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Effect of low pressure storage on the quality of green capsicums (*Capsicum annum L.*)

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Abstract

Green capsicums (*Capsicum annum L.*) were stored under low pressure (4 kPa) at 10°C for 5 and 11 days with 100% RH. The results showed that the incidence of stem decay under low pressure storage for 5 and 11 days and storage at ambient atmosphere at 20°C for three days lower compared to fruits that were stored at regular atmosphere at 10°C. Fruit that had been stored at low pressure at 10°C had no symptoms of flesh rots for up to 11 days, whilst fruit which had been stored at regular atmosphere at 10°C had 6% flesh rots after 11 days storage at 10°C.There was no difference in flesh firmness and colour retention between fruits stored at low pressure and regular pressure at 10°C. Capsicums stored at low pressure had higher overall acceptability compared to fruit that were stored at regular atmosphere at 10°C. These results demonstrate the potential of low pressure storage as an effective technique to manage capsicum fruit quality, however there was no additional benefit when fruits were stored at low pressure for more than 5 days.

Keywords: *Capsicum annum L.*; low pressure; colour; firmness; flesh rots; stem decay

Introduction

Green capsicums or bell peppers *(Capsicum annum L.*) are harvested at fully mature green stage for fresh consumption. Green capsicum fruit are highly perishable and rapidly lose quality after harvest. The major limiting factors for the storage of green capsicums includes skin colour degreening, flesh shrivel and rots affecting both the flesh and calyx/stem. Shrivel is a result of moisture loss from the fruit and is a consequence of storage in low humidity and is exacerbated by the hollow centre of capsicum fruit (O'Donoghue et al., 2013). The calyx (stem) of capsicum fruit can also be affected by moisture loss where localized 'die-back' of the tissues can occur (O'Donoghue, et al., 2013). Another storage problem of capsicums is postharvest degreening of the green capsicums. This significantly downgrades consumer acceptance, as the retention of the green skin colour is a key determinant of consumer preference.

The recommended storage conditions for capsicums is 8°C with 95% relative humidity (RH) (Cantwell & Kasmire, 2011). Capsicums are susceptible to chilling damage at lower storage temperatures (< 7°C), although this is cultivar and ripeness dependent. However storage at higher storage temperatures, particularly at elevated humidity often results in the growth of postharvest pathogens (Lim et al., 2007). Both chilling injury and rot development are not often visible during storage, but develop after the fruit warms to room temperature (Balandrán-Quintana et al., 2003) and are responsible for important economic losses.

A range of pre-storage treatments prior to cold storage have been developed to maintain green capsicum quality. Current potential treatment methods to maintain the quality of green capsicums include coatings with chitosan (Xing et al., 2011), Semperfresh^{TM} (composed of sucrose esters of fatty acids, sodium carboxymethyl cellulose and mon-odiglycerides of fatty acids) (Özden & Bayindirli, 2002), and treatment with 1-methylcyclopropene (Fernández-Trujillo et al., 2009). Hot water treatment (50 – 53°C) was also reported as a method to improve the quality of capsicums (Fallik et al.1996; González-Aguilar et al., 2000), while (Elazar Fallik et al., 1999) further showed that capsicums brushed with hot water (55ºC), prevented fruit decay during transport.

Low pressure storage technology has been around for many years but it has recently re- emerged as a technique which can rapidly remove the heat, reduce the oxygen level and rapidly remove and manage the storage atmosphere (Wang et al., 2001). Unlike other physical treatments (such as heat, gamma irradiation and ultra violet, a potential advantage of pressure treatment is the homogeneity of application during treatment (Vigneault et al., 2012). Most modern low pressure systems utilise a method to maintain high humidity to lower water loss and wilting, where the low pressure treatment also lowers respiration, and ethylene production to delay fruit ripening during storage (Burg, 2004). Low pressure storage can also incorporate reliable adjustment of the storage temperature and atmospheric composition, which can effectively overcome disadvantages associated with atmospheric refrigeration and controlled atmosphere storage processes (Li et al., 2006).

