

# Retention of green colour of tomatoes marketed as a green vegetable at ambient conditions in Cambodia with modified atmosphere storage and fumigation with 1-methylcyclopropene (1-MCP)

Sonnthida Sambath<sup>1,2</sup>, R.B.H. Wills<sup>1,a</sup>, V.V.V. Ku<sup>1</sup> and S. Newman<sup>3</sup>

<sup>1</sup> School of Environmental and Life Sciences, University of Newcastle, Ourimbah NSW 2258, Australia

<sup>2</sup> Cambodian Agriculture Research and Development Institute, Phnom Penh, Cambodia

<sup>3</sup> NSW Department of Primary Industries, Ourimbah NSW 2258, Australia

## Summary

**Introduction** – Unripe, green tomatoes are marketed in Cambodia but have a short market life due to rapid ripening under tropical temperatures and the unavailability of refrigerated technology. This paper examines the potential for storage in modified atmospheres (MA) and/or fumigation with 1-MCP to extend the green life of tomatoes held at ambient tropical conditions. **Materials and methods** – Laboratory studies were conducted in Australia at 25 °C with ‘Neang Pich’ tomatoes to determine the optimum conditions for fumigation with 1-MCP and controlled atmospheres (CA) to delay loss of green colour. A commercial polyethylene film bag was identified to generate the desired atmosphere and field trials with the bag were then conducted in Cambodia under ambient conditions (25 to 35 °C) to determine the extension in green life of ‘Mongal’ and ‘Plai Bi’ tomatoes. **Results and discussion** – Maximum retention of green life was achieved either by fumigation with 1 µL L<sup>-1</sup> 1-MCP for 1 h, or by storage in 5% oxygen and 10% carbon dioxide, but no cumulative benefit combining the two treatments was observed. MA storage was considered the most viable technology in Cambodia and field trials in Cambodia found the green life increased from the 2–3 days when held in air to 7–9 days in the MA bag. **Conclusions** – Use of MA with a plastic bag offers an easy to implement technology for Cambodian farmers to delay the loss of green life of tomatoes and thereby greatly increase marketing options.

## Keywords

Cambodia, tomato, *Lycopersicon esculentum*, green life, plastic film bags, tropical climate conditions

## Résumé

Préservation de la couleur verte des tomates commercialisées en tant que légume vert dans des conditions ambiantes au Cambodge grâce au stockage sous atmosphère modifiée et fumigation au 1-méthylcyclopropène (1-MCP).

## Significance of this study

*What is already known on this subject?*

- Unripe, green tomato is an important market segment in Cambodia.
- Affordable technology is lacking to maintain green fruit under ambient tropical temperatures.

*What are the new findings?*

- Storage in MA composed of 5% oxygen and 10% carbon dioxide, and fumigation with 1 µL L<sup>-1</sup> 1-MCP extended tomato green life.
- Modified atmosphere storage alone was able to maximise tomato green life.

*What is the expected impact on horticulture?*

- Generation of a MA with a plastic bag is an affordable technology for Cambodian farmers to increase their marketing options for green tomatoes.

**Introduction** – Les tomates vertes non mûres sont commercialisées au Cambodge, mais leur durée de vie est courte en raison de la maturation rapide sous les températures tropicales et en l’absence de chaîne du froid. Cet article examine le potentiel de stockage sous atmosphère modifiée (MA) et/ou de fumigation au 1-MCP pour prolonger la durée de vie en vert des tomates conservées dans des conditions tropicales ambiantes. **Matériel et méthodes** – Des études de laboratoire ont été menées en Australie à 25 °C avec les tomates ‘Neang Pich’ pour déterminer les conditions optimales de fumigation au 1-MCP et les atmosphères contrôlées (CA) aptes à retarder la perte de couleur verte. Un sac commercial en polyéthylène a été identifié pour générer l’atmosphère souhaitée et des essais sur le terrain avec ce sac ont ensuite été effectués au Cambodge en conditions ambiantes (25 à 35 °C) pour déterminer l’extension de vie en vert des tomates ‘Mongal’ et ‘Plai Bi’. **Résultats et discussion** – La préservation maximale de vie en vert a été obtenue soit par fumigation avec 1 µL L<sup>-1</sup> 1-MCP pendant 1 h, soit par un stockage sous 5% d’oxygène et 10% de dioxyde de carbone, mais aucun avantage cumulatif combinant les deux traitements n’a été obtenu.

<sup>a</sup> Corresponding author: [ron.wills@newcastle.edu.au](mailto:ron.wills@newcastle.edu.au).

**Le stockage sous MA a été considéré comme la technologie la plus adaptée au Cambodge et les essais de terrain ont montré que la durée de vie verte passait de 2 à 3 jours dans le sac à MA. Conclusion – L'utilisation de MA avec un sac en plastique offre aux agriculteurs cambodgiens une technologie facile à mettre en œuvre pour retarder la sortie de vie en vert des tomates et augmenter ainsi considérablement les possibilités de commercialisation.**

#### Mots-clés

Cambodge, tomate, *Lycopersicon esculentum*, vie en vert, film plastique, conditions climatiques tropicales

## Introduction

Tomato (*Lycopersicon esculentum*) is a climacteric fruit that undergoes a marked ripening process that is initiated by ethylene. The most obvious change during ripening is the loss of green colour due to destruction of chlorophyll and development of a red colour due to synthesis of carotenoids such as lycopene (Wills and Golding, 2016). In most countries, tomatoes are consumed when ripe but in Cambodia there is a substantial local market for unripe green tomatoes that are consumed as a vegetable in salads, stir fries and soups. While the green tomatoes are generally marketed immediately after harvest, there is considerable price fluctuation due to varying volumes of supply, that ranges from 800 to 2,600 Riel kg<sup>-1</sup> (Sokhen *et al.*, 2004; MAFF, 2017). The ability to withhold produce from the market for up to a week while maintaining the green status of tomatoes would be of considerable value to farmers. Refrigerated storage would delay ripening but the technology is either not available or is too expensive to utilise. Hence, a simple, low cost technology that can be effective at the ambient tropical temperatures of 25 to 35 °C which prevail in Cambodia (Genova *et al.*, 2006; Hickey, 2010) would be beneficial. We considered that fumigation with 1-methylcyclopropene (1-MCP) and storage in a modified atmosphere (MA) had the potential to extend the green life of tomatoes stored at tropical ambient temperatures.

