The application of low pressure storage to maintain the quality of zucchinis

Penta Pristijono, Michael C. Bowyer, Christopher J. Scarlett, Quan V. Vuong, Costas E. Stathopoulos and John B. Golding

This is an Accepted Manuscript of an article published by Taylor & Francis in New Zealand Journal of Crop and Horticultural Science on 11th October 2017, available online: http://wwww.tandfonline.com/10.1080/01140671.2017.1383277

The application of low pressure storage to maintain the quality of zucchinis

Penta Pristijono[1*], Michael C. Bowyer[1], Christopher J. Scarlett[1], Quan V. Vuong[1], Costas E. Stathopoulos[2], and John B. Golding[1,3]

[1]School of Environmental and Life Sciences, University of Newcastle, Ourimbah, NSW 2258, Australia

[2]School of Science, Engineering and Technology, University of Abertay, Dundee DD1 1HG, UK

[3]NSW Department of Primary Industries, Gosford, NSW 2250, Australia

*Corresponding author: Dr Penta Pristijono; email: penta.pristijono@newcastle.edu.au; School of Environmental and Life Sciences, University of Newcastle, PO Box 127, Ourimbah - NSW 2258, Australia.

Abstract

Zucchini (*Cucurbita pepo* var. cylindrica) were stored at low pressure (4 kPa) at 10°C at 100% relative humidity (RH) for 11 days. Fruit quality was examined upon removal and after being transferred to normal atmosphere (101 kPa) at 20°C for three days. Zucchinis stored at low pressure exhibited a 50% reduction in stem-end browning compared with fruit stored at atmospheric pressure (101 kPa) at 10°C. The benefit of low pressure treatment was maintained after the additional three days storage at normal atmospheric pressure at 20°C. Indeed, low pressure treated fruit transferred to regular atmosphere 20°C for three days possessed a significantly lower incidence of postharvest rot compared to fruit stored at regular atmospheric pressure at 10°C. Zucchinis stored at low pressure showed higher levels of acceptability (28% and 36 % respectively) compared to fruit stored at regular atmospheres at 10°C for both assessment times.

Keywords: postharvest; storage; refrigeration; vegetables; stem-browning

Introduction

Zucchini, also known as courgette (*Cucurbita pepo* var. cylindrica) are an important vegetable crop around the world (Esquinas-Alcazar and Gulick, 1983). Zucchini is a non-climacteric fruit that is harvested at an immature stage, when the fruit reaches an average length of about 20 cm and the rind is still tender and edible (de Jesús Avena-Bustillos et al., 1994; Megías et al., 2015). The thin skin of the fruit offers little barrier to water loss, leading to desiccation and rapidly softening if not refrigerated (Occhino et al., 2011).

However to store many chilling sensitive fruits and vegetables at low but non-freezing temperatures induces fruit damage known as chilling injury (CI) (Sevillano et al., 2009). Zucchini fruit is particularly susceptible to this physiological disorder which is characterised by water loss, flesh rot, flesh softening and pitting of the fruit skin (Martínez-Téllezet al., 2002; Serrano et al., 1998). Carvajal et al. (2015) reported that zucchini fruits stored at 4ºC for 3 days showed skin damaged due to CI. A minimum temperature of 7^{o}C for commercial storage of zucchini is recommended to prevent significant economic loss (McCollum, 1990).

Low pressure treatment has been studied as a method for maintaining postharvest quality in fruits and vegetables (Burg 2004). Low pressure storage has been known for many years and is a re-emerging technique that is homogeneous in application (Vigneault et al., 2012) which can rapidly remove the heat and reduce the concentration of oxygen and other harmful gases from the immediate storage environment (Wang et al., 2001). Many modern low pressure treatment systems are now capable of maintaining high humidity levels within the treatment chamber, which reduces water loss and wilting in the produce and reduces respiration and endogenous ethylene production to delay fruit ripening (Burg, 2004). Low pressure storage can also reliably and consistently adjust the internal temperature and composition of the storage atmosphere (Li et al., 2006).

