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Abstract

Freshly harvested vine-ripened tomato (*Solanum lycopersicum* cv Neang Pich) were stored at low pressure (4 kPa) at 10°C for 11 days with 100% RH. Fruit quality was examined upon removal and after being transferred to normal atmosphere (101 kPa) at 20°C for 3 days. Fruits weight loss was significantly lower in fruits which stored at low pressure (4 kPa) than fruits that were stored at regular atmospheres (101 kPa) at 10°C. Fruits that were stored at low pressure (4 kPa) reduced calyx browning by 12.5% and calyx rots of 16% compared to fruits that were stored at regular atmospheres (101 kPa) at 10°C. Fruit firmness was not significantly different between fruits stored at low pressures (4 kPa) and the normal atmosphere (101 kPa) with the average firmness of 14 N after fruits were stored at 10°C for 11 days. There was no difference in SSC/TA ratio. The results suggest that low pressure of 4 kPa at 10°C has potential as an alternative, non-chemical postharvest treatment to improve tomato quality during storage.

Keywords: *Solanum lycopersicum*; postharvest; chilling injury; calyx; browning

Introduction

Tomatoes are important fresh vegetable in many countries. Tomatoes are perishable which are normally harvested before the climacteric rise to maintain good eating quality, to prolong shelf-life and reduce spoilage rate (Saltveit, 2005). Tomatoes are often harvested when fully ripen, then held at typical retail outlet display temperatures, which are around 20°C. Current storage methods to maintain to tomatoes include refrigeration storage and controlled atmospheric storage. Many of the modified atmosphere packaging systems are designed for tomatoes to be held at between 5°C and 10°C (Fagundes et al., 2015). However, most studies on control atmosphere (CA) or modified atmosphere packaging (MAP) have been done on tomatoes, where the fruit were harvested at the pre-climacteric stage and stored at the lowest temperature to minimise chilling injury (Salveit 1997). In recent study, D’Aquino et al. (2016) reported
that cherry tomatoes harvested at the red-ripe stage stored in different modified atmosphere at 20°C, and showed the micro-perforated films with moderate levels of CO₂ (2–4 kPa), O₂ partial pressures of 15–18 kPa O₂ with the RH close to 100 % reduced respiration rate and reduction in the rate of degradation of sugars.

Low pressure treatment has been studied to control postharvest decay of fruits and vegetables. Low pressure storage has been around for many years and is a re-emerging technique which can rapidly remove the heat, reduce the oxygen level and expel the harmful gases in sufficient time (Wang, et al. 2001). Most low pressure systems now utilise a method to maintain high humidity which lowers water loss and wilting, also lowers respiration and ethylene production to delay fruit ripening during storage (Burg, 2004). Low pressure storage can also adjust the inside temperature and composition of the atmosphere of horticultural produce reliably and consistently (Li et al., 2006), which can effectively overcome the disadvantages of refrigerated storage and controlled atmosphere storage.

Low pressure storage based on sub-atmospheric pressure and cold storage has exhibited potential for extending the shelf-life of many horticultural crops (Romanazzi et al., 2008, An et al., 2009, and Jiao et al., 2013). Low pressure storage has been reported to delay the ripening of bananas (Burg and Burg, 1966) and increase shelf life of mango (Apelbaum et al., 1977). In addition, An et al. (2009) reported that strawberries stored under low pressure conditions (50.7 kPa) retained higher levels of ascorbic acid and exhibited lower bacterial growth. Similarly, Chen et al. (2013a) founds that low pressure storage extended the postharvest life of Chinese bayberry and improved postharvest quality during storage. The objective of this study was to examine the effectiveness of low pressure storage (4kPa) at 10°C for 11 days with a short shelf-
life at regular pressure (101 kPa) at 20°C to maintain the quality of vine-ripened tomatoes.

Materials and methods

Fruits

Vine-ripened tomatoes (*Solanum lycopersicum* cv Neang Pich) with healthy calyces attached were harvested from the NSW Department of Primary Industries greenhouse (Ourimbah, NSW, Australia), and harvested in the cool of early morning to minimise temperature differences at harvest. Non-blemished tomatoes, with uniform shape and size were sampled and each fruit was labelled, then weighed and randomly allocated into experimental units. Each treatment unit consisted of 20 fruits. Experiments were replicated with six batches of fruit harvested on different occasions.