Low pressure storage based on sub-atmospheric pressure has been shown to extend the storage and shelf-life of many horticultural crops such as bananas (Burg & Burg , 1966), mango (Apelbaum et al., 1977), strawberries (An et al., 2009), Chinese bayberry (Chen et al., 2013) and tomato (Pristijono et al., 2017b). There are limited studies of the effect of low pressure storage on the quality of green capsicums. (Burg, 2004) reported that peppers tolerated two days exposure to a pressure of 2.67 kPa at 12-13°C, however longer exposure times have not been examined. This study examined the effectiveness of low pressure storage (4 kPa) at 10°C for 5 and 11 days with the addition of a three days shelf-life at regular pressure (101 kPa) at 20°C, to maintain the quality of green capsicums.

Materials and methods

Fruits

Local fresh green capsicum fruit (*Capsicum annum L.*), free from damage and uniform in size were obtained from the local wholesale market. Non-blemished fruit (260 - 270 g) were randomly selected, weighed and sorted into experimental units. The experimental design was completely randomized, consisting three treatment units (a) regular pressure of 101 kPa at 20°C, (b) regular pressure of 101 kPa at 10°C and (c) low pressure of 4 kPa at 10°C. Each experimental unit consisted of 16 fruits which was replicated three times for treatment and storage period (5 and 11 days).

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 the study. Low pressure was achieved using 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 ensure the inflowing rarefied air was humidified before entering the low pressure chamber. Relative humidity in the system was calculated by measuring 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 LP 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 cool rooms at 10°C, where three chambers were allocated to 5 days storage and three chambers for 11 days.

Experimental procedures of storage

Each treatment unit of 16 fruits was placed into an 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 maintained at 4 kPa, 10°C and 100 %, respectively. Each replicate used an independent separate low pressure chamber (total of 6 low pressure chambers). Two sets of control fruit which each consisted 16 fruits were placed onto a plastic tray at either 101 kPa 10°C or 20°C, and covered with a loose low density polyethylene (LDPE) plastic bag (66 cm x 58 cm) to maintain the RH of 97% around the produce during storage. Fruits were assessed immediately upon removal (after the fruit had warmed to room temperature) after 5 and 11 days from 10°C and after additional three days storage at 101 kPa 20°C. Calibrated loggers (TinyTag View 2) were used to monitor temperature and relative humidity within each treatment.

Fruit quality assessment

Fruit quality assessment included; weight loss, stem rots, colour, flesh rots, fruit firmness and overall acceptability. The weight loss was calculated as percentage based on the initial weight of capsicums and weight after storage.

Skin colour changing was assessed visual based on a grading scale from 1 to 4, where 1 = severe degreening mainly orange or red; 2 = 50 – 75% green; 3 = more than 75 % green; and 4 = 100 % green (Figure 1). The skin colour changing index was expressed as : colour changing index (%) = ∑[(degreening level) × (number of fruit at this level)]/(highest level × total number of fruit in the treatment) × 100.

The incidence of flesh rots were visually assessed and scored based on the percentage of total flesh area containing the number of black rots, using the following scores; 1 = severe rots or > 50 % affected; 2 = moderate rots, two spots or large lesion; 3 = slight rots or noticeable black rots of one to two spots; and 4 = fresh with no symptoms of rots. The flesh rots rate was calculated according to Wang et al. (2015) with some slight modifications. The calculation as calyx rots index (%) = ∑[(rot score) × (number of fruit at this level)] / (highest level × total number of fruit in the treatment) × 100.

Stem decay was subjectively evaluated using an subjective grading scale from 1 to 4, where 1 = severe decay or > 50 % rotten; 2 = moderate decay, soft, water soaked lesions, noticeable or 25 -50% stem rotten; 3 = slight, small spots, affecting < 25 % stem decay; and 4 = no symptoms of stem decay. The stem decay was calculated according to Pristijono et al. (2017b) with some slight modifications. The stem decay index was expressed as: stem decay index (%) = ∑[(decay level) × (number of fruit at this level)]/(highest level × total number of fruit in the treatment) × 100.

Green capsicums firmness was measured according to Pristijono et al. (2017a), with some slight modifications, where the firmness determined as the maximum force (Lloyd Texture Analyser, Fareman, UK), required to push a 68 mm^2 flat probe into the fruit flesh to a depth of 7 mm. The average of two reading points from each side of the fruit was taken three cm from calyx-end. The firmness results were expressed in

Newton (N). The overall acceptability index was estimated based on the fruit freshness combination of the level of skin discoloration, stem and flesh rotted, scoring from 1 to 4, where, score 1= poor, consumer would throw away; 2 = not saleable but edible, acceptable for cooking; 3 = less than 20 % skin degreening and with slight stem and flesh rots; and 4 = fresh with no symptom of stem and flesh rots and discolouration. The fruits overall acceptability index was assessed according to Pristijono et al. (2017a), with some slight modifications. The acceptability index was expressed as: acceptability index (%) = ∑[(acceptable level) × (number of fruit at this level)]/(highest level × total number of fruit in the treatment) × 100.