There is limited scientific literature regarding the effect of low pressure storage on the quality of zucchinis. However, there are reports on the effect of low pressure storage on the quality of Cucurbitacea of which zucchini is a family member. For example, low pressure treatment improved the quality of "Acorn" squash (McKeown & Lougheed, 1981) and cucumbers (Burg, 2004). However Burg (2004) observed that there was no quality improvement for "Yellow crookneck" squash stored at low pressure. The objective of this study was to examine the effectiveness of low pressure storage (4kPa) at 10°C for 11 days with an additional short shelf-life at regular pressure (101 kPa) at 20°C to maintain zucchini fruit quality postharvest.

Materials and methods

Fruits

Fresh, locally grown zucchini fruit (*Cucurbita pepo* var. cylindrica*)* free from damage and uniform in shape and size were obtained from a local commercial grower. Fruits between 20 and 22 cm in length and non-blemished were randomly selected, weighed and sorted into treatment units of 12 fruits.

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 equipped with an air flow controller to adjust the air exchange rate to prevent build-up of metabolic gases such as ethylene. A humidifier was used to ensure that inflowing air was correctly humidified before entering the low pressure chamber. 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 recorded. The six different chambers were located inside two different cool rooms held at 10°C.

Experimental procedures of storage

Individual experiments consisted of three different treatments; (a) control of fruit placed on a plastic tray at 101 kPa at 20°C and 96% RH, (b) control of fruit placed on a plastic tray at 101 kPa at 10°C and 94% RH and (c) placed in an unsealed plastic container (45 cm x 20 cm x 15 cm) stored in the low pressure chamber at 4 kPa, 10°C and 100 % RH. Controls (a) and (b) were covered with a loose low density polyethylene (LDPE) plastic bag (66 cm x 58 cm) to maintain RH around the produce during storage. Temperature and RH were monitored with calibrated TinyTag View 2 loggers. The experiment was replicated three times, where each replicate used a different independent low pressure chamber. The fruit was assessed immediately upon removal from storage after 11 days and again after additional three days storage in air at regular pressure (101 kPa) and temperature (20°C).

Fruit quality assessment

Fruit quality assessments parameters included; weight loss, stem-end browning, colour, blossom-end rot, fruit firmness and overall acceptability. Weight loss was calculated as a percentage based on the initial weight of zucchinis and weight after storage.

The incidence of flesh (blossom end) rot was assessed visually and scored (1-5) based on the percentage of total blossom end area affected by black or white rot;1 = severe rot (> 50 % affected); 2 = moderate rot (noticeable white or black rot of 30 – 50 %); 3 = slight rot (noticeable white or black rot of 10 – 30 %);4 = slight rot (small white or black spot); and 5 = no rot. Flesh rot index was calculated according to Wang et al., (2015), with slight modifications as shown in Equation 1.

$$Rots\ index\ (\%)\ =\ \left(\frac{Rot\ score\ in\ each\ fruit \times number\ of\ fruit\ at\ the\ same\ rot\ score}{highest\ rot\ score \times number\ of\ fruit\ in\ the\ treatment}\right) \times 100 \qquad (1)$$

Stem-end discolouration was subjectively evaluated using a grading scale from 1 to 5, where 1 = severe browning (> 60 % browned); 2 = moderate browning affecting 20 – 60 % stem; 3 = browning affecting < 20 % stem; 4 = slight browning (no longer bright); and 5 = no browning. Stem-end browning was calculated according to Pristijono et al. (2017), with slight modifications, as shown in Equation 2.

$$Browning\ index\ (\%) = \left(\frac{Browning\ level\ in\ each\ fruit\ \times number\ of\ fruit\ at\ the\ same\ browning\ level}{Highest\ browning\ level\ \times\ total\ number\ of\ fruit\ in\ the\ treatment}\right) \times 100 \qquad (2)$$

Zucchini firmness was determined using a texture analyser (Lloyd Texture Analyser, Fireman, UK) and estimated as the average maximum force (Newton) required to push a 7 mm probe into the fruit flesh to a depth of 2 mm. The average was gained from 2 reading points taken from each side of the fruit at a distance of 5 cm from the blossom-end.