Low pressure storage system

A laboratory scale low pressure system (VivaFresh™) with six identical low pressure aluminium chambers (0.61 L × 0.43 W × 0.58 H m$^3$) was used in this study. Low pressure was achieved with a two-stage rotary vacuum pump (Model 2005I, Alcatel Adixen, USA) regulated by a compact proportional solenoid valve controlled by a proportional/integral/derivative (PID) computer control system. The system was equipped with an air flow controller to adjust the air exchange rate, which was used to prevent build-up of metabolic gases given off by the fruit. A humidifier was used to make sure the inflowing air was humidified before entering the low pressure chamber. The relative humidity was measured with a wet-bulb and dry-bulb temperatures using calibrated YSI 55000 Series GEM thermistors. Sensors inside the low pressure chambers were used to record the temperature, humidity and pressure during treatment.
All data from temperature and pressure sensors in the low pressure system were digitised and sent to a computer control box and recording system via ethernet cable port. The six different chambers were located inside two different cool rooms of 10°C. Detailed information about the low pressure storage system and instrumentation are described by Jiao et al. (2012).

**Experimental procedures of storage**

Each treatment unit of 20 fruits were placed into a loose unsealed plastic container (45 cm x 20 cm x 15 cm) and placed into the low pressure chamber, where the pressure, temperature and humidity were 4 kPa, 10°C and 100 %, respectively. Each replicate used a different low pressure chamber with two different cool rooms. Two sets of control fruit which consist of each 20 fruits were put in plastic tray at 101 kPa 10°C and 20°C, and covered with a loose low density polyethylene (LDPE) plastic bag (66 cm x 58 cm) to maintain the high relative humidity (95 % RH) around the produce during storage and logging the temperature and RH with calibrated TinyTag View 2 loggers. Fruits were assessed immediately upon removal at 11 days from 10°C and after additional 3 days storage at 101 kPa 20°C.

**Fruit quality assessment**

Fruit quality assessment included; weight loss, calyx detachment, calyx rots, calyx discolouration, chilling injury (CI), fruit firmness, soluble solid content (SSC) and titratable acidity (TA).

The weight loss was calculated as percentage based on the initial weight of tomatoes and weight after storage. Calyx detachment was assessed based on the scoring of its attachment to the fruit (1) or detachment (0). The incidence of calyx rots were assessed visually based on the percentage of total calyx area containing the number of
(black or white) rots, using the following scores: 1 = severe rots or > 50 % affected; 2 = moderate rots or noticeable white or black rots of 30 – 50 %; 3 = slight rots or small white or black spots; and 4 = no rots. The calyx rots rate was calculated according to Wang et al. (2015), with slight modifications. The calculation as calyx rots index (%) = \[\sum_{i=1}^{i} ((\text{rot score}) \times (\text{number of fruit at this level})) / (\text{highest level} \times \text{total number of fruit in the treatment}) \times 100.\] A total of six replicates (n = 20) were performed for each treatment.

Calyx discolouration was subjectively evaluated using a grading scale from 1 to 4, where 1 = severe browning or > 60 % browned and shrivelled; 2 = moderate browning affecting 20 – 60 % stem and calyx; 3 = slight browning or shrivelling or no longer bright; and 4 = no browning. The calyx browning index was expressed as: browning index (%) = \[\sum_{i=1}^{i} ((\text{browning level}) \times (\text{number of fruit at this level})) / (\text{highest level} \times \text{total number of fruit in the treatment}) \times 100.\]

Tomato firmness was determined as the maximum force (Lloyd Texture Analyser, Fareman, UK), required to push a 7 mm probe into the fruit flesh to a depth of 2 mm. The average of 2 reading points from each side of the fruit was taken. The firmness results were expressed in Newton (N). The soluble solid content (SSC), expressed as a percentage on the Brix scale, was measured from the juice of fruit by means of a digital refractometer (ATAGO Inc., Bellevue, WA, USA) at room
temperature. A representative drop from well-shaken juice was placed on dry and clean refractometer prism, and readings were taken directly. Titratable acidity (TA) expressed as % citric acid, was determined by titrating 3 mL tomato supernatant to pH 8.2 with a 0.1 N NaOH solution using an automatic titrator (Mettler Toledo T50, Australia).

Statistical analysis

Statistical analysis to determine differences between treatments was performed using Statistical Analysis System - version 9.4 (SAS Institute, Cary, NC, USA), with the one-way ANOVA and least significant difference (LSD) at $P = 0.05$ used to determine significant differences between individual treatments.