Fumigation with 1-MCP delays the time to ripen of a wide range of climacteric fruit by inhibiting the action of ethylene (Watkins, 2015). The initial report of 1-MCP delaying ripening of tomatoes was by Wills and Ku (2002) and the effect has been confirmed in numerous subsequent papers. Most studies with tomatoes have been at ambient temperate or low storage temperatures of ≤20 °C but Paul *et al.* (2010) did report 1-MCP increased time to ripen of eight Indian cultivars stored at 25 and 30 °C and Mostofi *et al.* (2003) showed a similar effect with 'Rapsodie' tomatoes held at 25 °C. However, time to ripen is different to the time fruit remain green due to time that fruit are at the breaker, turning and pink colour stages before becoming ripe (USDA, 2013). No studies have reported on the time that fruit remained green at any storage temperature.

The use of modified atmospheres, where the carbon dioxide level is increased and the oxygen level is reduced, was established by Kidd and West in the U.K. in the 1920s and is now known to inhibit ripening of many fruits (Wills and Golding, 2016). Modified atmosphere storage was recognised as a low cost technology when Scott and Roberts (1966) demonstrated that enclosing bananas in a polyethylene film bag self-generated a MA that delayed ripening. However, there

has been little attention given to the effect of MA on the ripening of green tomatoes at tropical temperatures.

The combined treatment of produce with 1-MCP and a MA was reported recently by Zewter *et al.* (2012) who found delayed ripening of bananas and Sabir (2012), who showed greater delay in loss of green colour of broccoli stored at 0 °C than for produce with a single treatment. This study examined the ability of fumigation with 1-MCP, storage in a controlled atmosphere of carbon dioxide and oxygen (CA), or as a combination 1-MCP/CA treatment to extend the time tomatoes remained green (designated at the green life) in laboratory trials in Australia at 25 °C. Trials were also conducted in Australia at 25 °C on a range of commercially available plastic film bags to select a bag that generated the desired atmosphere and the ability of this bag was evaluated in field trials conducted at ambient tropical conditions in Cambodia.

## Materials and methods

### Tomatoes

Experiments in the Australian laboratory were conducted with 'Neang Pich' (or 'CLN2498A') tomatoes. This cultivar is a heat tolerant variety developed by the World Vegetable Center (formerly AVRDC) and released for use in Cambodia by the Cambodian Agricultural Research and Development Institute (CARDI, 2007). Tomatoes for all experiments in Australia were grown in a greenhouse at the New South Wales Department of Primary Industries, Ourimbah, NSW. Mature green tomatoes were harvested at 8 to 11 am and immediately transferred to the laboratory. Fruit without defects and of similar size and skin colour were selected for experiments. For controlled each batch of tomatoes, the fruit were randomly distributed into the required number of treatment units with five fruit per treatment.

Field trials in Cambodia were conducted with current commercial cultivars of 'Mongal' and 'Plai Bi' tomatoes grown in the Kean Svay district, Kandal province. Fruit was sourced from the local commercial market or harvested directly from farms. For farm-sourced fruit, mature green tomatoes were harvested between 7 am and 10 am from commercial farms. Good quality green tomatoes were then selected from the harvested fruit in a shady area on the farm and transported to the nearby Kbal Koh Vegetable Research Station (about 15 min by road). For each batch of tomatoes, the fruit were randomly distributed into the required number of treatment units with 10 kg fruits per treatment.

### 1-MCP fumigation

Experiments were designed as a factorial randomised completed block with four batches of five fruit for each treatment. SmartFresh™ powder (0.14% active ingredient) (AgroFresh, Australia) was used to release 1-MCP gas for fumigation. Produce was placed in an air-tight plastic container (10 L) fitted with a fan for gas circulation. The desired weight of SmartFresh™ was placed into a glass bottle (125 mL), distilled water (2 mL) was added into the bottle and the lid immediately closed. The bottle was shaken for 2 min to release the 1-MCP and then placed into the plastic container. The bottle lid was opened and the container immediately sealed for 1–4 h. After fumigation, the drums were flushed with humidified air containing 0.1 μL L<sup>-1</sup> ethylene at 500 mL min<sup>-1</sup> (Li *et al.*, 2017) to simulate a commercial environment for ethylene (Wills *et al.*, 2000) and to avoid accumulation of carbon dioxide in the drum.

### Storage temperature

Laboratory experiments in Australia were conducted at 25 °C in a temperature controlled room while experiments conducted in Cambodia were at ambient conditions. Temperature-humidity data loggers, Tinytag View 2, (Hasting Data Logger, Port Macquarie, Australia), were placed in the Australian storage room while in Cambodian field trials, HOBO Onset UX 100-003, (Massachusetts) loggers were placed in packages of produce.