Skin colour (Hue angle, °Hue) was measured with a Minolta colorimeter (Minolta CR-400, Osaka) using the average of four point measurements taken at a distance of 5 cm from blossom end of the fruit.

The acceptability index was estimated based on the fruit freshness combination of the level of stem-end browned, blossom-end flesh rotted and skin discolouring, scoring from 1 to 4, where, score 1= poor, not edible; 2 = not saleable but edible, acceptable for cooking; 3 = saleable, good marketable; and 4 = excellent fresh with no symptoms of flesh rots and discolouration. The overall acceptability index of fruit was assessed according to Pristijono et al. (2017), with slight modifications as shown in Equation 3.

$$Acceptability\ index\ (\%) = \left(\frac{Acceptability\ in\ each\ fruit \times number\ of\ fruit\ at\ the\ same\ acceptable\ level}{Highest\ acceptable\ level \times number\ of\ fruit\ in\ the\ treatment}\right) \times 100 \quad (3)$$

Statistical analysis

Statistical analysis was performed using Statistical Analysis System - version 9.4 (SAS Institute, Cary, NC, USA) and SPSS (ver 23, IBM, USA). One-way ANOVA was used to analyse the data. The mean values were evaluated by using least significant differences (LSD) test with $p < 0.05$ as statistical significance.

Results and discussion

Colour

Fruit colour was assessed upon removal from low pressure storage and again after being stored at atmospheric pressure (101 kPa) at 20°C for three days. There was no significant difference in peel colour between fruit subject to low pressure storage (4kPa) 10ºC and fruit stored under regular atmospheric pressure (101 kPa) either at

10°C or 20°C storage temperature (data not shown). Hue angle did not change significantly during storage at low pressure (4 kPa) and regular pressure (101 kPa) at 10°C for 11 days, remaining at a constant value of 122. These observations are in agreement with previous studies by Burg (2004) who showed that the peel of “Acorn” squash remained green after fruits were stored at low pressure of 7.33 – 8 kPa for 11 days at 7ºC.

Weight loss

Weight loss is a complex phenomenon propagating from mechanical, biological and physical interactions. Weight loss can lead to wilting and shrivelling, both of which reduce market value and consumer acceptability. Postharvest weight loss in vegetables is usually due to the loss of water through transpiration (Znidarcic et al., 2010). After 11 days storage zucchinis stored at regular atmospheric pressure (101 kPa) at 20°C resulted in greater weight loss than fruit were stored at 10°C at pressures of 4 and 101 kPa (Table 1). The results are in agreement with studies by De Castro et al. (2006) who demonstrated that weight loss in tomato fruits stored at different temperatures was proportional to the storage temperature.

The results presented in Table 1 show that water loss from the fruit stored in the low pressure storage (4 kPa, 10°C) was higher than those stored at regular atmosphere (101 kPa) at 10°C upon removal. This finding is in agreement with previous research by Laurin et al. (2006) who reported that low pressure treatment of “Alpha-type” cucumbers (70 kPa for 6 hours) increased weight loss. However it is very important to consider all the variables assiociated with water loss and vapour presseure deficit, and care should be taken when comparing studies.

In this study after an additional storage for three days at normal pressure (101 kPa) at 20°C, the fruit previously stored at low pressure did not show significant differences in weight loss to zucchinis that were stored at regular atmosphere at 10°C. This observation is similar to report by Hashmi et al. (2013) who observed that the low pressure treatment did not affect the weight loss of strawberries. However, these observations contradict previous reports by Burg (2004) who reported that “Acorn” squash stored under pressure of 7.33 – 8 kPa at 7ºC and 90-95% RH for 11 days resulted in loss of 4.2 % its weight.

