Ethylene biosynthesis enzyme activities in the pulp and peel of partially ripe 1-MCP-treated Bananas

Andreas Kleiber¹, Margaret Sedgley ², Nancy Bagnato³ and Farid Moradinezhad⁴*

1, 3 Department of Horticultural Science, University of Adelaide, Australia
2, School of Environmental and Rural Science, University of New England, Armidale, New South Wales, Australia
4, Department of Horticultural Science, University of Birjand, Birjand, Iran

Purpose: The study of effects of 1-Methylcyclopropene (1-MCP) on ripening of bananas is still an important issue for commercial application of 1-MCP on bananas. Research Method: Mature green bananas were treated with ethylene only (100 µL L⁻¹ for two consecutive days) and ethylene the same treatment followed by 1-MCP (300 nL L⁻¹) for 24 h to evaluate the ethylene and 1-MCP effects on ethylene biosynthesis enzyme activities. Ethylene production of whole banana fruit, ACC synthase (ACS) and ACC oxidase (ACO) activities in the pulp and peel of samples were measured. Findings: The result showed that ethylene production rate by the control fruit was significantly greater than the ethylene production rate by the 1-MCP-treated fruit at days 4, 8 and 10. However, changes in ethylene production were similar in both control and 1-MCP-treated bananas. The banana peel and pulp show different patterns of ethylene production during ripening. At the onset of ripening pulp tissues showed higher levels of ACS, and lower levels of ACO activity than peel. Assays of ACO and ACS activities in ethylene-treated fruit showed that the peel had higher levels of ACO activity than the pulp. The ACO and ethylene production were inhibited by 1-MCP treatment whereas ACS increased following 1-MCP application. Research limitations: Evaluation of ACS and ACO activities during different seasons. Originality/value: Pulp and the peel of bananas respond differently to ethylene and 1-MCP treatment with a greater impact on peel than the pulp. The findings of this study allow 1-MCP to be used in a more commercially reliable manner.
INTRODUCTION

The plant hormone ethylene participates in most stages of plant growth and development including seed germination, fruit ripening, senescence, abscission and various stresses (Abeles, 1985). In climacteric fruits like bananas, ethylene not only induces the ripening process but also regulates its progression (Lelievre et al., 1997). Ethylene production during ripening is highly regulated by two key enzymes, ACC synthase (ACS) and ACC oxidase (ACO), both of which are responsive to exogenous ethylene (Oetiker & Yang, 1995). Exogenous ethylene exerts a negative feedback regulation on ethylene production in immature climacteric fruit such as figs and banana (known as System 1), but is auto-stimulatory in mature climacteric fruit (known as System 2) (Lelievre et al., 1997).

1-Methylcyclopropene (1-MCP), which is an ethylene antagonist compound is in keen interest of postharvest researchers for past two decades, acts by binding, apparently irreversibly, to the ethylene receptors (Jiang et al., 2002) affecting the banana fruit ripening process (Macnish et al., 2000) suggesting that 1-MCP is potentially an ideal tool to provide a better understanding of ethylene biosynthesis regulation in banana fruit ripening. Although the 1-MCP application for delaying the ripening and maintaining the quality of banana has been also studied by researchers, but inconsistent responses reported for its effects in limiting the commercialization of 1-MCP application for bananas (Trivedi, 2012). Hence, further research is needed to study 1-MCP effects on bananas with focus on ethylene biosynthesis enzymes for establishing its commercial application.

The pattern of ethylene production during ripening in banana fruit is different from that in most other climacteric fruits (Mitra, 1997) suggesting the regulation of ethylene biosynthesis in banana may also be different. In addition, previous reports regarding the role of banana pulp in triggering the ripening of the whole fruit provide conflicting data. Dominguez and Vendrell (1993) suggested that a rise in ACO activity of the pulp before similar activity in the peel was proof that ACO activity of the pulp plays a role in initiating autocatalytic ethylene production during ripening. In contrast, a further study reported that changes in ACO activity during ripening did not support a role for the pulp as a trigger for the ripening of the whole fruit (Moya-Leon & John, 1994).