### Storage in modified atmosphere

Controlled atmosphere laboratory trials were conducted in Australia to determine desirable concentrations of oxygen and carbon dioxide for the storage of green tomatoes. This was followed by trials with polyethylene bags that might generate a MA in the range of the selected gas composition. For controlled atmosphere trials, green tomatoes were placed into a sealed drum (40 L) and a range of gas mixtures were passed through the drums at 85 mL min<sup>-1</sup>. The gas mixtures were obtained by blending compressed air, carbon dioxide gas from cylinder (BOC, Gosford, Australia) and nitrogen gas generated from a nitrogen generator to obtain the required atmosphere. All gas mixtures were bubbled through water to raise the humidity to about 95%. Controlled atmosphere experiments were a factorial randomised complete block design with four batches of fruit each with five tomatoes treated independently as a treatment unit of each batch. For the MA trials in Australia, seven types of polyethylene bag that are currently used for commercial fruit and vegetable storage, albeit not at the elevated temperatures used in this study, were supplied by Amcor, Australia. Green tomatoes (2 kg or 10 kg) were sealed into a bag and the oxygen and carbon dioxide concentrations generated in the packages during storage at 25 °C were determined with an oxygen and carbon dioxide analyser (Novatech 1737, Australia). A gas sample was obtained automatically when the needle of the gas analyser was inserted into the plastic bag and the concentration of gas was recorded.

MA trials in Cambodia used the optimum plastic bag determined from the Australian studies and the plastic bag (30 × 55 cm bag with 18 × 8 mm holes) that Cambodian farmers currently use to pack tomatoes (designated as Bag CB). It was expected that Bag CB would not generate a MA due to the substantial number of large holes in the film and the bags being unsealed. A treatment unit was 10 kg green tomatoes which were placed into a plastic bag which was held in the fibreboard package that is currently used in Cambodia and stored at ambient conditions. The atmosphere that was generated into the sealed plastic bags during storage was determined by the Novatech gas analyser.

### Skin colour assessment

The skin colour of tomatoes was assessed subjectively based on the USDA tomato colour chart (USDA, 2013), where the colour was ranked from score 1 to 6, with 1 = green, 2 = breaker (≤10% orange), 3 = turning, 4 = pink, 5 = light red and 6 = red. At the commencement of each experiment, all tomatoes were green with a score of 1.0. The green life of each treatment unit was designated as the average time from harvest for each fruit in a unit to reach breaker stage (score 2.0).

### Statistical analysis

Statistical procedures were performed using GenStat 16<sup>th</sup> edition. Factorial analysis of variance of colour score and green life was conducted to assess the treatment effect. The

least significant difference (LSD) at  $P=0.05$  for means were calculated to determine significant differences between individual treatments. To analyse the proportion of green tomatoes in the Cambodian study, the data was transformed to a general linear mixed model with a binomial distribution but the data was adjusted so that 100% green fruit was changed to 99.9% and 0% green fruit to 0.1% to avoid  $\pm \infty$  which cannot be modelled.

## Results and discussion

### Fumigation with 1-MCP

Mature green 'Neang Pich' tomatoes were fumigated with 0, 1, 5 and 10  $\mu\text{L L}^{-1}$  1-MCP for one and four hours at 25 °C followed by storage in humidified air containing 0.1  $\mu\text{L L}^{-1}$  ethylene. The fruit were assessed for green life (time to reach breaker stage, colour score 2). The 1-MCP treatment significantly ( $P<0.001$ ) extended the green life over untreated produce by about four days, although there was no significant difference between the three 1-MCP concentrations (Table 1). There was no significant effect of fumigation time hence only the mean values for each 1-MCP concentration are presented. The 1-MCP extended green life by about four days.

### Storage in reduced oxygen

Green 'Neang Pich' tomatoes were stored at 25 °C in 2, 5, 10 and 21% oxygen. Fruit were removed from the controlled atmospheres after 2, 3, and 5 days and scored for skin colour. There was a significant effect ( $P<0.001$ ) of oxygen concentration on fruit colour with a greater delay in loss of green colour increasing as the oxygen concentration was reduced (Table 2). The green life was 2–3 days for tomatoes held in 21% oxygen (ambient air) and about five days in 10% oxygen. Tomatoes held in 5% and 2% oxygen showed no significant change in colour over the five days. However, some fruit stored in 2% oxygen developed typical anaerobic injury with the appearance of translucent watery areas which often showed fungal decay. The use of 5% oxygen thus seemed to be an acceptable concentration to maximise green life without injury.

### Combined 1-MCP fumigation and storage in low oxygen

Green 'Neang Pich' tomatoes were fumigated with 1-MCP at 1  $\mu\text{L L}^{-1}$  for one hour and then held in 5, 10 and 21% oxygen for five days. Fruit were scored for skin colour on removal from the modified atmosphere then stored in air and the

**TABLE 1.** Effect of 1-MCP fumigation on green life of 'Neang Pich' tomatoes during subsequent storage at 25 °C in air containing 0.1  $\mu\text{L L}^{-1}$  ethylene. Each value is the mean of 80 fruits (5 fruits × 4 replicates × 2 batches of produce × 2 fumigation times).

1-MCP concentrations ( $\mu\text{L L}^{-1}$ )	Green life <sup>y</sup> (days)
0 (control)	7.0 <sup>a</sup>
1	11.7 <sup>b</sup>
5	11.4 <sup>b</sup>
10	11.2 <sup>b</sup>
LSD <sup>z</sup>	0.95

<sup>y</sup> Values with the same superscript letter are not significantly different at  $P=0.05$ .

<sup>z</sup> Least significant difference between means at  $P=0.05$ .

