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This is the accepted manuscript of an article published by Taylor & Francis in the Journal of *Horticultural Science and Biotechnology* on 10 April 2017 available online: http://dx.doi.org/10.1080/14620316.2017.1301222

1 Use of Low Pressure Storage to Improve the Quality of Tomatoes

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14 Abstract

15 Freshly harvested vine-ripened tomato (Solanum lycopersicum cv Neang 16 Pich) were stored at low pressure (4 kPa) at 10°C for 11 days with 100 % RH. 17 Fruit quality was examined upon removal and after being transferred to normal 18 atmosphere (101 kPa) at 20°C for 3 days. Fruits weight loss was significantly 19 lower in fruits which stored at low pressure (4 kPa) than fruits that were stored at 20 regular atmospheres (101 kPa) at 10°C. Fruits that were stored at low pressure (4 21 kPa) reduced calyx browning by 12.5 % and calyx rots of 16 % compared to 22 fruits that were stored at regular atmospheres (101 kPa) at 10°C. Fruit firmness 23 was not significantly different between fruits stored at low pressures (4 kPa) and 24 the normal atmosphere (101 kPa) with the average firmness of 14 N after fruits 25 were stored at 10°C for 11 days. There was no difference in SSC/TA ratio. The 26 results suggest that low pressure of 4 kPa at 10°C has potential as an alternative, 27 non-chemical postharvest treatment to improve tomato quality during storage.

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

29 Introduction

30 Tomatoes are important fresh vegetable in many countries. Tomatoes are 31 perishable which are normally harvested before the climacteric rise to maintain good 32 eating quality, to prolong shelf-life and reduce spoilage rate (Saltveit, 2005). Tomatoes 33 are often harvested when fully ripen, then held at typical retail outlet display 34 temperatures, which are around 20°C. Current storage methods to maintain to tomatoes 35 include refrigeration storage and controlled atmospheric storage. Many of the modified 36 atmosphere packaging systems are designed for tomatoes to be held at between 5° C and 37 10°C (Fagundes et al., 2015). However, most studies on control atmosphere (CA) or 38 modified atmosphere packaging (MAP) have been done on tomatoes, where the fruit 39 were harvested at the pre-climacteric stage and stored at the lowest temperature to 40 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.

45 Low pressure treatment has been studied to control postharvest decay of fruits 46 and vegetables. Low pressure storage has been around for many years and is a re-47 emerging technique which can rapidly remove the heat, reduce the oxygen level and 48 expel the harmful gases in sufficient time (Wang, et al. 2001). Most low pressure 49 systems now utilise a method to maintain high humidity which lowers water loss and 50 wilting, also lowers respiration and ethylene production to delay fruit ripening during 51 storage (Burg, 2004). Low pressure storage can also adjust the inside temperature and 52 composition of the atmosphere of horticultural produce reliably and consistently (Li et 53 al., 2006), which can effectively overcome the disadvantages of refrigerated storage and 54 controlled atmosphere storage.

55 Low pressure storage based on sub-atmospheric pressure and cold storage has 56 exhibited potential for extending the shelf-life of many horticultural crops (Romanazzi 57 et al., 2008, An et al., 2009, and Jiao et al., 2013). Low pressure storage has been 58 reported to delay the ripening of bananas (Burg and Burg, 1966) and increase shelf life 59 of mango (Apelbaum et al., 1977). In addition, An et al. (2009) reported that 60 strawberries stored under low pressure conditions (50.7 kPa) retained higher levels of 61 ascorbic acid and exhibited lower bacterial growth. Similarly, Chen et al. (2013a) 62 founds that low pressure storage extended the postharvest life of Chinese bayberry and 63 improved postharvest quality during storage. The objective of this study was to examine 64 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.

67 Materials and methods

68 Fruits

69 Vine-ripened tomatoes (*Solanum lycopersicum cv* Neang Pich) with healthy 70 calyxes attached were harvested from the NSW Department of Primary Industries 71 greenhouse (Ourimbah, NSW, Australia), and harvested in the cool of early morning to 72 minimise temperature differences at harvest. Non-blemished tomatoes, with uniform 73 shape and size were sampled and each fruit was labelled, then weighed and randomly 74 allocated into experimental units. Each treatment unit consisted of 20 fruits. 75 Experiments were replicated with six batches of fruit harvested on different occasions.

