



# Apparent synergism between the positive effects of 1-MCP and modified atmosphere on storage life of banana fruit

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## ABSTRACT

Fruit of cv. Gros Michel banana were treated with 1-MCP (1000 nL L<sup>-1</sup> for 4 h at 25 °C) and then packed in non-perforated polyethylene (PE) bags for modified atmosphere storage (MAP). The bags were placed in corrugated cardboard boxes and stored at 14 °C. Fruit were removed from cool storage and ripened at room temperature using ethephon. The length of storage life was determined by the change in peel color to yellow, after this ethephon treatment. Fruit treated with 1-MCP+MAP had a storage life of 100 days. The storage life of control fruit (no 1-MCP and no MAP) was 20 days. Fruit held in PE bags without 1-MCP treatment had a 40 day storage life, and the same was found in fruit treated with 1-MCP but without PE bags. 1-MCP is an inhibitor of ethylene action, but also inhibited ethylene production, mainly through inhibition of ACC oxidase activity in the peel. MAP inhibited ethylene production mainly through inhibition of ACC oxidase, both in the peel and pulp. The combination of 1-MCP treatment and MAP storage resulted in much lower ethylene production due to inhibition of both ACC synthase and ACC oxidase activity.

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## 1. Introduction

In green banana fruit, an increase in ethylene production causes yellowing of the peel and pulp, the increase in pulp softness, and the improvement of the eating quality of the pulp (Jiang et al., 1999). These processes can be affected by inhibiting ethylene synthesis or ethylene action.

1-Methylcyclopropene (1-MCP) is an inhibitor of ethylene action (Watkins, 2006). 1-MCP delays postharvest ripening of several fruit (Mworio et al., 2010; Piriavinit et al., 2011), including banana (Golding et al., 1998). 1-MCP blocks the ethylene receptor, but also inhibits ethylene production if this production is autocatalytic. Autocatalytic ethylene production becomes increased through perception of ethylene concentrations at the receptor level (Watkins, 2006).

Modified atmospheres (MA; low oxygen and high carbon dioxide concentrations) generally reduce the rate of respiration (Hertog et al., 1998) and inhibit the onset of the autocatalytic increase

in ethylene production (Kader, 1995). Low oxygen concentrations might reduce ethylene formation at the level of ACC oxidase, which requires oxygen (Kader, 1995). High carbon dioxide also reduces ethylene production. It possibly also acts by affecting the conversion from ACC to ethylene by ACC oxidase (de Wild et al., 2003). MA has been applied to banana fruit packed in polyethylene bags containing an ethylene absorbent (Scott et al., 1970). Ketsa et al. (2000) reported that cv. Sucrier bananas, placed in polyethylene bags containing an ethylene absorbent and a carbon dioxide scrubber, which were then stored at 14 °C, had a storage life of up to 6 weeks.

Currently most bananas are exported from Thailand using air transport. The purpose of our work was to find ways to use sea transport. Markets in Europe and the USA can only be reached by sea after about 4–6 weeks. The storage life of banana fruit should therefore exceed 6 weeks. Cv. Gros Michel, locally named 'Hom Thong Taiwan', is one of the main banana cultivars in Thailand. Like fruit of other banana cultivars, it has a short shelf-life at ambient temperature. Additionally, fruit of this cultivar is susceptible to chilling injury when stored below 10 °C (Hassan et al., 1990).

The objective of our research was to investigate if it was possible to increase the storage life of cv. Gros Michel banana fruit using 1-MCP treatment in combination with MAP treatment.

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## 2. Materials and methods

### 2.1. Plant material

Cv. Gros Michel banana (*Musa acuminata*, AAA Group) hands at 80% maturity (i.e. commercial maturity) were obtained from an orchard in eastern Thailand. The hands were placed in corrugated boxes and transported by refrigerated truck (25 °C) to the laboratory, where they arrived within 2 h of harvest. Hands were dipped for 3 min in 500 mL prochloraz (Sportak) fungicide solution, and then allowed to air-dry at ambient temperature (28–30 °C). Fruit were sorted for freedom from visual defects and uniformity of weight and shape. Fruit were randomly divided into lots for each of the treatments (control, 1-MCP, MA, 1-MCP+MA) and stored at low temperature. Fruit were removed from cool storage and ripened at room temperature, using ethephon as a ripening agent.

### 2.2. 1-MCP

In preliminary experiments, we fumigated green banana fruit, picked at the commercial harvest stage, with 1000, 1500 and 2000 nL L<sup>-1</sup> 1-MCP. Fruit were then stored at 14 °C. Storage life was not affected by the concentration used, but fruit treated with 1500 or 2000 nL L<sup>-1</sup> 1-MCP had lower eating quality than those treated with 1000 nL L<sup>-1</sup>. Eating quality was reduced by a relatively hard texture of the fruit center. We therefore used 1000 nL L<sup>-1</sup> 1-MCP in the present experiment. Replications containing four to six hands were treated with 1-MCP (EthylBloc®, USA) for 4 h at 25 °C, as described previously (Piriyaivinit et al., 2011; Phetsirikoon et al., 2012). Controls were not treated with 1-MCP.

### 2.3. Modified atmosphere

Replications containing 5–6 hands were randomly placed in corrugated cardboard boxes (40 cm × 48 cm × 22 cm) without plastic lining. These served as the MA control fruit. Other batches of 5–6 hands were randomly placed in non-perforated PE bags (75 cm wide × 110 cm × 0.11 mm) which were closed tight. Boxed bananas were stored at 14 °C and 85–95% RH.