**TABLE 2.** Skin colour score (1–6 scale) of ‘Neang Pich’ tomatoes during storage in low oxygen at 25 °C. Each value is the mean of 80 fruits (5 fruits × 4 replicates × 4 batches of produce).

% Oxygen	Skin colour scores <sup>y</sup> after		
	2 days	3 days	5 days
21	1.8 <sup>bc</sup>	2.3 <sup>c</sup>	4.4 <sup>d</sup>
10	1.4 <sup>ab</sup>	1.4 <sup>ab</sup>	2.2 <sup>c</sup>
5	1.4 <sup>ab</sup>	1.3 <sup>ab</sup>	1.3 <sup>ab</sup>
2	1.4 <sup>ab</sup>	1.4 <sup>ab</sup>	1.0 <sup>a</sup>
<i>LSD</i> <sup>z</sup>	0.59		

<sup>y</sup> Values with the same superscript letter are not significantly different at  $P=0.05$ .

<sup>z</sup> Least significant difference between means at  $P=0.05$ .

colour score monitored daily to determine the green life. The control (no 1-MCP and stored in air) tomatoes significantly ( $P<0.001$ ) changed colour over the initial five days, whereas fruit in all other treatments had not significantly changed from the initial colour score of 1.0 after five days (Table 3).

During subsequent storage in air, prior storage in 5 and 10% oxygen significantly ( $P<0.001$ ) extended green life over the air control by about two days. Fumigation with 1-MCP significantly extended green life by about five days compared to untreated tomatoes but there was no significant difference between oxygen concentrations.

**Combined 1-MCP fumigation and storage in elevated carbon dioxide**

Green ‘Neang Pich’ tomatoes were fumigated with 1 μL L<sup>-1</sup> 1-MCP for one hour at 25 °C and held in air and 2, 5 and 10% carbon dioxide for five days. Fruit were assessed for skin colour after five days and then stored in air and the colour score monitored daily to determine the green life. After five days, control fruit stored in air and 2% and 5% carbon dioxide showed a significant ( $P<0.001$ ) loss of green colour but all fruit fumigated with 1-MCP and control fruit stored in 10% carbon dioxide retained the original green colour (Table 4). During subsequent storage in ambient air there was a significant ( $P<0.001$ ) difference in green life between treatments (Table 4). For non-fumigated fruit, green life increased as the percentage of carbon dioxide increased. All fruit fumigated with 1-MCP had even longer green life by about five days compared to control fruit but there was only a small but significant difference in green life between carbon oxide concentrations.

**Combined 1-MCP fumigation and storage in low oxygen and high carbon dioxide**

The colour of green ‘Neang Pich’ tomatoes was examined using the previously determined treatments of fumigation with 1 μL L<sup>-1</sup> 1-MCP for one hour followed by storage in 5% oxygen with 10% carbon dioxide. Tomatoes were assessed for colour score at 3-day intervals up to 15 days in the controlled atmospheres and then stored in air when green life

**TABLE 3.** Colour score of ‘Neang Pich’ tomatoes fumigated with 1-MCP then stored in a reduced oxygen atmosphere at 25 °C for five days and green life during subsequent storage in air. Each value is the mean of 80 fruits (5 fruits × 4 replicates × 4 batches of produce).

Treatments		% Oxygen	Colour scores <sup>y</sup> on removal from controlled atmosphere	Green life (days)
Fumigation				
Control		21	1.7 <sup>c</sup>	9.1 <sup>a</sup>
		10	1.0 <sup>a</sup>	11.2 <sup>b</sup>
		5	1.0 <sup>a</sup>	10.8 <sup>b</sup>
1-MCP		21	1.0 <sup>a</sup>	14.3 <sup>c</sup>
		10	1.0 <sup>a</sup>	14.2 <sup>c</sup>
		5	1.0 <sup>a</sup>	14.2 <sup>c</sup>
<i>LSD</i> <sup>z</sup>			0.23	1.52

<sup>y</sup> Values in the same column with the same superscript letter are not significantly different at  $P=0.05$ .

<sup>z</sup> Least significant difference between means at  $P=0.05$ .

**TABLE 4.** Colour score of ‘Neang Pich’ tomatoes fumigated with 1-MCP then stored in an elevated carbon dioxide atmosphere at 25 °C for five days and green life during subsequent storage in air.

Treatments		% CO <sub>2</sub>	Colour scores <sup>y</sup> on removal from controlled atmosphere	Green life (days)
Fumigation				
Control		0	1.8 <sup>c</sup>	5.8 <sup>a</sup>
		2	1.6 <sup>b</sup>	6.4 <sup>b</sup>
		5	1.6 <sup>b</sup>	6.9 <sup>b</sup>
		10	1.1 <sup>a</sup>	7.5 <sup>c</sup>
1-MCP		0	1.1 <sup>a</sup>	11.3 <sup>d</sup>
		2	1.0 <sup>a</sup>	11.9 <sup>ef</sup>
		5	1.1 <sup>a</sup>	11.5 <sup>de</sup>
		10	1.0 <sup>a</sup>	12.3 <sup>f</sup>
<i>LSD</i> <sup>z</sup>			0.15	0.53

<sup>z</sup> Least significant difference between means at  $P=0.05$ .

<sup>y</sup> Values in the same column with the same superscript letter are not significantly different at  $P=0.05$ .



**TABLE 5.** Colour score of ‘Neang Pich’ tomatoes fumigated with 1-MCP then stored in modified atmosphere (MA = 5% oxygen and 10% carbon dioxide) at 25 °C and green life during subsequent storage in air. Values are the means of 80 fruits (5 fruits × 4 replicates × 4 batches of produce).