76 Low pressure storage system

77 A laboratory scale low pressure system (VivaFreshTM) with six identical low 78 pressure aluminium chambers (0.61 L \times 0.43 W \times 0.58 H m³) was used in this study. 79 Low pressure was achieved with a two-stage rotary vacuum pump (Model 2005I, 80 Alcatel Adixen, USA) regulated by a compact proportional solenoid valve controlled 81 by a proportional/integral/derivative (PID) computer control system. The system was 82 equipped with an air flow controller to adjust the air exchange rate, which was used to 83 prevent build-up of metabolic gases given off by the fruit. A humidifier was used to 84 make sure the inflowing air was humidified before entering the low pressure chamber. 85 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 86 87 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).

93

Experimental procedures of storage

94 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 95 96 pressure, temperature and humidity were 4 kPa, 10°C and 100 %, respectively. Each 97 replicate used a different low pressure chamber with two different cool rooms. Two sets 98 of control fruit which consist of each 20 fruits were put in plastic tray at 101 kPa 10°C 99 and 20°C, and covered with a loose low density polyethylene (LDPE) plastic bag (66 100 cm x 58 cm) to maintain the high relative humidity (95 % RH) around the produce 101 during storage and logging the temperature and RH with calibrated TinyTag View 2 102 loggers. Fruits were assessed immediately upon removal at 11 days from 10°C and after 103 additional 3 days storage at 101 kPa 20°C.

104 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).

108 The weight loss was calculated as percentage based on the initial weight of 109 tomatoes and weight after storage. Calyx detachment was assessed based on the scoring 110 of its attachment to the fruit (1) or detachment (0). The incidence of calyx rots were 111 assessed visually based on the percentage of total calyx area containing the number of

112 (black or white) rots, using the following scores : 1 = severe rots or > 50 % affected; 2 113 = moderate rots or noticeable white or black rots of 30 - 50 %; 3 = slight rots or small 114 white or black spots; and 4 = no rots. The calyx rots rate was calculated according to 115 Wang et al. (2015), with slight modifications. The calculation as calyx rots index (%) = 116 $\sum[(\text{rot score}) \times (\text{number of fruit at this level})] / (\text{highest level} \times \text{total number of fruit in}$ 117 the treatment) × 100. A total of six replicates (n = 20) were performed for each 118 treatment.

119 Calyx discolouration was subjectively evaluated using a grading scale from 1 to 120 4, where 1 = severe browning or > 60 % browned and shrivelled; 2 = moderate 121 browning affecting 20 - 60 % stem and calyx; 3 = slight browning or shrivelling or no 122 longer bright; and 4 = no browning. The calyx browning index was expressed as: 123 browning index (%) = $\sum [(browning level) \times (number of fruit at this level)]/(highest)$ 124 level \times total number of fruit in the treatment) \times 100. The CI index was estimated based 125 on the percentage of total fruit surface area containing the number of spot or dot sunken 126 lesions or surface pitting, score 1 = many spots or large lesions (\geq 50 %); 2 = moderate 127 or 4 - 8 small spots or lesion ≤ 0.1 cm² (30 - 50 %); 3 = slight or 1-3 spots (10 - 30 %); 128 and 4 = fresh with no symptom of chilling injury. The CI index was expressed as: CI 129 index (%) = $\sum [(CI \text{ level}) \times (\text{number of fruit at this level})]/(\text{highest level} \times \text{total number})$ 130 of fruit in the treatment) \times 100. 131 Tomato firmness was determined as the maximum force (Lloyd Texture 132 Analyser, Fareman, UK), required to push a 7 mm probe into the fruit flesh to a depth of 133 2 mm. The average of 2 reading points from each side of the fruit was taken. The 134 firmness results were expressed in Newton (N). The soluble solid content (SSC), 135 expressed as a percentage on the Brix scale, was measured from the juice of fruit by 136 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).