Firmness

Fruit firmness was assessed both immediately after the zucchinis were removed from low pressure storage (10°C, 11 days) and again three days after transfer to storage atmosphere (101 kPa) and 20°C. Fruit stored at 10°C under low pressure maintained higher firmness values than fruit stored at regular atmosphere (101 kPa) at 20°C (Table 1). The maintenance of fruit firmness was more obvious after the additional shelf-life storage at 20°C for three days, with the low pressure treated fruit exhibiting significantly greater firmness ($p<0.05$). However there was no difference in firmness between fruits stored at low pressure (4 kPa, 10°C) and regular pressure (101 kPa) at 10°C. The findings are in agreement with previous work by Hashmi et al. (2016) who found that low pressure treatment (50 kPa) of strawberries had no beneficial effect of fruit firmness. In this study, the differences in fruit firmness between low pressure (4 kPa, 10°C) and regular pressure (101 kPa, 20°C) treatments maybe a result of difference in water loss.

Blossom-end flesh rots

Zucchini fruits are highly perishable where postharvest decay such as blossom-end flesh rots, fungal decay including black rot, cottony leak and bacterial soft rots are the principal factors contributing to spoilage (Burg, 2004). Low pressure treatment of other horticultural produce such as cucumbers and bananas have been shown to improved freshness, taste and flavour and reduced the incidence of deterioration attributable to bacterial and fungal infection (Burg, 2004). In this study, zucchini fruit exposed to low temperature reduced the incidence of blossom-end rot (Figure 1). Further, the incidence of rot in the low pressure treated fruit stored for an additional three days at atmospheric pressure (101 kPa) and 20°C was significantly lower than control fruit stored at 101 kPa and 10°C. The findings are in agreement reports by Wang et al. (2015) who found that honey peaches stored at low pressure (10-80 kPa) at 0°C for 30 days produced a significantly lower incidence of fruit rot. Hashmi et al. (2016) also reported similar findings for strawberries treated at 50 kPa at 5ºC for 4 hours and subsequently stored at 20ºC.

Differing levels of flesh rot between treatments stored at atmospheric and low pressure at 10°C after removal to 20°C may be due to reduced oxygen availability during low pressure treatment, where the oxygen (O_2) levels at 4 kPa are approximately 1 % O_2 (v/v). Burg (2004) has previously reported that low oxygen storage conditions (0.1 – 0.25% O_2) have significantly inhibitory effects on pathogen and spore germination.

Stem-end browning

The fresh appearance of the stem-end of zucchini fruit is a major determinant in assessing fruit quality and acceptability. Low pressure storage at 10°C resulted in significantly lower levels of stem-end browning compared to storage at 10°C under normal atmospheric pressure (101 kPa), which were further significantly lower than storage at 20°C (Figure 2). These observations were similar immediately upon removal and after an additional three days storage at 20°C, where the additional time resulted in an increase in stem-end browning, but the differences between the treatments remained the same. These findings are consitent with Gao et al. (2006) who observed that low pressure storage conditions (40 – 50 kPa, 4ºC for 49 days) significantly reduced the incidence of browning in loquat fruit. However further mechanistic studies are required to determine whether a similar or different pathway for low pressure storage action occurs in reducing browning in stem-end of zucchinis.

Acceptability index

The overall acceptability of the zucchini fruit was visually assessed based on a combination of flesh rots and stem discolouration. Fruit stored at low pressure for 11 days had higher overall aceptability levels than fruit stored at atmospheric pressure for the same time period, either at 10°C or 20°C (Figure 3). Further, zucchinis previously stored at low pressure for 11 days at 10ºC, followed by subsequent storage of the atmospheric pressure (101 kPa) for a further three days at 20°C showed the highest acceptability index (79 %) of all experimental treatments.These overall acceptability results were associated with reduced stem-end browning during storage and lower levels of blossom-end flesh rot. These results show that zucchini fruit stored at low pressure (4 kPa) combined with temperature storage of 10°C improved fruit quality by maintaining overall freshness and acceptability.