Statistical analysis

Statistical analysis was performed using Statistical Analysis System - version 9.4 (SAS Institute, Cary, NC, USA) and SPSS (ver 23, IBM, USA). All data were analysed for homogeneity of variance and then subjected to one-way analysis of variance (ANOVA). The mean values were evaluated by using least significant differences (LSD) test with $p < 0.05$ as the level of statistical significance.

Results and discussions

Weight loss

Weight loss is an important indicator of capsicum quality deterioration, as weight loss can lead to wilting and shrivelling which reduces both market value and consumer acceptability.Shrivel is due to moisture loss, and is a consequence of low storage humidity and is further exacerbated by the hollow nature of capsicum fruit (O'Donoghue, et al., 2013). Results in Table 1 show that after 11 days storage, capsicums stored in regular atmosphere pressure (101 kPa) at 20°C had significantly greater weight loss than fruits stored at 10°C under either low pressure (4 kPa) or regular pressure (101 kPa). The results are in accordance with previous research conducted on tomato which found weight loss to vary in proportion to storage temperature (De Castro et al., 2006).

In this study, low pressure storage did not significantly affect weight loss of capsicums stored at regular atmosphere at 10°C for 5 or 11 days. These findings are in agreement with previous findings by Hashmi et al. (2013) who reported that low pressure treatment did not affect the weight loss of strawberries. However these observations contradict findings reported by Hughes et al.,(1981) who found that weight loss in 'Bellboy' peppers stored in low pressures (5.1, 10.1 and 20.3 kPa) at 8.8°C (storage time not specified) was at least five times greater than control fruit stored under regular pressure conditions but the RH of this experiment were not reported. Laurin et al. (2006) who also reported that low pressure treatment (71 kPa, 6 hours, 20°C) increased weight loss of Alpha-type cucumbers. Further, (Burg, 2004) also reported that 'Acorn' squash stored at 7.33 – 8 kPa at 7ºC and 90-95% RH for 11 days experienced a weight loss of 4.2 %.

As expected in terms of storage time, fruit stored for 5 days resulted in significantly lower in weight loss than 11 days storage for fruits stored either at regular pressure at 20ºC or low pressure and regular pressure at 10°C. The results show that fruit stored at 20°C resulted in significantly higher weight loss than that stored at with low pressure or regular pressure atmosphere at 10°C and that the longer storage time increased weight loss regardless the pressures treatment during storage.

Colour

Skin colour is an important postharvest quality attribute for green capsicums as their quality is often determined based on appearance including skin colour. In this study, initial skin colour of green capsicums was uniformly dark green with a Hue angle of 121.0 (high hue value corresponds to dark green). However during storage, the skin colour turned partly yellow. This colour change was difficult to objectively assess using a colorimeter because of the non-uniformity of colour change, therefore skin colour change was assessed based on the grading scale (Figure 1).

The fruit's skin colour was assessed both immediately after capsicums were removed from low pressure treatment of 4 kPa at 10°C for 5 or 11 days, and after the fruit were transferred to 20°C at regular atmosphere (101 kPa) for 3 days. There was a significant difference between regular pressure at 20°C and low pressure storage (4 kPa) at 10°C after capsicums were stored for 5 and 11 days (Table 1). As expected the skin colour changes were greater when the fruit were stored subsequently for the additional 3 days at regular pressure 20°C. However there was no significant difference in colour changes observed between fruit stored at low pressure (4 kPa) and regular atmosphere pressure (101 kPa) at 10°C for both storage times of 5 and 11 days upon removal and after being transferred 3 days at regular pressure at 20°C. This observation is similar with previous study by Burg (2004) who reported that 'Neusiedler Ideal' peppers remained green after treatment at 10 kPa for 23 days at 10-12°C and 'Acorn' squash peel also remained green after fruit storage at low pressure of 7.33 – 8 kPa for 11 days at 7ºC.