Treatments	Number of days of treatment				
	3	6	9	12	15
<i>Colour scores<sup>y</sup> on removal from controlled atmosphere</i>					
Control (air)	1.5 <sup>bc</sup>	3.1 <sup>d</sup>	4.2 <sup>e</sup>	5.0 <sup>f</sup>	5.7 <sup>g</sup>
MA	1.0 <sup>a</sup>	1.1 <sup>ab</sup>	1.0 <sup>a</sup>	1.0 <sup>a</sup>	1.1 <sup>ab</sup>
1-MCP + Air	1.1 <sup>ab</sup>	1.0 <sup>a</sup>	1.5 <sup>bc</sup>	1.9 <sup>c</sup>	3.5 <sup>d</sup>
1-MCP + MA	1.0 <sup>a</sup>	1.0 <sup>a</sup>	1.0 <sup>a</sup>	1.0 <sup>a</sup>	1.0 <sup>a</sup>
<i>LSD<sup>y</sup></i>	<i>0.50</i>				
<i>Green life<sup>y</sup> (days)</i>					
Control (air)	6.0 <sup>a</sup>	– <sup>x</sup>	–	–	–
MA	7.6 <sup>b</sup>	9.7 <sup>c</sup>	11.9 <sup>de</sup>	14.3 <sup>g</sup>	17.2 <sup>i</sup>
1-MCP + Air	11.5 <sup>d</sup>	11.2 <sup>d</sup>	11.5 <sup>d</sup>	13.5 <sup>fg</sup>	–
1-MCP + MA	10.8 <sup>cd</sup>	12.9 <sup>ef</sup>	13.7 <sup>fg</sup>	15.9 <sup>h</sup>	17.8 <sup>i</sup>
<i>LSD<sup>z</sup></i>	<i>1.20</i>				

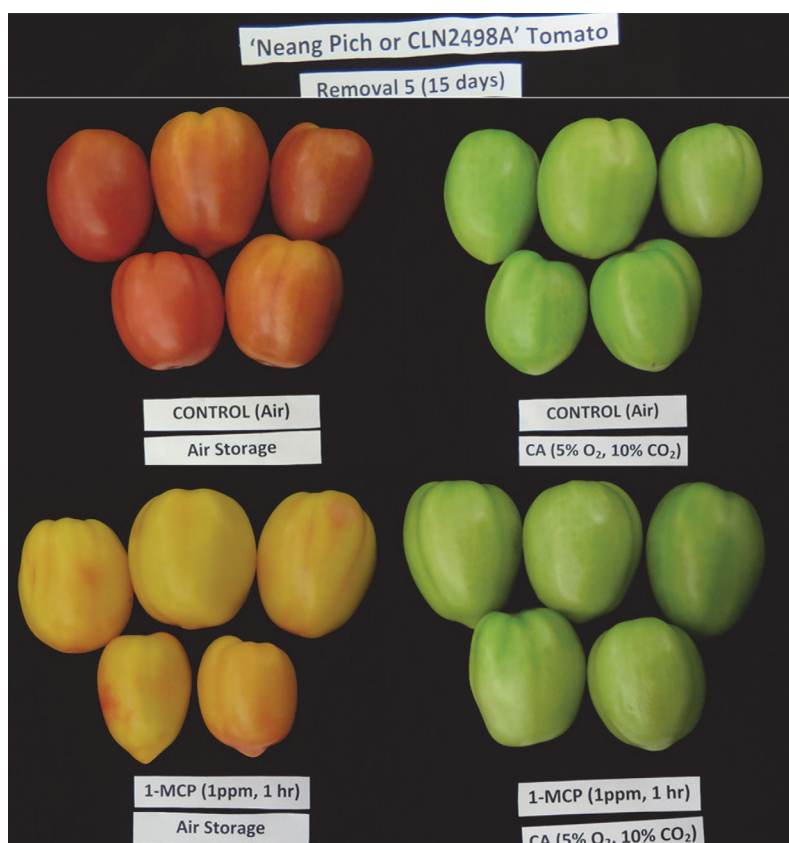
<sup>z</sup> Least significant difference between means at  $P=0.05$ .

<sup>y</sup> Values with the same superscript letter are not significantly different at  $P=0.05$ .

<sup>x</sup> Indicates that fruit had attained its green life before the removal day.

was determined. There was a significant ( $P<0.001$ ) effect of 1-MCP and storage atmosphere on the colour of tomatoes during the 15 days when fruit were held in the controlled atmosphere (Table 5). The control (air stored) tomatoes showed loss of green colour (score  $\geq 2.0$ ) after six days while fruit fumigated with 1-MCP and stored in air had lost green colour after about 12 days. Tomatoes stored in the controlled atmosphere with or without fumigation with 1-MCP still retained a green colour after 15 days. The differential effect on fruit colour of the various treatments at 15 days can be seen in Figure 1.

All fruits held in the controlled atmosphere lost their green colour during subsequent storage in air but the green life was significantly different ( $P<0.001$ ) between treatments (Table 5). Tomatoes not fumigated with 1-MCP lost green colour at 2–3 days after being transferred to storage in air, regardless of the time the fruit had been held in the controlled atmosphere. This contrasted with 1-MCP-fumigated tomatoes where fruit stored in the controlled atmosphere for three days had a longer green life by about three days over non-fumigated fruit but the difference in green life between the fumigated and non-fumigated fruit decreased the longer



**FIGURE 1.** Difference in skin colour of ‘Neang Pich’ tomatoes treated with the various combinations of 1-MCP fumigation and storage in 5% oxygen and 10% carbon dioxide at 25 °C for 15 days.

tomatoes were held in the controlled atmosphere and after 15 days there was no significant difference in green life.