Conclusions

In conclusion, the low pressure storage of 4 kPa at 10°C for 11 days maintained the quality of zucchinis during storage by reducing flesh rots, stem-end browning and increased acceptability. This benefit was maintained with a subsequent shelf life assessment for three days at 20°C in regular atmosphere (101 kPa).The low pressure storage also maintained firmness, colour and weight loss, similar to regular atmosphere storage. Thus, the results of this experiment support the application of low pressure storage for horticultural produce, but large scale experiments are required to be conducted for the commercial validation and optimisation of low pressure storage.

Acknowledgements

The author would like to thank John Archer, David Cruikshank and Christine Cruickshank at NSW DPI for assistance with the running and maintenance of the low pressure chambers.

Funding

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 Centre for Food and Beverage Supply Chain Optimisation (IC140100032).

References

Burg SP 2004. Postharvest physiology and hypobaric storage of fresh produce. E-Book. CABI Publisher.

Carvajal F, Palma F, Jamilena M, Garrido D 2015. Cell wall metabolism and chilling injury during postharvest cold storage in zucchini fruit. Postharvest Biology and Technology 108: 68-77. doi: http://dx.doi.org/10.1016/j.postharvbio.2015.05.013.

De Castro L, Cortez L, Vigneault C 2006. Effect of sorting, refrigeration and packaging on tomato shelf life. Journal of Food, Agriculture and Environment 4: 70-74.

de Jesús Avena-Bustillos R, Krochta JM, Saltveit ME, de Jesús Rojas-Villegas R, Sauceda-Pérez J 1994. Optimization of edible coating formulations on zucchini to reduce water loss. Journal of Food Engineering 21: 197-214. doi: http://dx.doi.org/10.1016/0260-8774(94)90186-4.

Esquinas-Alcazar JT, Gulick. PJ 1983. Genetic resources of cucurbitaceae. International Board for Plant Genetic Resources, Rome, Italy.

Gao HY, Chen HJ, Chen WX, Yang YT, Song LL, Jiang YM, Zheng YH 2006. Effect of hypobaric storage on physiological and quality attributes of loquat fruit at low temperature. Acta Horticulturae 712: 269-274. doi: 10.17660/ActaHortic.2006.712.29.

Hashmi MS, East AR, Palmer JS, Heyes JA 2013. Hypobaric treatment stimulates defence-related enzymes in strawberry. Postharvest Biology and Technology 85: 77-82. doi: http://dx.doi.org/10.1016/j.postharvbio.2013.05.002.

Hashmi MS, East AR, Palmer JS, Heyes JA 2016. Hypobaric treatments of strawberries: A step towards commercial application. Scientia Horticulturae 198: 407-413. doi: http://dx.doi.org/10.1016/j.scienta.2015.12.017.

Laurin É, Nunes MCN, Émond JP, Brecht JK 2006. Residual effect of low-pressure stress during simulated air transport on Beit Alpha-type cucumbers: Stomata behavior. Postharvest Biology and Technology 41: 121-127. doi: http://dx.doi.org/10.1016/j.postharvbio.2005.09.012.

Li W, Zhang M, Yu H 2006. Study on hypobaric storage of green asparagus. Journal of Food Engineering 73: 225-230. doi: http://dx.doi.org/10.1016/j.jfoodeng.2005.01.024.

Martínez-Téllez MA, Ramos-Clamont MG, Gardea AA, Vargas-Arispuro I. 2002. Effect of infiltrated polyamines on polygalacturonase activity and chilling injury responses in zucchini squash (Cucurbita pepo L.). Biochemical and Biophysical Research Communications 295: 98-101. doi: https://doi.org/10.1016/S0006-291X(02)00631-9.