141 Statistical analysis

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

146 **Results and Discussions**

147 Vine-ripened tomatoes with red skin colour and fresh green calyx were used in 148 this experiment. The colour values determined on the skin showed only slight 149 differences among the three batches used. The hue angle (°H), one of the appropriate 150 quality indexes did not show significant differences (p < 0.05) denoting homogeneity in 151 terms of tomatoes ripeness. The initial quality parameter at the beginning of the 152 experiment as follows; Hue value = 45.7 ± 0.8 , firmness = 15.0 ± 0.9 N, SSC = $3.2 \pm$ 153 0.2 °Brix and TA = 0.35 \pm 0.04 % citric acid.

154 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 assed 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.

164 Effect on weight loss

165 Weight loss of the tomatoes under the different treatments is presented in Figure 166 1b and shows weight loss was the greatest when tomatoes were constantly kept at 101 167 kPa 20°C. Low pressure storage resulted in the lowest water loss from the fruit, where 168 after 11 days storage, weight loss was much less in low pressure (4kPa) storage 169 compared with that at room temperature of 20°C (101 kPa) and refrigeration storage of 170 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 171 172 observations are contradictory with those previously observed by Hashmi et al. (2013a), 173 where the low pressure treatment did not affect the weight loss of strawberries, and 174 Laurin et al. (2006) who reported that low pressure treatment of 70 kPa for 6 hours 175 increased weight loss of Alpha-type cucumbers in subsequent storage. However this 176 may be due to the water vapour pressure and relative humidity maintained within the 177 test chambers (Jiao et al., 2012). In this experiment weight loss after low pressure 178 storage was kept to a minimum, as the incoming air was humidified to achieve high 179 relative humidity inside the chamber (Burg, 2004).

180 *Effect on chilling injury*

181 Tomatoes are usually stored at low temperature to delay ripening and extend 182 shelf life, but the tomatoes are also susceptible to chilling injury (CI) when continuously 183 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, discolouration, 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).

189 In this experiment, tomatoes stored under low pressure storage (4 kPa) for 11 190 days at 10°C produced significantly lower chilling injury symptoms compared to fruits 191 stored at regular atmosphere (101 kPa) at 10°C and these symptoms developed more 192 when the tomatoes were transferred to regular pressure (101 kPa) 20°C for 3 days 193 (Figure 2). This suggests low pressure plays role in enhancing the chilling tolerance of 194 mature ripe tomato fruit and are consitent with those previously reported by Burg 195 (2004) who observed that low presure storage (29.33 kPa) completely prevented rind 196 pitting due to CI in Persian limes. These effects of low pressure on CI maybe a result at 197 low O₂ level and nearly saturated humidity present during low pressure storage, as a 198 high humidity has been shown to ameliorate low temperature injuries in many fruits 199 and vegetables (Burg, 2004).

200 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 compare to those fruits were stored at 10°C for both presure 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

208 regular atmosphere (101 kPa) storage of 10°C, where the reduction of calyx browning 209 was 12.5 % lower after 11 days. A greater difference between the treatment and control 210 was observed after subsequent storage for 3 days at 20°C, whereupon calyx browning of 211 the low pressure treated fruit was 26 % lower. Although the calyx may act as an 212 independent entity, these results are consitent with those previously reported by Gao et 213 al. (2006) who observed that low pressure storage reduced the browning of logan fruits, 214 however further mechanism studies are required to determine whether a similar or 215 different pathway for low pressure storage action occurs in reducing browning in 216 tomatoes.

217 Effect on calyx rots

218 Tomato fruit are highly perishable and are susceptible to physiological 219 deterioration and fungal decay (Salveit, 2005). Burg et al., 2004 reported that low 220 pressure treatment retained freshness, taste and flavor as well as discouraged 221 commodity deterioration caused by bacteria and fungi in many fruits fruit and 222 vegetables. In this study, fruits stored at regular pressure (101 kPa) 20°C for 11 days 223 had highest rots compared to other treatments. While tomatoes exposed to low pressure 224 storage (4 kPa) at 10°C displayed significantly lower levels of calyx rots with the 225 reduction of 16 % compared to the control fruits (regular atmosphere, 10°C) (Figure 226 3b). This observation continued on fruit that were subsequently held at regular 227 atmosphere (101 kPa) 20°C for 3 days, with the further calyx rots reduction of 1 % at 228 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 *R. stolonifer* and *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).