Previous studies have shown that 1-MCP at 300 nL L$^{-1}$ (Bagnato et al., 2003) or 1000 nL L$^{-1}$ (Jiang et al., 1999) could be effective in delaying the ripening of bananas. However, there have been limited studies of the effect of 1-MCP on the ethylene biosynthesis enzymes of bananas. While ethylene production and the enzymes responsible for its synthesis have been measured in bananas (Pelayo et al., 2003), they have only been examined in bananas treated with 1-MCP in the pre-climacteric stage (when green) and not in partially ripened bananas (Pathak et al., 2003). However, in Inaba et al. (2007) report has been a comparison between banana pulp and peel in terms of the effect of 1-MCP on the ripening process and ethylene enzyme activities but they have only been examined in bananas treated with propylene followed by the 1-MCP application but not in an optimum concentration and time. These are two important factors from the postharvest point of view that influence ripening process because without consideration of them fruit will not ripen properly when 1-MCP is applied. In a recent paper on banana (Zhu et al., 2015) a combination of 50 μL L$^{-1}$ ethephon with 400 nL L$^{-1}$ 1-MCP significantly delayed the ripening and maintained the quality of banana fruit without detrimentally affecting normal ripening after ripening acceleration treatment. This treatment effectively delayed and decreased respiration rate and ethylene production and delayed the peak activity of ACC synthase and ACC oxidase.
The purpose of this study, therefore, was to evaluate the effect of 1-MCP on ripening-associated changes of bananas after application of ethylene in terms of ethylene production in the whole fruit, and also ethylene biosynthesis enzyme activities both in pulp and peel.

MATERIALS AND METHODS

Tissue material and experimental procedure
Mature green Cavendish bananas (Musa acuminata cv. Williams) were obtained during April and May from a commercial orchard in north Queensland, Australia. Fruits were harvested, transported and prepared as described in Moradinezhad et al. (2008). Eighty-four bananas were allocated to each of two treatments, ethylene only and ethylene followed by 1-MCP. Twelve fruits were placed into two 10 L plastic containers (six fruits in each container) for each sampling day. After the first sampling, fruit were treated with 100 µL L⁻¹ ethylene for two consecutive days at 22 ºC. Containers were ventilated for 20 minutes each day. Whole banana fruits were then sealed in the same plastic containers and exposed to 1-MCP (0 or 300 nL L⁻¹) for 24 h at 22 ºC. 1-MCP was prepared, applied and measured as previously described in Moradinezhad et al. (2008). Tissue was harvested before any treatment (day 1), during and after ethylene treatment (days 2 and 3) or after 1-MCP treatment (days 4, 6, 8 and 10). Pulp and peel tissues were separated from the middle section of fruit, cut into slices, frozen in liquid nitrogen and stored at –80 ºC until used.

The enzymatic experiment was conducted using a split-plot design with time (seven levels) in the main plot and 1-MCP (two levels) in sub-plots, with three replicates. Data were analysed with the Genstat 9 program (9th edition, 2009, Lawes Agricultural Trust, VSN International Ltd) using the split-plot design. A least significant difference test LSD at 0.05 was used to determine significant differences between means.

For quality assessments, six fruit from each treatment were used to measure ethylene production, and six fruit were used to assess ACS and ACO activities on each sampling day.

Ethylene measurement in whole banana fruit
Ethylene production was measured as described in Moradinezhad et al. (2008) using a gas chromatograph (Varian 3400, Varian Associates Inc., Mulgrave, Victoria) connected to a flame ionisation detector (GC-FID). The rate of ethylene production was expressed as µL kg FW⁻¹ h⁻¹.

In vivo ACC synthase (ACS) activity in pulp and peel
The ACS activity was measured in vivo according to the method of Kato et al. (2000) with some minor changes. Frozen discs of pulp or fragments of banana peel tissue (0.2 g) were homogenised with a pestle and mortar in 3 mL extraction buffer consisting of 0.1 M 4-(2-hydroxethyl)-1-piperazinepropanesulfonic acid (EPPS)-KOH buffer, pH 8.5, 10 mM 2-mercaptoethanol, and 10 µM pyridoxal phosphate at 2 ºC (Kato et al., 2000). The homogenate was centrifuged (Eppendorf 5810 R, Eppendorf AG, 22331 Hamburg, Germany) at 4000 × g for 10 minutes at 4 ºC.