Results and Discussions

Vine-ripened tomatoes with red skin colour and fresh green calyx were used in this experiment. The colour values determined on the skin showed only slight differences among the three batches used. The hue angle (ºH), one of the appropriate quality indexes did not show significant differences ($p < 0.05$) denoting homogeneity in terms of tomatoes ripeness. The initial quality parameter at the beginning of the experiment as follows; Hue value = 45.7 ± 0.8, firmness = 15.0 ± 0.9 N, SSC = 3.2 ± 0.2 °Brix and TA = 0.35 ± 0.04 % citric acid.

Effect on calyx detachment

Tomato fruits were stored under either at low pressure of 4 kPa or normal atmosphere (101 kPa) at 10°C for 11 days. Upon removal from the low pressure, the calyx was assessed based on whether it was detached or intact in every fruit. The different storage treatments did not affect calyx detachment, for the fruits stored at 20°C for 11 days had 97 % of the calyx remain intact, with the additional loss of 2 % with further
storage of 3 days at 20°C. While for tomatoes stored at 10°C both 101 kPa and 4 kPa
for 11 days and an additional storage at 20°C for 3 days, the calyx remained 100 %
intact (Figure 1a). These results suggest that refrigeration storage and low pressure
storage for 11 days maintained the calyx intact in tomatoes.

**Effect on weight loss**

Weight loss of the tomatoes under the different treatments is presented in Figure
1b and shows weight loss was the greatest when tomatoes were constantly kept at 101
kPa 20°C. Low pressure storage resulted in the lowest water loss from the fruit, where
after 11 days storage, weight loss was much less in low pressure (4kPa) storage
compared with that at room temperature of 20°C (101 kPa) and refrigeration storage of
10°C (101 kPa), and there were no significant differences between weight loss in room
temperature storage (101 kPa, 20°C) and refrigeration storage of 10°C (101 kPa). These
observations are contradictory with those previously observed by Hashmi et al. (2013a),
where the low pressure treatment did not affect the weight loss of strawberries, and
Laurin et al. (2006) who reported that low pressure treatment of 70 kPa for 6 hours
increased weight loss of Alpha-type cucumbers in subsequent storage. However this
may be due to the water vapour pressure and relative humidity maintained within the
test chambers (Jiao et al., 2012). In this experiment weight loss after low pressure
storage was kept to a minimum, as the incoming air was humidified to achieve high
relative humidity inside the chamber (Burg, 2004).

**Effect on chilling injury**

Tomatoes are usually stored at low temperature to delay ripening and extend
shelf life, but the tomatoes are also susceptible to chilling injury (CI) when continuously
exposed to temperatures below 12°C (Wang, 1993 and Zhang et al., 2010). Although
incipient CI in tomatoes is not generally apparent during storage at low temperatures, visible symptoms of CI, such as, surface lesion or indentations, discoloration, and increased decay develop when exposed to warmer temperatures. CI is an enormously complex phenomenon, with damage to the plasma membranes considered to be one of the most common primary causes of CI in fruit (Rui et al., 2010).

In this experiment, tomatoes stored under low pressure storage (4 kPa) for 11 days at 10°C produced significantly lower chilling injury symptoms compared to fruits stored at regular atmosphere (101 kPa) at 10°C and these symptoms developed more when the tomatoes were transferred to regular pressure (101 kPa) 20°C for 3 days (Figure 2). This suggests low pressure plays role in enhancing the chilling tolerance of mature ripe tomato fruit and are consistent with those previously reported by Burg (2004) who observed that low pressure storage (29.33 kPa) completely prevented rind pitting due to CI in Persian limes. These effects of low pressure on CI maybe a result at low O₂ level and nearly saturated humidity present during low pressure storage, as a high humidity has been shown to ameliorate low temperature injuries in many fruits and vegetables (Burg, 2004).