Firmness

In this study, fruit firmness was assessed both immediately after capsicums were stored under low pressure of 4 kPa at 10°C for 5 or 11 days, and transferred to

20°C under regular pressure (101 kPa) for 3 days. The results of the objective measurement of fruit firmness are presented in Table 1 and show the maintenance of firmness in fruit stored at 10°C (4 and 101 kPa) compare to those stored at regular pressure at 20°C. However there was no significant difference in fruit firmness between fruit stored at low pressure storage (4 kPa) 10ºC and regular pressure (101 kPa) at 10°C storage temperature for both storage time of 5 and 11 days upon removal and after being stored 3 days at regular pressure at 20ºC. These observations are consistent with those previously reported by (Burg, 2004) who found that 'Neusiedler Ideal' peppers remained firm after storage at 10-12°C under 10 kPa for 23 days. Similarly, Hashmi et al. (2016) found that low pressure treatment (50 kPa) of strawberries had no beneficial effect on fruit firmness, whilst Pristijono et al., (2017b) reported that tomatoes firmness did not change with low pressure treatment (4 kPa, 10°C, 11 days).

Comparing the storage time, there was no significant difference in fruit firmness between capsicums stored at low pressure at 10°C for 5 and 11 days. This also relates to the water loss data, where there was no difference between the different treatment times, however future study needs to consider a longer time of storage for capsicums if the firmness is considered as a major quality parameter.

Flesh rots

There was no effect on flesh rots following treatment with at low pressure 10°C for 5 days upon removal, however when green capsicums treated with low pressure storage (4 kPa) at 10°C for 11 days flesh rots were significantly lower levels in comparison with the control fruit stored at regular atmosphere at both 10°C and 20°C and subsequently held at regular atmosphere at 20°C for 3 days (Figure 2). The results are agreement with previous report by (J. Wang et al., 2015) which found that 'Honey' peaches stored at low pressure of 10-80 kPa resulted in significantly lower level of fruits rots after 30 days storage at 0°C. Romanazzi et al. (2001) also reported that strawberries were stored at low pressure of 25 kPa at 20°C for four hours significantly reduced the percentage of fruits affected by grey mould as compared to control. The difference in flesh rots between regular pressure and low pressure at 10°C may due to low level of oxygen availability during the storage (less than 1 % O_2) because pathogen and spore germination has been shown to be inhibited when the level of oxygen is between 0.1 – 0.25% (Burg, 2004). Therefore the development of rots after removal from low pressure storage is slower than fruits stored continuously at atmospheric pressure (Figure 2b).

Comparing the level of flesh rots between 5 and 11 days storage, the results showed that after fruit was stored at low pressure (4 kPa, 10°C) for 5 days, there was no differential effect between low pressure and atmospheric pressure treatments on flesh rot. By contrast, fruits stored at low pressure (4 kPa) and 10°C for 11 days showed significantly lower incidence of flesh rots compared with fruit stored at 10°C at regular pressure. This observation continued in the fruit that was removed from low pressure and subsequently stored at regular pressure for 3 days at 20°C. The results show that low pressure treatment exerts a significant positive effect on reducing capsicum flesh rots after 11 days storage.

Stem decay

Stem freshness is another important quality parameter for capsicum fruit. The effect of low pressure storage on the incidence of stem decay in green capsicum is presented in Figure 3. The results show that low pressure storage (4 kPa, 10°C) did not significantly reduce the incidence of stem decay compared with fruit stored at regular pressure (101 kPa, 10°C) after 5 and 11 days storage upon removal. However fruit stored at regular atmosphere at 20°C had significantly higher stem decay incidence compared with fruit stored at 10°C (4 or 101 kPa).

Fruit treated with low pressure (4 kPa,10°C) had 9 % lower stem rots than fruit treated at regular pressure (101 kPa, at 10°C) for 5 and 11 days and subsequently stored at regular pressure at 20°C for a further 3 days. The constant low rate of stem decay may be affected by the decay incidence when fruits were stored at low pressure due to low oxygen level, therefore when fruits were transferred to regular pressure at 20°C, the decay rate of fruits were stored at low pressure and control fruits resume to the normal rate where the untreated fruits had already higher decay rate than fruits were stored at low pressure. Burg (2004) reported that fungus growth resumed at the normal rate after the fungus were transfered from low pressure to regular pressure atmosphere. The findings of the current studies are consistent with a previous report by Pristijono et al. (2017b) who demonstrated that tomatoes stored at low pressure (4 kPa, 10°C) for 11 days reduced the incidence of calyx rots. While the findings around low pressure treatment are promising, further mechanistic studies are required to fully understand the mode of action associated with the reduction in stem decay.