The ACS activity was assayed in 12 × 75-mm test tubes in a reaction mixture that consisted of 50 mM EPPS-KOH buffer, pH 8.5, 50 µM S-(5’-adenosyl)-L-methionine chloride (SAM) and the crude extract enzyme in a total volume of 1 mL (Kato et al., 2000). The test tube containing the reaction mixture was sealed with a rubber stopper no. 17 (9.5 mm suba-seal) and incubated for 30 minutes at 30 ºC and then the reaction was stopped by adding 0.1 mL of 40 mM HgCl₂. Approximately 0.1 mL of a cold mixture of 5% NaOCl and
saturated NaOH (2:1, v/v) was injected through the stopper by means of a 1-mL syringe fitted with a 25-gauge needle. The reaction mixture was then agitated on a Vortex mixer for a period of 5 sec before placement on ice. The ACC formed in the reaction was assayed by the method of Lizada and Yang (1979) by ethylene measurement using a 1 mL sample of the head-space gas which was withdrawn and injected into a gas chromatograph (Varian 3400) as previously detailed (Moradinezhad et al. 2008). The ACS activity was expressed as mmol ACC formed kg FW\(^{-1}\) h\(^{-1}\).

**In vivo ACC oxidase (ACO) activity in pulp and peel**

The ACO activities were measured *in vivo* according to Pretel et al. (1995) with some minor changes. The ACO activity was determined in a test tube (16 mL) containing 2 g banana pulp or peel in a 10 mL volume that was incubated with 25 mM Hepes-Tris buffer (pH 7.5) containing 0.5 M sorbitol and 1 mM 1-aminocyclopropane-1-carboxylic acid (ACC). After 30 minutes, the test tubes containing the reaction mixture were sealed with rubber stoppers no. 25 (12.5 mm suba-seal) and incubated for 1 h at 30 °C with continuous shaking. Then a 1 mL gas sample of the head-space of the test tube was withdrawn and monitored for its ethylene content by gas chromatography. ACO oxidase activity was expressed as mmol ethylene produced kg FW\(^{-1}\) h\(^{-1}\).

**RESULTS**

The data of experiments in both months were not significantly different and therefore they were combined in the figures.

**Ethylene production during ripening in whole banana fruit**

Although ethylene production of control and 1-MCP-treated fruit stored in 10 L containers at 22 °C had similar trends to some extent, the ethylene production rate by the control fruit was significantly greater than the ethylene production rate by the 1-MCP-treated fruit at days 4, 8 and 10 (Fig. 1). However, changes in ethylene production were similar in both control and 1-MCP treated bananas at days 1, 2, 3 and 6. Two ethylene production peaks were observed at day 4 (one day after ethylene treatment) and day 10 for both control and 1-MCP-treated fruit (although these were lower in 1-MCP-treated fruit).

![Fig. 1. Changes in ethylene production of whole banana fruit treated with 100 µL L\(^{-1}\) ethylene for 48 h (control) or treated with 100 µL L\(^{-1}\) ethylene for 48 h followed by 300 nL L\(^{-1}\) 1-MCP for 24 h and stored at 22 °C. Each data point is mean ± S.E. from n=12.](image-url)
ACS activity during ripening in pulp and peel
In control fruit, ACS activity of banana pulp increased from the second day of ethylene treatment (day 3) to a peak at day 4 (Fig. 2, a). The ACS activity then declined to day 8 and subsequently increased on day 10. The ACS peak in the pulp was higher in 1-MCP-treated than in control fruit (Fig. 2, a). 1-MCP-treated fruit followed a smaller trend except that ACS activity increased at a lower rate between days 2 and 4 but kept increasing until day 6 and then declined.

Changes in ACS activity in banana peel in both 1-MCP-treated and control fruit was similar up to day 4 (Fig. 2, b). ACS activity increased much later in the peel with a significant peak from day 8 to day 10 in control fruit. The 1-MCP treated fruit had a significant increase in ACS activity to day 6 followed by a slight decline to day 8 and then increase to day 10.

ACO activity during ripening in pulp and peel
In control fruit, ACO activity in banana pulp increased during ethylene treatment up to a peak at day 3, however, it declined after ethylene treatment to day 4 and then two more peaks at days 6 and 10 were observed (Fig. 3, a). 1-MCP-treated bananas had the same trend except that ACO activity did not increase after day 8.