McCollum T G 1990. Gene B influences susceptibility to chilling injury in cucurbita pepo. Journal of the American Society for Horticultural Science 115: 618-622.

McKeown A, Lougheed E 1981. Low Pressure Storage of Some Vegetables. Acta Horticulturae 116: 83-100. doi: 10.17660/ActaHortic.1981.116.12.

Megías Z, Barrera A, Manzano S, Martínez C, Garrido D, Valenzuela JL, Jamilena M 2015. Physical and chemical treatments enhancing postharvest fruit quality in zucchini. Acta Horticulturae 1091: 141-146. doi : 10.17660/ActaHortic.2015.1091.17.

Occhino E, Hernando I, Llorca E, Neri L, Pittia P 2011. Effect of Vacuum impregnation treatments to improve quality and texture of zucchini (Cucurbita Pepo, L). Procedia Food Science 1: 829-835. doi: http://dx.doi.org/10.1016/j.profoo.2011.09.125.

Pristijono P, Scarlett CJ, Bowyer MC, Vuong QV, Stathopoulos CE, Jessup AJ, Golding JB 2017. Use of low pressure storage to improve the quality of tomatoes. The Journal of Horticultural Science and Biotechnology :1-8. doi: http://dx.doi.org/10.1080/14620316.2017.1301222.

Serrano M, Pretel MT, Martínez-Madrid MC, Romojaro F, Riquelme F 1998. CO_2 Treatment of zucchini squash reduces chilling-induced physiological changes. Journal of Agricultural and Food Chemistry 46: 2465-2468. doi: 10.1021/jf970864c.

Sevillano L, Sanchez-Ballesta MT, Romojaro F, Flores FB 2009. Physiological, hormonal and molecular mechanisms regulating chilling injury in horticultural species. Postharvest technologies applied to reduce its impact. Journal of the Science of Food and Agriculture 89: 555-573. doi: 10.1002/jsfa.3468.

Vigneault C, Leblanc DI, Goyette B, Jenni S 2012. Invited review: engineering aspects of physical treatments to increase fruit and vegetable phytochemical content. Canadian Journal of Plant Science 92: 373–397.

Wang J, You Y, Chen W, Xu Q, Wang J, Liu Y, Song L, Wu J 2015. Optimal hypobaric treatment delays ripening of honey peach fruit via increasing endogenous energy status and enhancing antioxidant defence systems during storage. Postharvest Biology and Technology 101: 1-9. doi: http://dx.doi.org/10.1016/j.postharvbio.2014.11.004.

Wang LP, Zhang P, Wang SJ 2001. Advances in research on theory and technology for hypobaric storage of fruit and vegetable. Storage and Process 5: 3-6.

Znidarcic D, Ban D, Oplanic M, Karic L, Pozrl T 2010. Influence of postharvest temperatures on physicochemical quality of tomatoes (Lycopersicon esculentum Mill.). Journal of Food, Agriculture and Environment 8: 21-25.

List of Tables

List of Figures

Table 1. Effect of low pressure storage on zucchinis' weight loss and firmness on different assessment day at 20°C.

Treatments	Weight loss (%)	Firmness (N)
Time zero	-	69.1
Upon removal		
101 kPa 20°C, 11 days	2.5	63.1
101 kPa 10°C, 11 days	1.5	65.3
4 kPa 10°C, 11 days	1.8	67.5
LSD (5%)	± 0.2	± 3.3
Additional storage 3 days at 101 kPa 20°C		
101 kPa 20°C, 11 days	3.0	52.9
101 kPa 10°C, 11 days	1.9	63.8
4 kPa 10°C, 11 days	2.1	68.0
LSD (5%)	± 0.4	± 7.5

Values are the mean of 3 replicates with 12 fruits in each replicate.

