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

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15

16 **Abstract**

17 Zucchini (*Cucurbita pepo* var. *cylindrica*) were stored at low pressure (4 kPa) at
18 10°C at 100% relative humidity (RH) for 11 days. Fruit quality was examined upon
19 removal and after being transferred to normal atmosphere (101 kPa) at 20°C for three
20 days. Zucchini stored at low pressure exhibited a 50% reduction in stem-end browning
21 compared with fruit stored at atmospheric pressure (101 kPa) at 10°C. The benefit of
22 low pressure treatment was maintained after the additional three days storage at normal
23 atmospheric pressure at 20°C. Indeed, low pressure treated fruit transferred to regular
24 atmosphere 20°C for three days possessed a significantly lower incidence of postharvest
25 rot compared to fruit stored at regular atmospheric pressure at 10°C. Zucchini stored at
26 low pressure showed higher levels of acceptability (28% and 36 % respectively)
27 compared to fruit stored at regular atmospheres at 10°C for both assessment times.

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

29 **Introduction**

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

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

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

54 reliably and consistently adjust the internal temperature and composition of the storage
55 atmosphere (Li et al., 2006).

56 There is limited scientific literature regarding the effect of low pressure storage
57 on the quality of zucchinis. However, there are reports on the effect of low pressure
58 storage on the quality of Cucurbitacea of which zucchini is a family member. For
59 example, low pressure treatment improved the quality of “Acorn” squash (McKeown &
60 Loughheed, 1981) and cucumbers (Burg, 2004). However Burg (2004) observed that
61 there was no quality improvement for “Yellow crookneck” squash stored at low
62 pressure. The objective of this study was to examine the effectiveness of low pressure
63 storage (4kPa) at 10°C for 11 days with an additional short shelf-life at regular pressure
64 (101 kPa) at 20°C to maintain zucchini fruit quality postharvest.

65

66 **Materials and methods**

67

68 *Fruits*

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

73

74 *Low pressure storage system*

75 A laboratory scale low pressure system (VivaFresh™) with six identical low
76 pressure aluminium chambers (0.61 L × 0.43 W × 0.58 H m³) was used in this study.
77 Low pressure was achieved with a two-stage rotary vacuum pump (Model 2005I,
78 Alcatel Adixen, USA) regulated by a compact proportional solenoid valve controlled by

79 a proportional/integral/derivative (PID) computer control system equipped with an air
80 flow controller to adjust the air exchange rate to prevent build-up of metabolic gases
81 such as ethylene. A humidifier was used to ensure that inflowing air was correctly
82 humidified before entering the low pressure chamber. Relative humidity was measured
83 with a wet-bulb and dry-bulb temperatures using calibrated YSI 55000 Series GEM
84 thermistors. Sensors inside the low pressure chambers were used to record the
85 temperature, humidity and pressure during treatment. All data from temperature and
86 pressure sensors in the low pressure system were recorded. The six different chambers
87 were located inside two different cool rooms held at 10°C.

88

89 *Experimental procedures of storage*

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

101

102 *Fruit quality assessment*

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

107 The incidence of flesh (blossom end) rot was assessed visually and scored (1-5)
108 based on the percentage of total blossom end area affected by black or white rot; 1 =
109 severe rot (> 50 % affected); 2 = moderate rot (noticeable white or black rot of 30 – 50
110 %); 3 = slight rot (noticeable white or black rot of 10 – 30 %); 4 = slight rot (small white
111 or black spot); and 5 = no rot. Flesh rot index was calculated according to Wang et al.,
112 (2015), with slight modifications as shown in Equation 1.

$$113 \quad \text{Rots index (\%)} = \left(\frac{\text{Rot score in each fruit} \times \text{number of fruit at the same rot score}}{\text{highest rot score} \times \text{number of fruit in the treatment}} \right) \times 100 \quad (1)$$

114 Stem-end discolouration was subjectively evaluated using a grading scale from 1
115 to 5, where 1 = severe browning (> 60 % browned); 2 = moderate browning affecting
116 20 – 60 % stem; 3 = browning affecting < 20 % stem; 4 = slight browning (no longer
117 bright); and 5 = no browning. Stem-end browning was calculated according to
118 Pristijono et al. (2017), with slight modifications, as shown in Equation 2.

$$119 \quad \text{Browning index (\%)} = \left(\frac{\text{Browning level in each fruit} \times \text{number of fruit at the same browning level}}{\text{Highest browning level} \times \text{total number of fruit in the treatment}} \right) \times 100 \quad (2)$$

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

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

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

$$136 \text{ Acceptability index (\%)} = \left(\frac{\text{Acceptability in each fruit} \times \text{number of fruit at the same acceptable level}}{\text{Highest acceptable level} \times \text{number of fruit in the treatment}} \right) \times 100 \quad (3)$$

137

138 *Statistical analysis*

139 Statistical analysis was performed using Statistical Analysis System - version
140 9.4 (SAS Institute, Cary, NC, USA) and SPSS (ver 23, IBM, USA). One-way ANOVA
141 was used to analyse the data. The mean values were evaluated by using least significant
142 differences (LSD) test with $p < 0.05$ as statistical significance.