Fig. 2. Changes in ACS activity of banana pulp (a) and peel (b) in fruit treated with 100 µL L⁻¹ ethylene for 48 h (control) or treated with 100 µL L⁻¹ ethylene for 48 h followed by 300 nL L⁻¹ 1-MCP for 24 h and stored at 22 ºC. Each data point is mean ± S.E. from n=12.
The ACO activity in banana peel of control fruit had the same trend as in pulp except it did not increase after day 8 (Fig. 3, b). ACO activity in the peel of 1-MCP treated-fruit increased during ethylene treatment up to a peak at day 3, and then steadily declined during and after 1-MCP treatment. The ACO activity was higher in banana peel than pulp, more than 3-fold greater in 1-MCP treated-fruit and approximately 4-fold greater in the control (Fig. 3). 1-MCP treatment generally decreased the activity of ACO in both banana pulp and peel.

**DISCUSSION**

The goal of these enzymatic studies was to broaden our knowledge of the physiology of the ripening process of both banana pulp and peel in particular the inhibitory effect of 1-MCP on these processes. Our results were consistent with the fact that banana peel and pulp show different patterns of ethylene production during ripening (Seymour, 1993), with the pulp tissue reported to be the principal source of ethylene production during ripening (Vendrell & McGlasson, 1971). At the onset of ripening pulp tissues showed higher levels of ACS, and lower levels of ACO activity than peel, as reported previously (Seymour, 1993).

![Fig. 3. Changes in ACO activity of banana pulp (a) and peel (b) in fruit treated with 100 µL L⁻¹ ethylene for 48 h (control) or treated with 100 µL L⁻¹ ethylene for 48 h followed by 300 nL L⁻¹ 1-MCP for 24 h and stored at 22 °C. Each data point is mean ± S.E. from n=12.](image-url)
The ethylene evolution pattern in 1-MCP-treated fruits was similar to that of control fruits. A sharp increase in ethylene evolution was observed at day 4 in both control and 1-MCP-treated fruit (the time that 1-MCP was applied to the fruit). This suggests that 1-MCP binds to the ethylene receptors incompletely in partially ripened bananas when applied during the climacteric period and does not suppress endogenous ethylene production, even though the 1-MCP treatment significantly decreased the ethylene production rate. Yan et al. (2011) also noted that application of 1-MCP suppressed the expression of genes associated with the ethylene-signalling pathway.

Application of 1-MCP after two days of ripening initiation, when autocatalytic ethylene production occurs (Golding et al., 1998), was apparently too late to suppress the ripening process, in agreement with the findings of Jiang et al. (1999) who noted that an extension of ripening is possible only when 1-MCP is applied within 24 h of ethylene treatment. This may be due to the reduced ability of 1-MCP to compete for the receptors (Sisler & Serek, 1997) or there may be fewer available ethylene binding sites.

Assays of ACO and ACS activities in our study of ethylene-treated fruit showed that the peel had higher levels of ACO activity than the pulp (expressed on a kg FW basis) and to a greater extent than found previously (Domínguez & Vendrell, 1993); (Moya-Leon & John, 1994). However, ACS activity in pulp increased to a greater extent than in the peel and its onset was earlier in the pulp, suggesting that ethylene production in banana fruit pulp triggers banana ripening, which supports the findings of Domínguez and Vendrell (1994) and is contrary to the findings of Moya-Leon and John (1994).

The ACO and ethylene production were inhibited by 1-MCP treatment whereas ACS increased following 1-MCP application. Because ethylene normally induces the ethylene climacteric in bananas by increasing ACO (Turner, 1997), the inhibition seen implies that 1-MCP also decreased the stimulatory effect of exogenous ethylene on ripening as reported by Zhu et al., (2015). However, the peel and pulp of Cavendish bananas behave differently in response to ethylene as indicated previously by Domínguez and Vendrell (1994) who stated that the peel is incapable of autocatalytic ethylene production (known as System 2). The pulp responds irreversibly to ethylene treatment. In addition, it has been reported that treatment with ethylene accelerates the ripening process and the climacteric peak is reached earlier (Domínguez & Vendrell, 1993; Inaba & Nakamura, 1986).

The ACO mRNA is detectable at all times in the pulp but only increases significantly in the peel at climacteric and post-climacteric stages (Lopez-Gomez et al., 1997) suggesting that the ripening process proceeds from the inside outwards (Domínguez and Vendrell, 1993; Tang et al., 1994). Under the conditions of this experiment a sharp peak of control pulp ACS activity during ripening was observed on day 4 that supports the view that pulp ethylene production triggers banana ripening, and subsequently, a peak in ACO activity in pulp was obtained at day 6 in control fruit. This shows that pulp may be triggering ripening as previously suggested (Domínguez & Vendrell, 1994). 1-MCP treatment increased ACS activity in both pulp and peel and subsequently the amount of ACO and ethylene production is increased.