Acceptability index

Overall acceptability of the fruit was visually assessed based on the combination of flesh rot, stem decay and skin discolouration. The impact of low pressure storage on overall visual acceptability of green capsicums is presented in Figure 3 and shows that green capsicums which were stored at 10°C (4 or 101 kPa) had higher overall aceptability levels than fruits which were stored at regular pressure (101 kPa) atmosphere 20°C after 5 and 11 days storage upon removal. The higher level of acceptability was found in fruit treated at low pressure (4 kPa, 10°C) and subsequently stored at regular pressure (101 kPa) at 20°C for 3 days, with the acceptability indices of 81 and 76 % for storage times of 5 and 11 days respectively. These results are consistent with (Burg, 2004) who reported that peppers stored at low pressure of 12.7 kPa at 7.2°C exhibited better fruit condition than fruit stored at regular pressure. In this study, overall acceptability results were associated with reduced stem decay, lower levels of flesh rots and skin degreening. These findings show that green capsicums stored at a pressure of 4 kPa combined and temperature of 10°C for at least 5 days improved fruit acceptability by maintaining overall freshness and acceptability.

Conclusions

In conclusion, the low pressure treatment of 4 kPa at 10°C for 5 or 11 days maintained the quality of capsicums during storage. Low pressure storage reduced the incidence of flesh rots, stems decay and increased acceptability. Low pressure treatment also maintained the fruit firmness and colour retention and reduced weight loss relative to regular atmosphere storage. These were also maintained with a subsequent shelf life assessment for three days at 20°C in regular atmosphere (101 kPa). However, except for the occurring flesh rots incidence, there was no further benefit to store green capsicums at low pressure more than 5 days at 10°C.

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Table 1. The weight loss, firmness and colour changes of green capsicums after stored at low pressure.

Treatments	Weight loss (%)	Firmness (N)	Colour Retention (%)
Upon removal			
101 kPa 20°C, 5 days	0.5^{a}	21.4^{a}	79^{a}
101 kPa 10°C, 5 days	0.3^{b}	25.4^{a}	94^{ab}
4 kPa 10°C, 5 days	0.5^{a}	22.5^{a}	98^{b}
Additional storage 3 days at 101 kPa 20°C			
101 kPa 20°C, 5 days	0.9^{a}	18.5^{a}	69^{a}
101 kPa 10°C, 5 days	1.0^{a}	25.8^{b}	94^{b}
4 kPa 10°C, 5 days	0.9^{a}	26.4^{b}	94^{b}
Upon removal			
101 kPa 20°C, 11 days	1.1^{a}	20.1^{a}	83^{a}
101 kPa 10°C, 11 days	1.0^{b}	23.0^{b}	94^{ab}
4 kPa 10°C, 11 days	0.7^{b}	22.0^{ab}	100^{b}
Additional storage 3 days at 101 kPa 20°C			
101 kPa 20°C, 11 days	3.0^{a}	17.5^{a}	66^{a}
101 kPa 10°C, 11 days	1.7^{b}	21.3^{b}	83^{b}
4 kPa 10°C, 11 days	1.4^{b}	21.5^{b}	91^{b}

Values are the mean of 3 replicates with 16 fruits in each replicate and the different letters indicate significant differences between treatments for each storage time ($p < 0.05$).