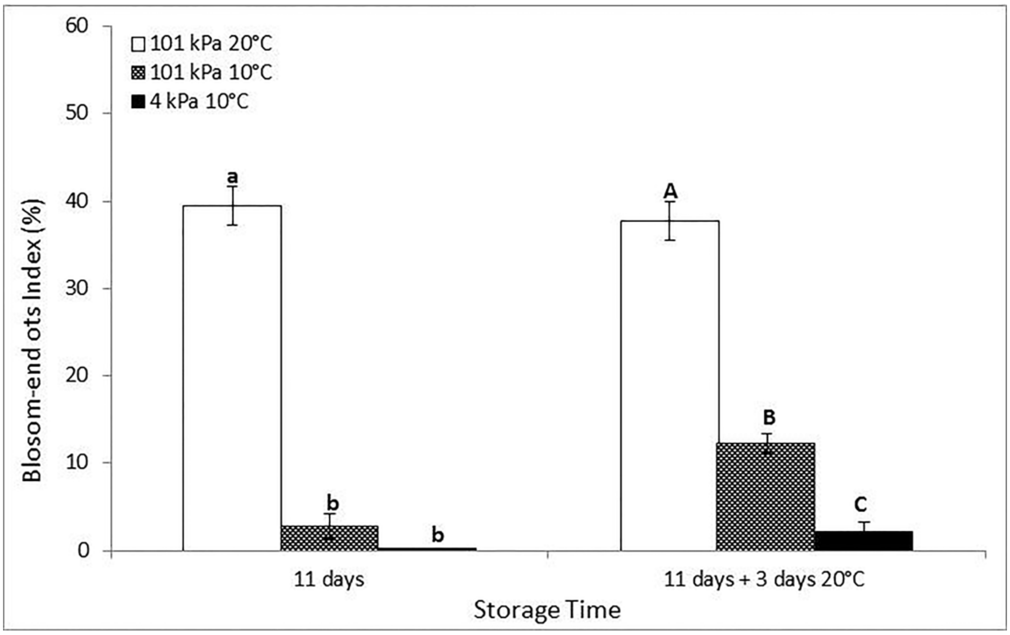

Figure 1. The blossom-end rotting index of zucchinis exposed to different treatments. The values are the mean of three replicates. The different letters indicate significant differences between treatments for each storage time ($p < 0.05$).