**Effect on calyx browning**

The fresh appearance of the calyx of vine riped tomatoes is a major component of the acceptability of these tomatoes type. A fresh green calyx is a major indicator of tomatoes freshness. The effect of low pressure storage on calyx browning in mature-red tomatoes are presented in Figure 3a. The results show that tomatoes were stored at 20°C for 11 days had significantly higher calyx browning compared to those fruits were stored at 10°C for both pressure of 4 kPa and 101 kPa. While for tomatoes stored at low pressure (4 kPa) storage of 10°C resulted in significantly less calyx browning than
regular atmosphere (101 kPa) storage of 10°C, where the reduction of calyx browning was 12.5% lower after 11 days. A greater difference between the treatment and control was observed after subsequent storage for 3 days at 20°C, whereupon calyx browning of the low pressure treated fruit was 26% lower. Although the calyx may act as an independent entity, these results are consistent with those previously reported by Gao et al. (2006) who observed that low pressure storage reduced the browning of logan fruits, however further mechanism studies are required to determine whether a similar or different pathway for low pressure storage action occurs in reducing browning in tomatoes.

**Effect on calyx rots**

Tomato fruit are highly perishable and are susceptible to physiological deterioration and fungal decay (Salveit, 2005). Burg et al., 2004 reported that low pressure treatment retained freshness, taste and flavor as well as discouraged commodity deterioration caused by bacteria and fungi in many fruits fruit and vegetables. In this study, fruits stored at regular pressure (101 kPa) 20°C for 11 days had highest rots compared to other treatments. While tomatoes exposed to low pressure storage (4 kPa) at 10°C displayed significantly lower levels of calyx rots with the reduction of 16% compared to the control fruits (regular atmosphere, 10°C) (Figure 3b). This observation continued on fruit that were subsequently held at regular atmosphere (101 kPa) 20°C for 3 days, with the further calyx rots reduction of 1% at the end of experiment.

These results are consistent with those previously reported by Wang et al. (2015), who showed that low pressure storage (10-20 kPa for 30 days) reduced incidence decay on Honey peach. Similarly, Romanazzi et al. (2001) reported that low
pressure storage reduced diseases incidence caused by \textit{R. stolonifer} and \textit{B. cinerea} in sweet cherries, strawberries and table grapes. Hashmi et al. (2013b) also observed that low pressure treatment (50 kPa, 4 hours) delayed rot development in strawberries subsequently stored at 20°C for 7 days. The reduction in postharvest decay by low pressure treatment has been attributed to modified low oxygen levels and reduced respiration (Dilley, 2003) as well as eliciting a stress response within the tissues that enhances natural disease resistance (Romanazzi et al., 2001).

The current study indicates that application of low pressure (4 kPa) storage in combination with low temperature (10°C) improves the storage life of tomatoes by reducing calyx rots. However, the magnitude of calyx rot reduction in this study was only 17 %, as compared to control treatment (101 kPa 10°C). It should be noted that this study was conducted on fully ripe tomatoes. A previous study reported that strawberries harvested at three-quarter maturity had lower rots than fully ripe fruit (Nunes et al., 2002). Guidarelli et al. (2011) suggested that the mode of action of low pressure treatment is the induction of fruit resistance, and fruit resistance is higher during the development stage of fruit ripeness. Therefore early application of low pressure storage may stimulate the defence system before the fully ripe stage. Hence the use of less ripe tomato fruits for low pressure storage may further improve its efficacy.

\textbf{Effect on firmness}

Fruit firmness is an important quality parameter of tomatoes, as loss of sensory quality in tomatoes is often associated with firmness changes during storage (Grierson and Kader, 1986). In this study, fruit firmness was assessed after tomatoes were stored under low pressure of 4 kPa at 10°C for 11 days, and transferred to 20°C at regular atmosphere (101 kPa) for 3 days. Figure 4 shows the effect of low pressure storage on
firmness in tomatoes, where fruits were stored at pressure storage of 4 kPa for 11 days at 10°C and after 3 days at 20°C did not have any effect on fruit firmness, meaning that the tissue structure of the produce remained intact. These observations are consistent with those previously reported by Hashim et al. (2016) who reported that low pressure treatment (50 kPa) did not affect the firmness of strawberries. However, in this study, control fruits that were stored at regular atmosphere (101 kPa) at 20°C, followed by additional storage for 3 days at the same storage conditions resulted in significantly softer compared to fruits that were stored at low pressure (4kPa) or regular pressure (101 kPa) 10°C, this observation may caused by severe water loss during storage and development of postharvest rots.