**Summary of controlled atmosphere and 1-MCP trials**

Maximum green life at 25 °C was achieved by holding ‘Neang Pich’ tomatoes in 5% oxygen and 10% carbon dioxide with a 2–3 day life as a green tomato for subsequent marketing in air. A green life of 17 days was achieved when fruit were held in the atmosphere for 15 days compared to a six-day green life for tomatoes stored in air. This atmosphere differs from the recommended commercial atmosphere of 5% oxygen and 3% carbon dioxide for tomatoes stored at 15 to 20 °C (Sargent and Moretti, 2016) and undoubtedly reflects differing ripening triggers at the higher temperature. However, the atmosphere is not dissimilar to the 5% oxygen and 8% carbon dioxide achieved by Mathooko and Nabawanuka (2003) for ‘Cal-J’ tomatoes held in a polyethylene film bag at 26 °C, where the green skin colour was retained for 12 days.

The use of 1-MCP with or without the controlled atmosphere resulted in a stronger initial retention of green life but its effect declined over time so that by 15 days it offered no additional inhibition to loss of green colour over the controlled atmosphere. If a short term delay in marketing was required the simplest treatment was fumigation with 1-MCP and storage in air while for maximum retention of green life use of the controlled atmosphere alone was sufficient. The dual use of 1-MCP plus the controlled atmosphere did not have any added benefit for either short or long term retention of green life.

**Selection of modified atmosphere package (MAP)**

Experiments were conducted in the Australian laboratory at 25 °C on seven commercially available film bags (Amcor, Australia) that are used for other horticultural produce. This was to identify a bag that allowed generation of an atmosphere approximating the desirable composition for green ‘Neang Pich’ tomatoes of 5% oxygen and 10% carbon dioxide. From an initial short-term trial with 2 kg green tomatoes,

the selected bag (Amcor bag L056, designated as Bag A) generated an atmosphere of about 10% oxygen and 5% carbon dioxide. A longer duration trial over 14 days with a larger Bag A containing 10 kg tomatoes generated an atmosphere of about 5% oxygen and 6% carbon dioxide after a few days in the bag. Bag A is marketed in Australia for the storage of leek at 0–2 °C and has gas transmission rates of about 7 L oxygen bag<sup>-1</sup> day<sup>-1</sup> and 12 L CO<sub>2</sub> bag<sup>-1</sup> day<sup>-1</sup> (Kumar Sellakanthan, Amcor, pers. commun.). Thus, Bag A showed potential to generate the desired atmosphere in field trials to be undertaken in Cambodia under the prevailing ambient conditions.

**MAP evaluation in ambient Cambodian conditions**

Bag A was evaluated against the polyethylene film bag that Cambodian farmers currently use to pack tomatoes (designated as Bag CB). Mature green ‘Mongal’ and ‘Plai Bi’ tomatoes purchased from the Kean Svay market were enclosed in each of two bags of Bag A (10 kg in each bag) and held for 5 days at ambient conditions in an open shed in which conditions during the trial ranged from 27 to 30 °C and 65 to 75% relative humidity (RH). The atmosphere in Bag A was too extreme with the oxygen concentration during storage period of about 1% but carbon dioxide increased to 18% after two days and then declined to about 11% at five days. Condensation appeared in Bag A after one day but gradually decreased over the storage period with no rotting occurring. There was no condensation or rotting in Bag CB. In order to generate an atmosphere better aligned with the desired 5% oxygen and 10% carbon dioxide, an experiment was conducted with 16 needle-size holes in Bag A. The atmosphere generated in Bag A over the five days was 2 to 4% oxygen and 6 to 9%, carbon dioxide which was close to the desired MA.

The ability of the MA generated in a sealed Bag A with 16 needle-size holes to inhibit ripening of mature green tomatoes (10 kg) was examined in comparison to the current practice of holding tomatoes in an unsealed Bag CB. ‘Mongal’ and ‘Plai Bi’ tomatoes were harvested from three and two farms, respectively, with nine bags for each treatment from

**TABLE 6.** Colour of ‘Mongal’ and ‘Plai Bi’ green tomatoes held in a modified atmosphere (Bag A) and air (Bag CB) during storage at ambient temperature in Cambodia. Each value for a farm is the mean of 10 kg fruit from 3 bags. MA: Modified atmosphere (5% oxygen and 10% carbon dioxide).

Treatments	Cultivar per farm	Skin colour scores <sup>y</sup> after			
		0 days	5 days	10 days	15 days
Air (Bag CB)	Mongal /1	1.0	3.5	5.1	4.7
	Mongal /2		1.4	3.4	5.4
	Mongal /3		3.2	4.7	4.6
	Plai Bi /1		3.5	4.9	5.3
	Plai Bi /2		3.0	4.4	5.1
	Mean			2.9 <sup>c</sup>	4.5 <sup>d</sup>
MA (Bag A)	Mongal /1		1.2	3.1	2.4
	Mongal /2		1.0	1.1	1.1
	Mongal /3		1.0	1.3	1.0
	Plai Bi /1		1.1	1.9	2.9
	Plai Bi /2		1.0	1.7	2.3
	Mean			1.1 <sup>a</sup>	1.8 <sup>b</sup>
LSD <sup>z</sup>				0.57	

<sup>z</sup> Least significant difference between means at *P*=0.05.