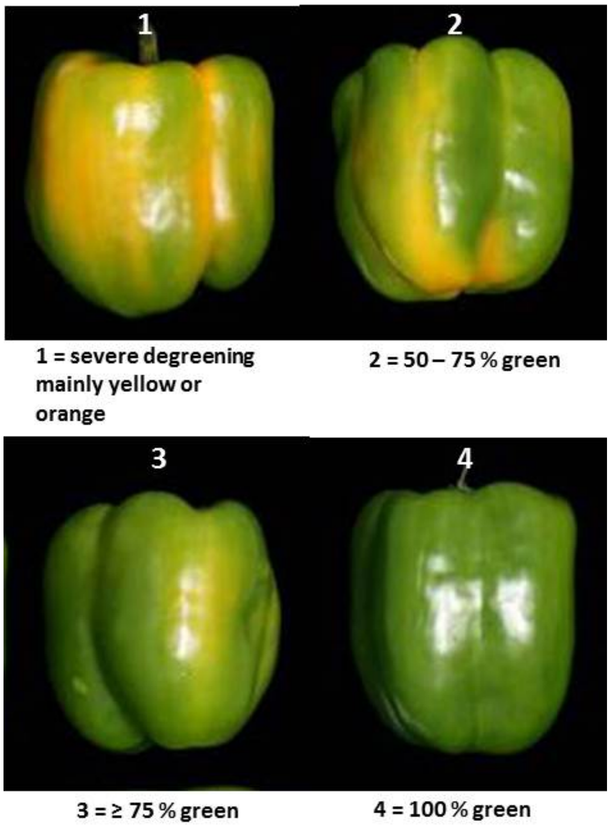

Figure 1. The green capsicums grading scale for skin degreening.

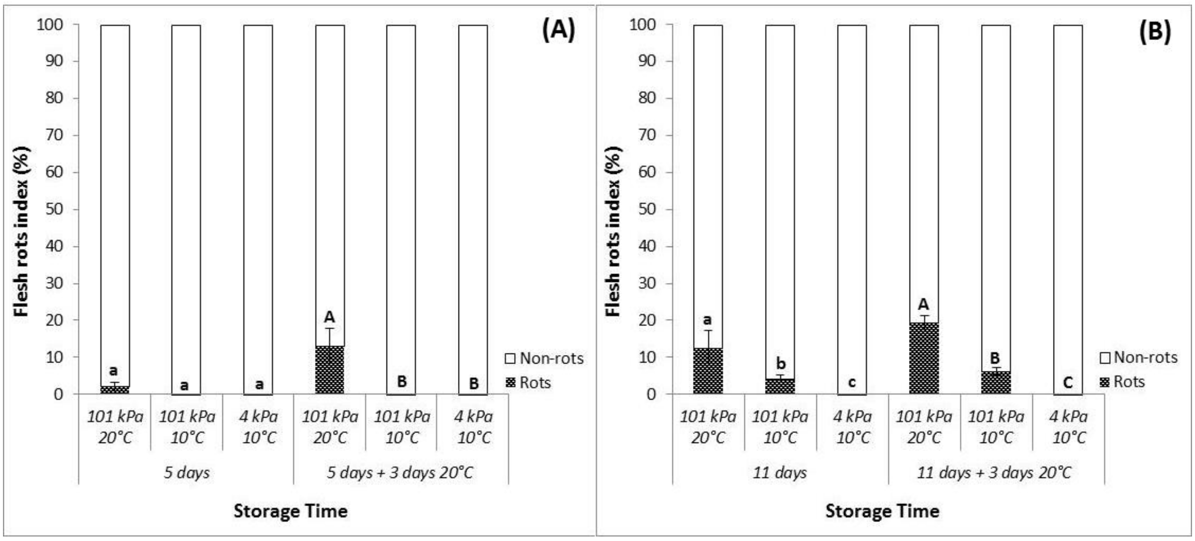

Figure 2. The capsicums flesh rots after stored for (A) 5 and (B) 11 days at different pressure and temperature. The values are the mean of three replicates and the different letters indicate significant differences between treatments for each storage time ($p < 0.05$).