239 The current study indicates that application of low pressure (4 kPa) storage in 240 combination with low temperature (10°C) improves the storage life of tomatoes by 241 reducing calyx rots. However, the magnitude of calyx rot reduction in this study was 242 only 17 %, as compared to control treatment (101 kPa 10°C). It should be noted that this 243 study was conducted on fully ripe tomatoes. A previous study reported that strawberries 244 harvested at three-quarter maturity had lower rots than fully ripe fruit (Nunes et al., 245 2002). Guidarelli et al. (2011) suggested that the mode of action of low pressure 246 treatment is the induction of fruit resistance, and fruit resistance is higher during the 247 development stage of fruit ripeness. Therefore early application of low pressure storage 248 may stimulate the defence system before the fully ripe stage. Hence the use of less ripe 249 tomato fruits for low pressure storage may further improve its efficacy.

250 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

256 firmness in tomatoes, where fruits were stored at pressure storage of 4 kPa for 11 days 257 at 10°C and after 3 days at 20°C did not have any effect on fruit firmness, meaning that 258 the tissue structure of the produce remained intact. These observations are consitent 259 with those previously reported by Hashim et al. (2016) who reported that low pressure 260 treatment (50 kPa) did not affect the firmness of strawberries. However, in this study, 261 control fruits that were stored at regular atmosphere (101 kPa) at 20°C, followed by 262 additional storage for 3 days at the same storage conditions resulted in significantly 263 softer compared to fruits that were stored at low pressure (4kPa) or regular pressure 264 (101 kPa) 10°C, this observation may caused by severe water loss during storage and 265 development of postharvest rots.

266 Effect on SSC, TA, SSC/TA ratio

267 The results of the effect of low pressure storage on soluble solids content (SSC), 268 titratable acidity (TA) and SSC/TA ratio in tomato are presented in Table 1 and shows 269 that SSC and TA did not change after storage at low pressure (4 kPa) for 11 days at 270 10°C and with an additional storage at normal atmosphere (101 kPa) at 20°C for 3 days. 271 These results are consistent with those previously reported by Jiao et al., (2013) who 272 observed that SSC and TA did not change in 'Red Delicious' apples after stored at low 273 pressure (33 kPa) 10°C for 15 days. Similarly, Wang et al. (2015) reported that low 274 pressure storage of 10 - 20 kPa for 30 days at 0 °C and 85–90 % RH maintained high 275 level of TSS in honey peach. However, other reports have been shown that low 276 pressure storage reduced the TA of logan (Gao et al., 2006), and Li et al. (2006) 277 showed lower SSC in asparagus during storage at low pressure atmosphere (35-40 kPa, 278 3°C) for 60 days. These differences may be due to maturation and the type of produce 279 used in each experiment and the duration of storage times under low pressure.

280 The SSC/TA, or sugar to acid ratio is an important taste factor and an indicator 281 of maturity, ripeness, or both in some mature fruit-type vegetables such as tomato 282 (Malundo et al., 1995). Loss of sensory quality in tomatoes is associated with reduction 283 of sweetness and acidic taste (Grierson and Kader, 1986). In this observation, similarly 284 the SSC/TA, or sugar/acid ratio showed no significant difference between the fruits 285 stored under low pressure storage (4 kPa) and regular pressure (101 kPa) at 10°C 286 (Table 1). These results suggest that low pressure storage did not have any effect on 287 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 288 289 low pressure of 12 kPa for 18 days at 2.8°C.

290 Conclusions

291 These results showed that low pressure storage under 4 kPa at 10°C for 11 days 292 maintained the quality of vine-ripened tomatoes during storage. Low pressure storage 293 significantly reduced calyx rots, calyx discolouration, weight loss and decreased 294 chilling injury symptoms. The low pressure storage also maintained the fruit's firmness, 295 SSC and TA, equally to regular atmosphere storage. These observations supports the 296 importance of low pressure storage, but large scale experiments are required to be 297 conducted for the commercial validation and optimisation of low pressure storage. 298 Further work is also required to look at less mature fruit to examine of low pressure can 299 maintain quality and ripen normally.