The increase in ACS activity first in the pulp (day 2) and then in the peel (day 6) in control fruit also support the assumption that banana ripening is from the inside out. While 1-MCP delayed slightly ACS activity and peak of ethylene in the pulp as reported by Zhu et al. (2015) on banana, its impact is greater in the peel. This supports the findings of Inaba et al. (2007) and Pelayo et al. (2003) who also observed an increase in ACS activity in 1-MCP-treated fruit concurrent with a reduction in respiration rate. It could be concluded that this
decrease in respiration rate is a result of 1-MCP blocking normal feedback regulation and increasing transcription. It has been previously shown that transcript accumulation and activity of ACS in tomato fruits is affected by 1-MCP treatment (Nakatsuka et al., 1997). Similar results were reported by Golding et al. (1999) who suggested that 1-MCP may block the normal feedback regulation of ethylene production and that the transcription of ACS in bananas may possibly be enhanced. By day 10, fruit treated with 1-MCP had much lower levels of ACS and ACO activity. This reduction in ACS and ACO activity suggests that transcription has decreased and normal feedback is re-established. This would be possible if the fruit has synthesised new receptors (Jiang et al., 1999).

CONCLUSION

The results of this research support previous studies that noted the ripening of peel was affected significantly by exogenous ethylene and that the peel ripened earlier than the pulp. In addition, it is concluded that the fruit pulp has a more important role in triggering banana ripening than the peel, as peel and pulp respond differently to exogenous ethylene in terms of ACO and ACS activity and to some extent in ethylene production.

REFERENCES


فعالیت آنزیم‌های بیوسنتز اتیلن در گوشت و پوست موز انرژی تیمار شده با 1-
متیل سیکلوپروپان

چکیده:
مطالعه اثرات 1-متیل سیکلوپروپان (1-ام سی پی) در رسیدگی موز هنوز یک مورد مهم برای تجاری سازی 1-ام سی پی در موز است. از این رو، در این تحقیق موز سیبی بالغ فقط با اتیلن (100 میکرو لیتر در لیتر برای دو روز متوالی) تیمار شد و تیمار با همان اتیلن به دنبال 1-ام سی پی (300 نانو لیتر در لیتر) به مدت 24 ساعت برای ارزیابی اثرات 1-ام سی پی در فعالیت آنزیم‌های بیوسنتز اتیلن انجام شد. تولید اتیلن در میوه موز کامل، فعالیت‌های ACC سنتتاز و ACC اکسیداز در نمونه‌های گوشت و پوست اندازه‌گیری شد. نتایج نشان داد که میزان تولید اتیلن توسط میوه شاهد به طور معنی‌داری بیشتر از میزان تولید اتیلن در میوه تیمار شده با 1-ام سی پی در روزهای 4، 8 و 10 بود. با وجود این، تغییرات در تولید اتیلن در هر دو موز گوشت و پوست شاهد و تیمار شده با 1-ام سی پی مشابه بود. گوشت و پوست موز انرژی متوقف شدند که تیمار ACC اکسیداز در میوه‌های تیمار شده با اتیلن نشان دادند. از روش فعالیت‌های ACC سنتتاز و ACC اکسیداز توسط تیمار 1-ام سی پی ACC اکسیداز نسبت به گوشت دارد. تولید اتیلن و ACC اکسیداز در میوه‌های تیمار 1-ام سی پی متعلق شد، در حالی که ACC سنتتاز در دنبال کاربرد 1-ام سی پی افزایش یافته، نتیجه اینکه، گوشت و پوست موز به تیمار اتیلن و 1-ام سی پی پاسخ مختلف دادند. با اثر بی‌شیوه در پوست نسبت به گوشت. این آزمون‌ها برای اندازه‌گیری میزان 1-
mطلاعه اجراه می‌دهد که 1-ام سی پی با یک رفتار قابل اعتماد تجاری بی‌شیوه استفاده شود.

کلمات کلیدی: ACC سنتتاز، ACC اکسیداز، 1-متیل سیکلوپروپان ACC Musa acuminata

Kleiber et al.