**Effect on SSC, TA, SSC/TA ratio**

The results of the effect of low pressure storage on soluble solids content (SSC), titratable acidity (TA) and SSC/TA ratio in tomato are presented in Table 1 and shows that SSC and TA did not change after storage at low pressure (4 kPa) for 11 days at 10°C and with an additional storage at normal atmosphere (101 kPa) at 20°C for 3 days. These results are consistent with those previously reported by Jiao et al., (2013) who observed that SSC and TA did not change in ‘Red Delicious’ apples after stored at low pressure (33 kPa) 10°C for 15 days. Similarly, Wang et al. (2015) reported that low pressure storage of 10 - 20 kPa for 30 days at 0 °C and 85–90 % RH maintained high level of TSS in honey peach. However, other reports have been shown that low pressure storage reduced the TA of logan (Gao et al., 2006), and Li et al. (2006) showed lower SSC in asparagus during storage at low pressure atmosphere (35-40 kPa, 3°C) for 60 days. These differences may be due to maturation and the type of produce used in each experiment and the duration of storage times under low pressure.
The SSC/TA, or sugar to acid ratio is an important taste factor and an indicator of maturity, ripeness, or both in some mature fruit-type vegetables such as tomato (Malundo et al., 1995). Loss of sensory quality in tomatoes is associated with reduction of sweetness and acidic taste (Grierson and Kader, 1986). In this observation, similarly the SSC/TA, or sugar/acid ratio showed no significant difference between the fruits stored under low pressure storage (4 kPa) and regular pressure (101 kPa) at 10°C (Table 1). These results suggest that low pressure storage did not have any effect on SSC, TA or SSC/TA in tomato, which is consistent with the results reported by Burg (2004) where the tomatoes flavour remained unchanged after fruits were stored under low pressure of 12 kPa for 18 days at 2.8°C.

Conclusions

These results showed that low pressure storage under 4 kPa at 10°C for 11 days maintained the quality of vine-ripened tomatoes during storage. Low pressure storage significantly reduced calyx rots, calyx discolouration, weight loss and decreased chilling injury symptoms. The low pressure storage also maintained the fruit’s firmness, SSC and TA, equally to regular atmosphere storage. These observations supports the importance of low pressure storage, but large scale experiments are required to be conducted for the commercial validation and optimisation of low pressure storage. Further work is also required to look at less mature fruit to examine of low pressure can maintain quality and ripen normally.

Acknowledgements

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References


Table 1. Effect of low pressure storage on soluble solids content (SSC), titratable acidity (TA), and SSC/TA (or sugar/acid) ratio on different assessment day at 20°C.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>SSC (°Brix)</th>
<th>TA (% citric acid)</th>
<th>SSC/TA ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upon removal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>101 kPa 20°C, 11 days</td>
<td>2.8</td>
<td>0.31</td>
<td>9.0</td>
</tr>
<tr>
<td>101 kPa 10°C, 11 days</td>
<td>2.9</td>
<td>0.35</td>
<td>8.3</td>
</tr>
<tr>
<td>4 kPa 10°C, 11 days</td>
<td>3.1</td>
<td>0.42</td>
<td>7.4</td>
</tr>
<tr>
<td><em>LSD (5%)</em></td>
<td>± 0.5</td>
<td>± 0.08</td>
<td>± 1.7</td>
</tr>
<tr>
<td>Additional storage 3 days at 101 kPa 20°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>101 kPa 20°C, 11 days</td>
<td>3.5</td>
<td>0.32</td>
<td>10.7</td>
</tr>
<tr>
<td>101 kPa 10°C, 11 days</td>
<td>3.4</td>
<td>0.34</td>
<td>10.2</td>
</tr>
<tr>
<td>4 kPa 10°C, 11 days</td>
<td>3.1</td>
<td>0.34</td>
<td>9.2</td>
</tr>
<tr>
<td><em>LSD (5%)</em></td>
<td>± 0.5</td>
<td>± 0.05</td>
<td>± 1.8</td>
</tr>
</tbody>
</table>

*Values are the mean of 6 replicates with 20 fruits in each replicate.*
Figure 1. The percentage of calyx intact (a) and weight loss of tomatoes (b) exposed to different treatments. The values are the mean of six replicates. Different superscript letter at each storage time show significant different at p <0.05.
Figure 2. The chilling injury index of tomatoes exposed to different treatments. The values are the mean of six replicates. Different superscript letter at each storage time show significant different at p <0.05.
Figure 3. The calyx browning index (a) and calyx rot incidence (b) of tomatoes exposed to different treatments. The values are the mean of six replicates. Different superscript letter at each storage time show significant different at p <0.05.
Figure 4. The firmness of tomatoes exposed to different treatments. The values are the mean of six replicates. Different superscript letter at each storage time show significant different at p < 0.05.