<sup>y</sup> Mean values with the same superscript letter are not significantly different at *P*=0.05.

each site which allowed for quality assessment of three bags after 5, 10 and 15 days storage. There were also five additional bags of Bag A for each farm for daily measurement of oxygen and carbon dioxide concentration. The mean oxygen concentration in the sealed bags during the first four days was about 3% but over the following 11 days it increased to about 6%. A similar trend was found for carbon dioxide with about 8% in the first six days and then increased to about 15%. Throughout the storage period, the daily temperature ranged from about 27 to 37 °C and the relative humidity from 50 to 90%. The fluctuation in temperature could have led to condensation in Bag A while there was no condensation in Bag CB.

There was a significant ( $P < 0.001$ ) effect of the MA on the external skin colour of tomatoes (Table 6). Tomatoes held in Bag CB (air) showed loss of green colour by five days and reached the red ripening colour after 10 days. Fruit in the MA retained the original green colour after five days and after 15 days the mean colour score was still  $< 2.0$ , which is considered acceptable in Cambodia for marketing as green tomatoes. However, of more importance to Cambodian farmers and traders is the proportion of green tomatoes remaining in the sample. The derived equations from the logit transformation of the percent of green fruits in a sample for the two bag types were significantly different ( $P < 0.001$ ) with:

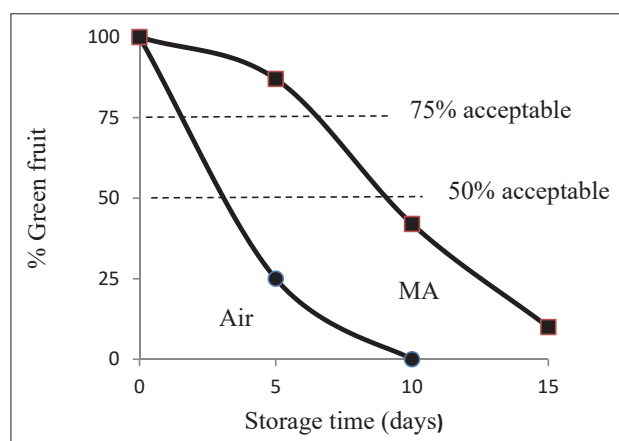
the green life of Bag CB (days) =  $\log \left[ \frac{p}{1-p} \right] - 3.725 / (-0.802)$ ; and

the green life of Bag A (days) =  $\log \left[ \frac{p}{1-p} \right] - 3.725 / (-0.403)$

where  $p$  is the percentage of green fruits that is acceptable for marketing. On the assumption that 75% green tomatoes in a bag would be a satisfactory outcome, tomatoes held in Bag A had a green life of about seven days compared to about two days in Bag CB. Rotting developed on some fruit but this only occurred on fruit that had started to ripen (colour score  $> 2.0$ ). If 50% green tomatoes was an acceptable outcome then the green life in Bag A was about nine days compared to about three days in Bag CB. These effects are illustrated in Figure 2.

Overall, the trials in Cambodia under ambient conditions with the selected plastic film bag generated a green life of about seven days compared to about two days for tomatoes held in the unsealed plastic bag used by Cambodian farmers. The MA generated in the bag varied, being 2–7% oxygen and 7–13% carbon dioxide. The variation was considered due to ambient temperature daily fluctuation of  $\sim 10$  °C (26–36 °C) and position of the bag in the stack of packages. Notwithstanding, the MA range generated in Bag A was sufficient to substantially extend the green life of tomatoes.

The extension in green life of about five days at 30 °C in Cambodia was shorter than the 11 days obtained from laboratory studies in Australia at a constant 25 °C. A previous study under Cambodian ambient conditions with tomatoes stored in a chamber fitted with an evaporative cooler showed a reduced temperature of about 1 °C and 10 °C during the night and day hours, respectively, so the temperature was about 25 °C at all times (Vanndy *et al.*, 2008). Use of an evaporative cooler where fruit are held at about 25 °C could be a feasible lower cost temperature management system than conventional refrigeration to extend the green life of tomatoes.



**FIGURE 2.** Proportion of green tomatoes (colour score  $\leq 2.0$ ) in a modified atmosphere (MA) (Bag A) and air (Bag CB) during storage at ambient temperature in Cambodia. Fruit held in MA (■) and air (●). Each value is the mean of 10 kg fruits from 15 bags (5 farms  $\times$  3 bags per farm).

Nonetheless, the additional five days of green life, based on retention of 75% green tomatoes in a bag met farmer satisfaction, is considered worthy of widespread evaluation with tomatoes in Cambodia and other countries with similar climate and stage of development. The market price on green tomatoes in Cambodia fluctuates greatly during the peak season even within a day (Sokhen *et al.*, 2004; Genova *et al.*, 2006) and the 7-day green life would seem to be sufficient to allow farmers to withhold tomatoes from the market until prices become more acceptable. The use of a plastic bag to generate the MA was considered to be a feasible option in rural Cambodia where small-scale farmers are familiar with using plastic bags as a packaging material and it would be relatively simple process to change to a different bag and to seal bags for generation of a MA.

The use of a plastic bag to generate a MA was evaluated in Cambodia even though fumigation with 1-MCP was found to extend the green life of tomatoes to a similar extent but it was considered that small farmers would find it more difficult logistically to use 1-MCP. However, it is recognised that larger commercial farms in Cambodia and other developing countries may find it more convenient to use 1-MCP rather than MAP.

### Acknowledgments

SS wishes to thank the Australian Centre for International Agricultural Research (ACIAR) for the scholarship that enabled her to undertake postgraduate studies. The authors thank Dr. Matthew Adkins and Dr. Hannah James at Agro-Fresh, Australia for supplying the SmartFresh™, Mr. Kumar Sellakanthan at Amcor Australia for supplying plastic film bags for assessment and assistance in bag selection through sharing technical information on the bags, Mrs. Lorraine Spohr at NSW Department of Primary Industries and Dr. Paul Rippon at the University of Newcastle for consulting on experimental design and data analysis, and Dr. Peter Hanson at AVRDC for supplying ‘Neang Pech’ seeds used for the Australian studies.