300 Acknowledgements

This work was supported supported by NSW Department of Primary Industries,
 Horticulture Innovation Australia and AusVeg (Project VG13043). The project was also
 supported by the University of Newcastle and the Australian Research Council Training

- 304 Centre for Food and Beverage Supply Chain Optimisation (IC140100032). Special
- 305 thanks go to Josh Jarvis at NSW Department of Primary Industries for growing the
- 306 tomato fruit used for this study.

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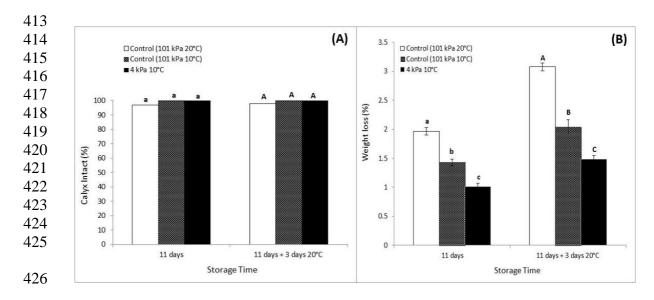
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408 Table 1. Effect of low pressure storage on soluble solids content (SSC), titratable acidity

409	(TA), and SSC/TA	(or sugar/acid) ratio	o on different assessment day at 20°C.
107	(111), and 55 0/ 111	(or sugar, acra) racio	on anierene assessment aug at 20°0.

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

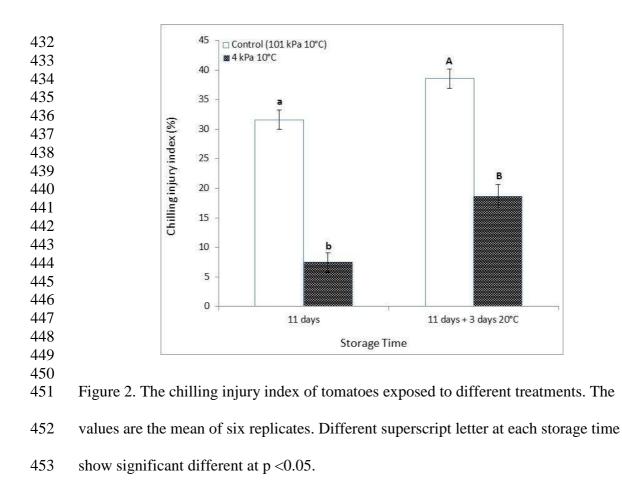
Values are the mean of 6 replicates with 20 fruits in each replicate.

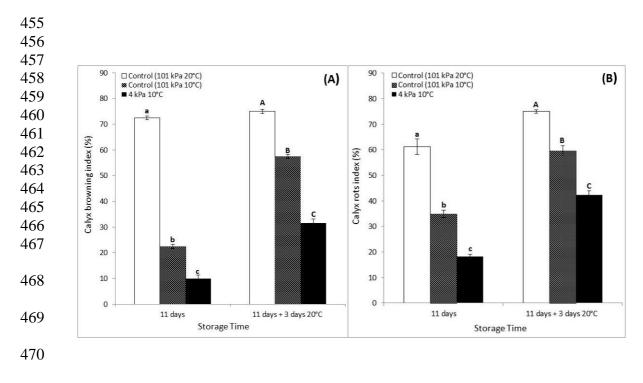


428 Figure 1. The percentage of calyx intact (a) and weight loss of tomatoes (b) exposed to

429 different treatments. The values are the mean of six replicates. Different superscript

430 letter at each storage time show significant different at p < 0.05.





471 Figure 3. The calyx browning index (a) and calyx rot incidence (b) of tomatoes exposed
472 to different treatments. The values are the mean of six replicates. Different superscript
473 letter at each storage time show significant different at p <0.05.