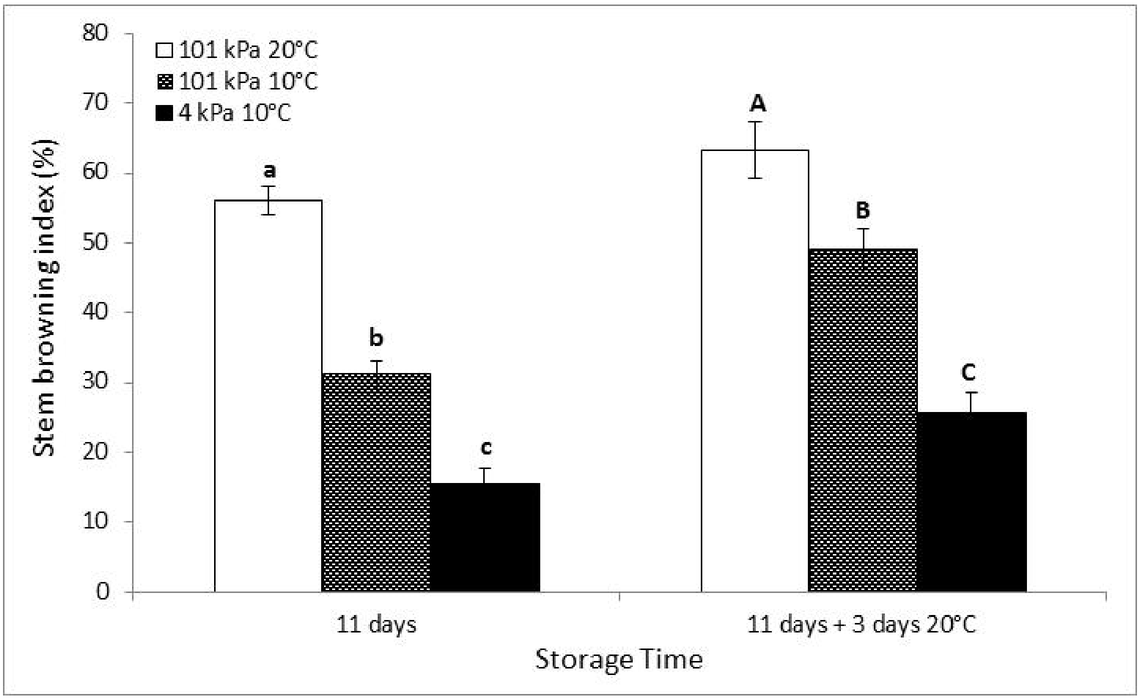

Figure 2. The stem-end browning index of zucchinis exposed to different treatments. The values are the mean of three replicates. The different letters indicate significant differences between treatments for each storage time ($p < 0.05$).

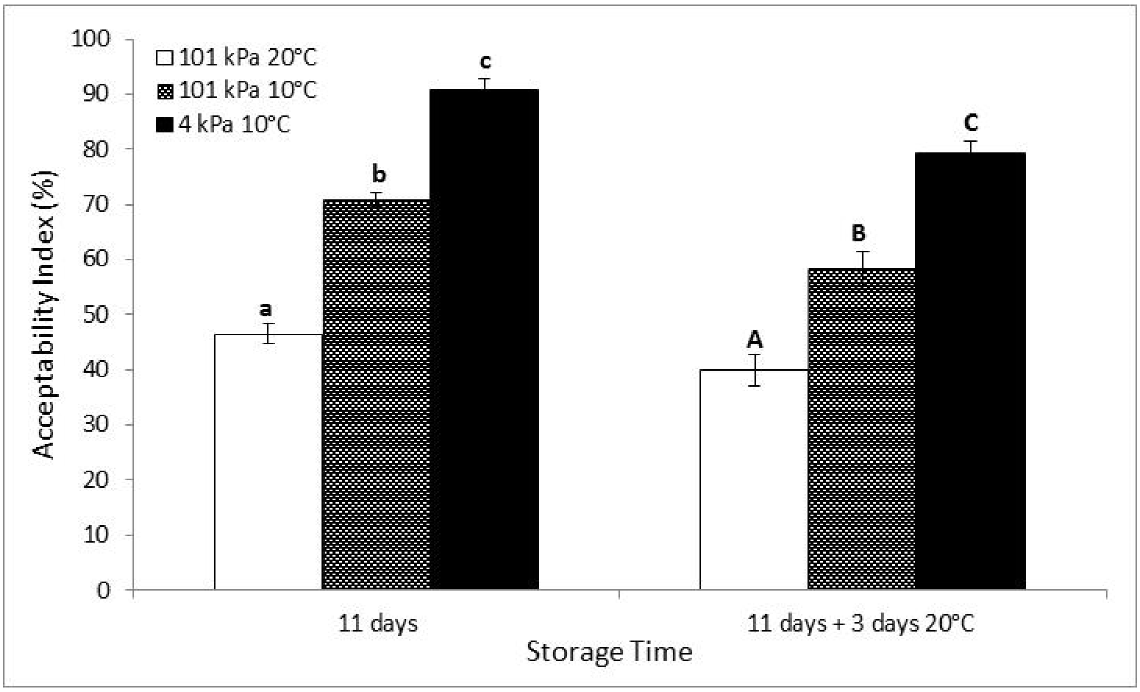

Figure 3. The acceptability index of zucchinis exposed to different treatments. The values are the mean of three replicates. The different letters indicate significant differences between treatments for each storage time ($p < 0.05$).