## References

- CARDI (2007). Tomato variety Neang Pech. Cambodian Agricultural Research and Development Institute. [www.cardi.org.kh/?page=document&&cateid=71&lg=en](http://www.cardi.org.kh/?page=document&&cateid=71&lg=en).
- Genova, C., Weinberger, K., Sokhom, S., Vanndy, M., and Yarath, C.E. (2006). Postharvest loss in the supply chain for vegetables: The case of tomato, yardlong bean, cucumber and Chinese kale in Cambodia. AVRDC Working Paper No. 16 (Shanhua, Taiwan: World Vegetable Center).
- Hickey, M. (2010). Improvement of vegetable production and postharvest practice in Cambodia and Australia. Final report for project HORT/2003/045 (Canberra: ACIAR). <http://aciar.gov.au/publication/fr2010-07>.
- Li, Y., Wills, R.B.H., and Golding, J.B. (2017). Storage at elevated ambient temperature and reduced ethylene delays degreening of Persian limes. *Fruits* 72, 288–291. <https://doi.org/10.17660/th2017/72.5.4>
- MAFF (2017). Daily Price of Agricultural Products. (Phnom Penh, Cambodia: Ministry of Agriculture, Food and Fisheries). <http://www.maff.gov.kh/>.
- Mathooko, F.M., and Nabawanuka, J. (2003). Effect of film thickness on postharvest ripening and quality characteristics of tomato (*Lycopersicon esculentum* L.) fruit under modified atmosphere packaging. *J. Agric. Sci. Technol.* 5, 39–60.
- Mostofi, Y., Toivonen, P.M.A., Lessani, H., Babalar, M., and Lu, C. (2003). Effects of 1-methylcyclopropene on ripening of greenhouse tomatoes at three storage temperatures. *Postharv. Biol. Technol.* 27, 285–292. [https://doi.org/10.1016/S0925-5214\(02\)00113-8](https://doi.org/10.1016/S0925-5214(02)00113-8).
- Paul, V., Pandey, R., and Srivastava, G.C. (2010). Ripening of tomato (*Solanum lycopersicum* L.). Part I: 1-methylcyclopropene mediated delay at higher storage temperature. *J. Food Sci. Technol.* 47, 519–526. <https://doi.org/10.1007/s13197-010-0090-5>.
- Sabir, F.K. (2012). Postharvest quality response of broccoli florets to combined application of 1-methylcyclopropene and modified atmosphere packaging. *Agric. Food Sci.* 21, 421–429. <https://doi.org/10.23986/afsci.6387>.
- Sargent, A.A., and Moretti, C.L. (2016). Tomato. In *The commercial storage of fruits, vegetables, and florist and nursery stocks*. Agriculture Handbook Nr. 66, K.C. Gross, C.Y. Wang, and M. Saltveit, eds. (Beltsville, MD: USDA-ARS).
- Scott, K.J., and Roberts, E.A. (1966). Polyethylene bags to delay ripening of bananas during storage. *Aust. J. Expt. Agric. Anim. Husb.* 33, 1029–1036.
- Sokhen, C., Kanika, D., and Moustier, P. (2004). Vegetable market flows and chains in Phnom Penh. (Montpellier: CIRAD). <http://agritrop.cirad.fr/544875/>.
- USDA (1975). Color classification requirements in tomatoes. USDA Visual aid TM-L-1. [http://ucanr.edu/sites/Postharvest\\_Technology\\_Center\\_/files/223147.jpg](http://ucanr.edu/sites/Postharvest_Technology_Center_/files/223147.jpg).
- Vanndy, M., Buntong, B., Chanthasombath, T., Sanatem, K., Acedo, A., and Weinberger, K. (2008). Evaporative cooling storage of tomato in Cambodia and Laos. *Acta Hort.* 804, 565–570. <https://doi.org/10.17660/ActaHortic.2008.804.83>.
- Watkins, C.B. (2015). Advances in the use of 1-MCP. In *Advances in Postharvest Fruit and Vegetable Technology*, R.B.H. Wills, and J.B. Golding, eds. (Boca Raton, FL: CRC Press), pp. 117–145. <https://doi.org/10.1201/b18489-7>.
- Wills, R.B.H., and Ku, V.V.V. (2002). Use of 1-MCP to extend the time to ripen of green tomatoes and postharvest life of ripe tomatoes. *Postharv. Biol. Technol.* 26, 85–90. [https://doi.org/10.1016/S0925-5214\(01\)00201-0](https://doi.org/10.1016/S0925-5214(01)00201-0).
- Wills, R.B.H., and Golding, J.B. (2016). *Postharvest: an Introduction to the Physiology and Handling of Fruit and Vegetables*, 6<sup>th</sup> edn. (Sydney: UNSW Press). <https://doi.org/10.1079/9781786391483.0000>.
- Wills, R.B.H., Warton, M.A., and Ku, V.V.V. (2000). Ethylene levels associated with fruit and vegetables during marketing. *Aust. J. Expt. Agric.* 40, 485–490.
- Zewter, A., Woldetsadik, K., and Workneh, T.S. (2012). Effect of 1-methylcyclopropene, potassium permanganate and packaging on quality of banana. *Afr. J. Agric. Res.* 7, 2425–2437. <https://doi.org/10.5897/AJAR11.1203>.

Received: May 13, 2018

Accepted: Sep. 21, 2018