### 2.4. Determination of ethylene, carbon dioxide and oxygen

Concentrations of ethylene, carbon dioxide and oxygen were determined in cardboard boxes and PE bags, using syringes to take samples. Air in the bags was sampled by piercing the syringe through the plastic film. The hole was then covered with tape. Samples were injected into a gas chromatograph equipped with a flame ionization detector (Shimadzu GC-14, Shimadzu, Tokyo, Japan) for ethylene or a thermal conductivity detector (Shimadzu GC-RIA, Shimadzu, Tokyo, Japan) for carbon dioxide and oxygen.

### 2.5. Quality assessments

At 10-day intervals, three boxes of each treatment were randomly sampled for determination of peel color, firmness, total soluble solids (TSS), titratable acidity (TA), storage life and eating quality. Peel color was determined with a color difference meter (Dr Lang Tricolor FLM 3, Berlin, Germany) to record *L*\*, *a*\* and *b*\* values of banana peel and pulp. Firmness was determined using an Effegi (Alfonsine, Italy) tester with a spherical 1.1 cm diameter plunger. The plunger was inserted to a depth of 5 mm in fruit, with and without peel. Total soluble solids (TSS) and titratable acidity (TA) of the pulp determined using a hand refractometer (Atago, Japan) and titration with 0.1 N NaOH, respectively.

To determine eating quality, stored bananas were ripened at 10 day intervals, by placing them in an aqueous 500 μL L<sup>-1</sup> ethephon solution at 25 °C for 48 h. Fruit were then transferred to ambient temperature (28–30 °C) for further ripening for 3 days. The peel then reached color index 6 on the CSIRO chart (Anon., 1972).

Eating quality of ripened bananas was assessed by a panel of 4–5 experienced panelists, using a scale of 1–5 whereby 1 = extremely low quality and 5 = extremely high quality. Data are means of three boxes in each test. The rate of peel yellowing was expressed in weeks to reach the breaker stage (color index no. 2 on the CSIRO chart).

Storage life depended on several criteria. Storage until a certain date was considered acceptable only if the peel did not contain clear blemishes at that time. Furthermore it was acceptable only if yellowing after ethylene treatment did not occur earlier than 3–4 days after the end of the ethylene treatment and not later than 5–6 days after the treatment. Finally, the storage life was only acceptable if the eating quality was adequate (more than 3.5 on average).

### 2.6. ACC concentration; activities of ACS and ACO

Ethylene is produced from methionine. The conversion of methionine to S-adenosyl methionine (SAM) is catalyzed by S-adenosyl methionine transferase. SAM is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase. ACC is converted to ethylene by ACC oxidase (Yang and Hoffman, 1984). ACC synthase and ACC oxidase are the enzymes controlling ethylene biosynthesis in plants.

The ACC concentration was measured using the method of Hoffman and Yang (1982), with slight modification. Frozen samples were homogenized in 9% trichloroacetic acid (TCA) (5 mL g<sup>-1</sup>) and incubated at 4 °C for 24 h. The extract was centrifuged at 13,870 × g for 40 min. The supernatant was adjusted to pH 7–8 with 1 N NaOH. The sample was placed in capped 12 mL vials which contained, apart from the sample solution (500 μL), 10 mM HgCl<sub>2</sub> (100 μL) and distilled water (300 μL). The internal standard solution contained sample (500 μL), 10 mM HgCl<sub>2</sub> (100 μL), 0.04 mM ACC (50 μL) and distilled water (250 μL). The ACC concentration was measured by gas chromatography of the ethylene produced after incubation for 3 min at 4 °C with 100 μL of saturated NaOH and 5.25% NaOCl.

ACS activity was also measured using the method of Hoffman and Yang (1982), with slight modification. Material was placed in homogenization buffer (1 mL g<sup>-1</sup> for fruit flesh and 2 mL g<sup>-1</sup> containing 100 mM N-(2-hydroxyethyl) piperazine-N'-3-propane-sulfonic acid (EPPS)), 0.5 μM pyridoxal phosphate and 4 mM dithiothreitol (DTT); pH 8.5 with KOH. The extract was centrifuged at 13,870 × g for 40 min. The supernatant was dialyzed overnight at 4 °C with dialysis buffer solution (pH 8.5) containing 2 mM EPPS, 0.2 μM pyridoxal phosphate, and 0.1 mM DTT. The supernatant volume was recorded volume. ACC synthase activity was determined in a reaction mixture containing 400 μL enzyme solution, 600 mM EPPS (50 μL) (pH 8.5), and distilled water (90 μL). After incubation for 3 h at 30 °C, 0.5 mM S-adenosyl methionine (SAM; 60 μL) was added to the solution. The samples were held in capped 12 mL vials containing 100 μL sample, 10 mM HgCl<sub>2</sub> (100 μL) and distilled water (200 μL). The internal standard contained sample (100 μL), 10 mM HgCl<sub>2</sub> (100 μL), 0.04 mM ACC (50 μL) and distilled water (150 μL). The ACC concentration was measured by gas chromatography, by determination of the ethylene produced after incubation for 3 min at 4 °C with 100 μL of saturated NaOH and 5.25% NaOCl. The protein concentration was determined using the Bradford method, using bovine serum albumin as a standard.

ACO activity was measured by modifying the method of Kato and Hyodo (1999). Samples were homogenized in a mortar with

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