate was centrifuged at $12{,}000 \times g$ for 25 min at 4 °C. The TSS was measured in the supernatant, using a hand-held refractometer (Atago, Tokyo, Japan) and multiplied by 3 to account for the dilution.

Total carotenoid concentrations, and concentrations of individual carotenoids, were determined as described in Wisutiamonkul et al. (2015).

2.5. Statistical analysis

Six replicates per treatment and 18 fruit per treatment were used in all experiments. Data were analyzed using ANOVA and least significant difference (LSD) at $P \leq 0.05$ for firmness, TSS, colour, respiration, ethylene production, and total and individual carotenoids. Data of carotenoids were also compared using Duncan's new multiple range test (DMRT), at $P \leq 0.05$.

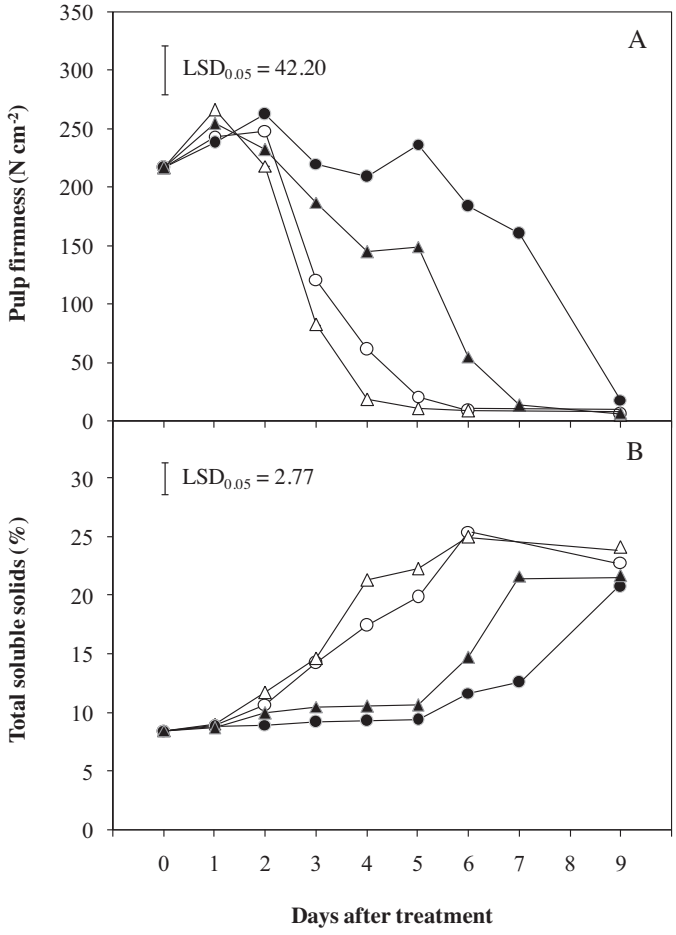

Fig. 1. Pulp firmness (A) and total soluble solids (B) in the pulp of durian (*Durio zibethinus* Murr.) cv. Chanee fruit during ripening at 25 °C. Treatments are controls (○), ethephon (Δ), 1-MCP (●) and 1-MCP + ethephon (▲). Data are means of six replications, each containing 3 fruit. LSD is indicated by a bar.