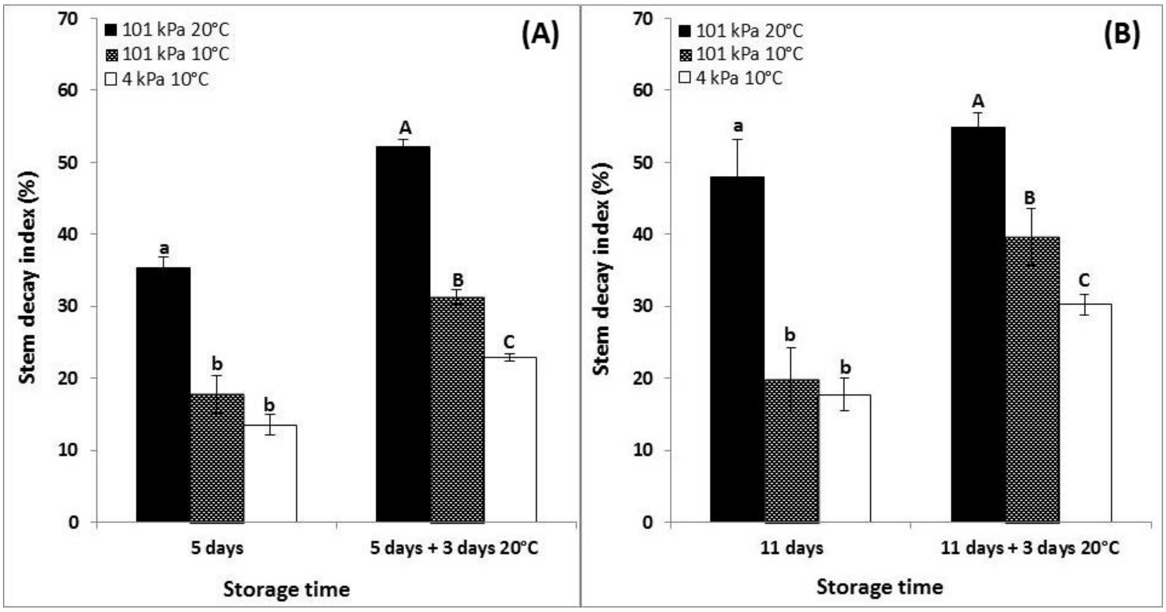

Figure3. The stem decay index of green capsicums after stored for (A) 5 and (B) 11 days at different pressure and temperature. The values are the mean of three replicates and the different letters indicate significant differences between treatments for each storage time ($p < 0.05$).

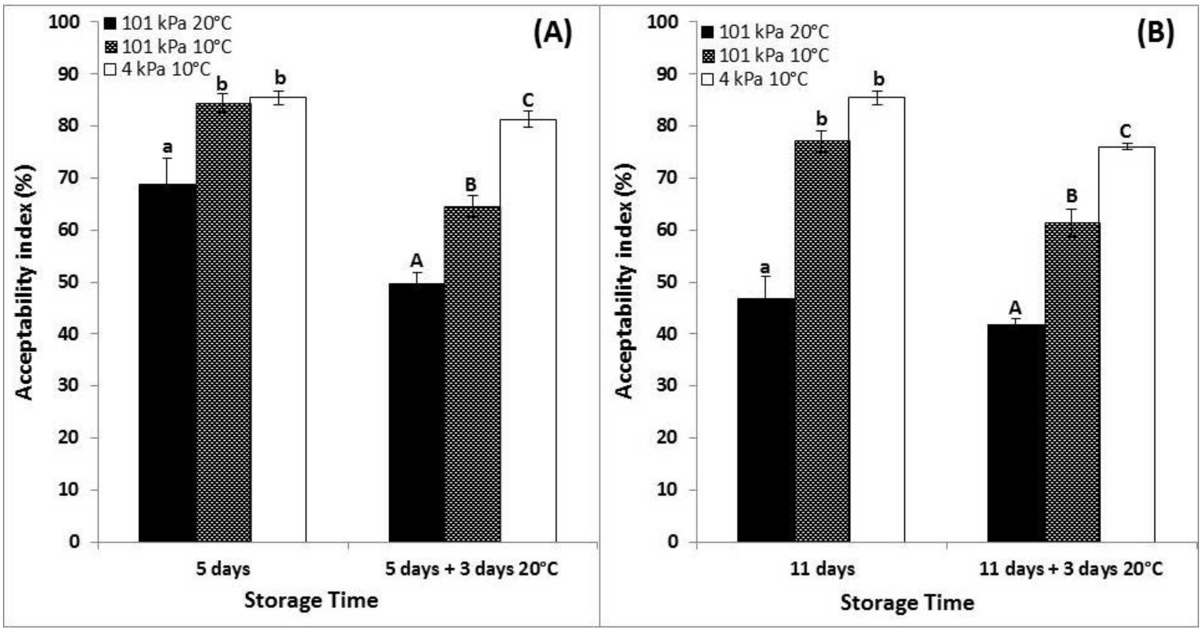

Figure 4. The overall acceptability index of green capsicums after stored for (A) 5 and (B) 11 days at different pressure and temperature. The values are the mean of three replicates and the different letters indicate significant differences between treatments for each storage time ($p < 0.05$).