143

144 **Results and discussion**

145

146 *Colour*

147 Fruit colour was assessed upon removal from low pressure storage and again
148 after being stored at atmospheric pressure (101 kPa) at 20°C for three days. There was
149 no significant difference in peel colour between fruit subject to low pressure storage
150 (4kPa) 10°C and fruit stored under regular atmospheric pressure (101 kPa) either at

151 10°C or 20°C storage temperature (data not shown). Hue angle did not change
152 significantly during storage at low pressure (4 kPa) and regular pressure (101 kPa) at
153 10°C for 11 days, remaining at a constant value of 122. These observations are in
154 agreement with previous studies by Burg (2004) who showed that the peel of “Acorn”
155 squash remained green after fruits were stored at low pressure of 7.33 – 8 kPa for 11
156 days at 7°C.

157

158 *Weight loss*

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

168 The results presented in Table 1 show that water loss from the fruit stored in the
169 low pressure storage (4 kPa, 10°C) was higher than those stored at regular atmosphere
170 (101 kPa) at 10°C upon removal. This finding is in agreement with previous research by
171 Laurin et al. (2006) who reported that low pressure treatment of “Alpha-type”
172 cucumbers (70 kPa for 6 hours) increased weight loss. However it is very important to
173 consider all the variables associated with water loss and vapour pressure deficit, and
174 care should be taken when comparing studies.

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

183

184 *Firmness*

185 Fruit firmness was assessed both immediately after the zucchinis were removed
186 from low pressure storage (10°C, 11 days) and again three days after transfer to storage
187 atmosphere (101 kPa) and 20°C. Fruit stored at 10°C under low pressure maintained
188 higher firmness values than fruit stored at regular atmosphere (101 kPa) at 20°C (Table
189 1). The maintenance of fruit firmness was more obvious after the additional shelf-life
190 storage at 20°C for three days, with the low pressure treated fruit exhibiting
191 significantly greater firmness ($p < 0.05$). However there was no difference in firmness
192 between fruits stored at low pressure (4 kPa, 10°C) and regular pressure (101 kPa) at
193 10°C. The findings are in agreement with previous work by Hashmi et al. (2016) who
194 found that low pressure treatment (50 kPa) of strawberries had no beneficial effect of
195 fruit firmness. In this study, the differences in fruit firmness between low pressure (4
196 kPa, 10°C) and regular pressure (101 kPa, 20°C) treatments maybe a result of difference
197 in water loss.

198

199 *Blossom-end flesh rots*

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

214 Differing levels of flesh rot between treatments stored at atmospheric and low
215 pressure at 10°C after removal to 20°C may be due to reduced oxygen availability
216 during low pressure treatment, where the oxygen (O₂) levels at 4 kPa are approximately
217 1 % O₂ (v/v). Burg (2004) has previously reported that low oxygen storage conditions
218 (0.1 – 0.25% O₂) have significantly inhibitory effects on pathogen and spore
219 germination.

220

221 *Stem-end browning*

222 The fresh appearance of the stem-end of zucchini fruit is a major determinant in
223 assessing fruit quality and acceptability. Low pressure storage at 10°C resulted in
224 significantly lower levels of stem-end browning compared to storage at 10°C under

225 normal atmospheric pressure (101 kPa), which were further significantly lower than
226 storage at 20°C (Figure 2). These observations were similar immediately upon removal
227 and after an additional three days storage at 20°C, where the additional time resulted in
228 an increase in stem-end browning, but the differences between the treatments remained
229 the same. These findings are consistent with Gao et al. (2006) who observed that low
230 pressure storage conditions (40 – 50 kPa, 4°C for 49 days) significantly reduced the
231 incidence of browning in loquat fruit. However further mechanistic studies are required
232 to determine whether a similar or different pathway for low pressure storage action
233 occurs in reducing browning in stem-end of zucchinis.

234

235 *Acceptability index*

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

247

248 **Conclusions**

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

257

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262

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268

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337

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341 different assessment day at 20°C.

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345 The values are the mean of three replicates. The different letters indicate significant
346 differences between treatments for each storage time ($p < 0.05$).

347 Figure 2. The stem-end browning index of zucchinis exposed to different treatments.

348 The values are the mean of three replicates. The different letters indicate significant
349 differences between treatments for each storage time ($p < 0.05$).

350 Figure 3. The acceptability index of zucchinis exposed to different treatments. The

351 values are the mean of three replicates. The different letters indicate significant

352 differences between treatments for each storage time ($p < 0.05$).

353

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

356

| Treatments | Weight loss (%) | Firmness (N) |
|--|-----------------|--------------|
| <u>Time zero</u> | - | 69.1 |
| <u>Upon removal</u> | | |
| 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 |
| <i>LSD (5%)</i> | ± 0.2 | ± 3.3 |
| <u>Additional storage 3 days at 101 kPa 20°C</u> | | |
| 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 |
| <i>LSD (5%)</i> | ± 0.4 | ± 7.5 |

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

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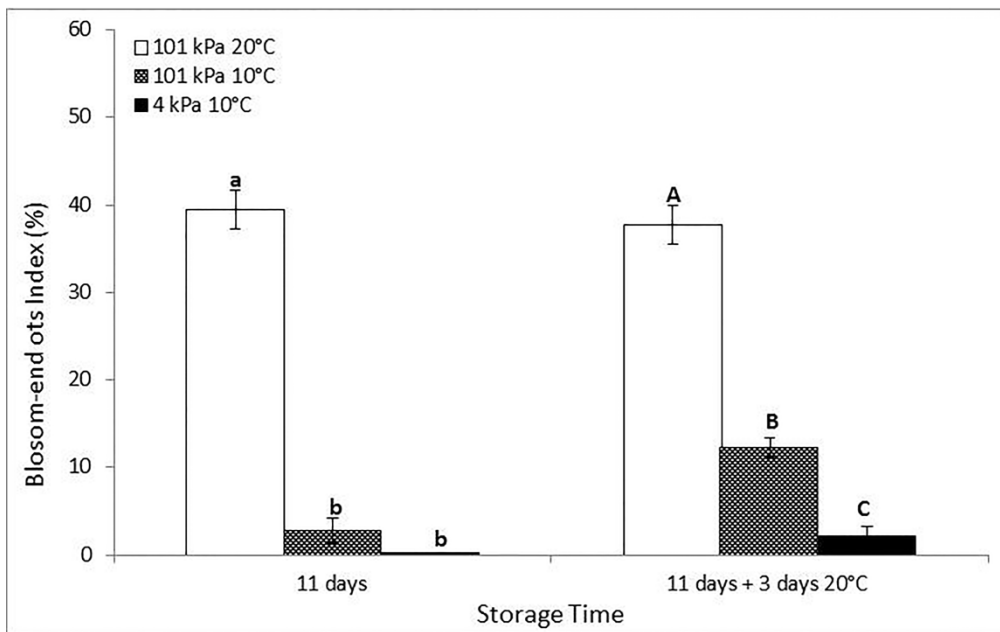
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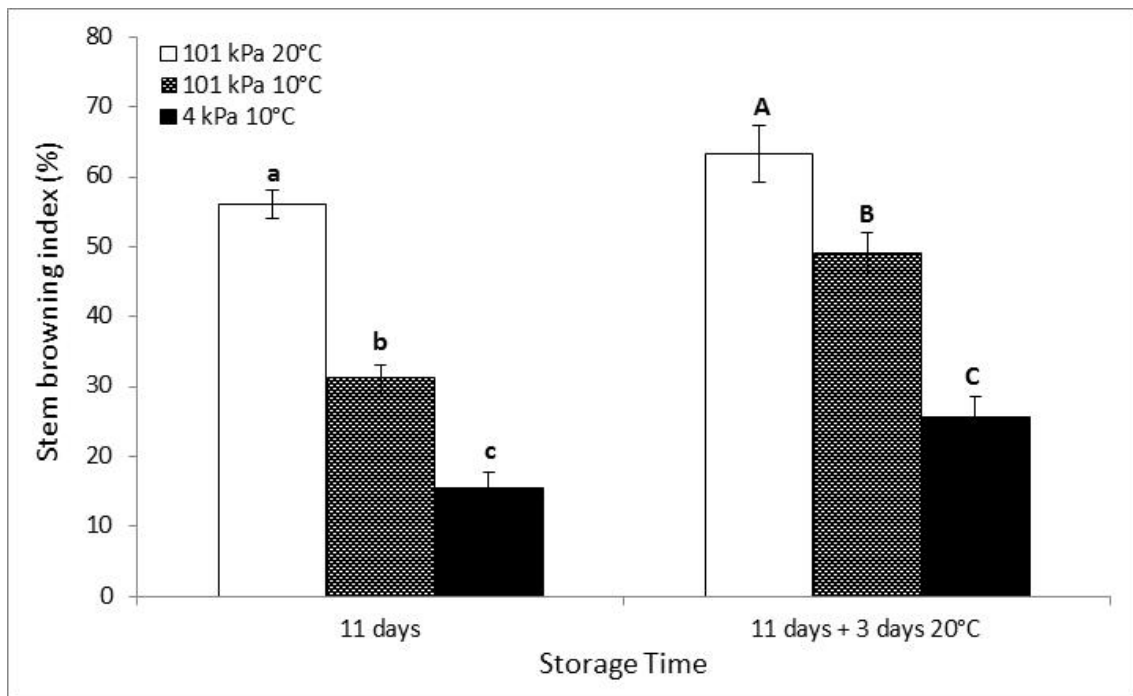
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Figure 2. The stem-end browning index of zucchinis exposed to different treatments.

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The values are the mean of three replicates. The different letters indicate significant

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differences between treatments for each storage time ($p < 0.05$).

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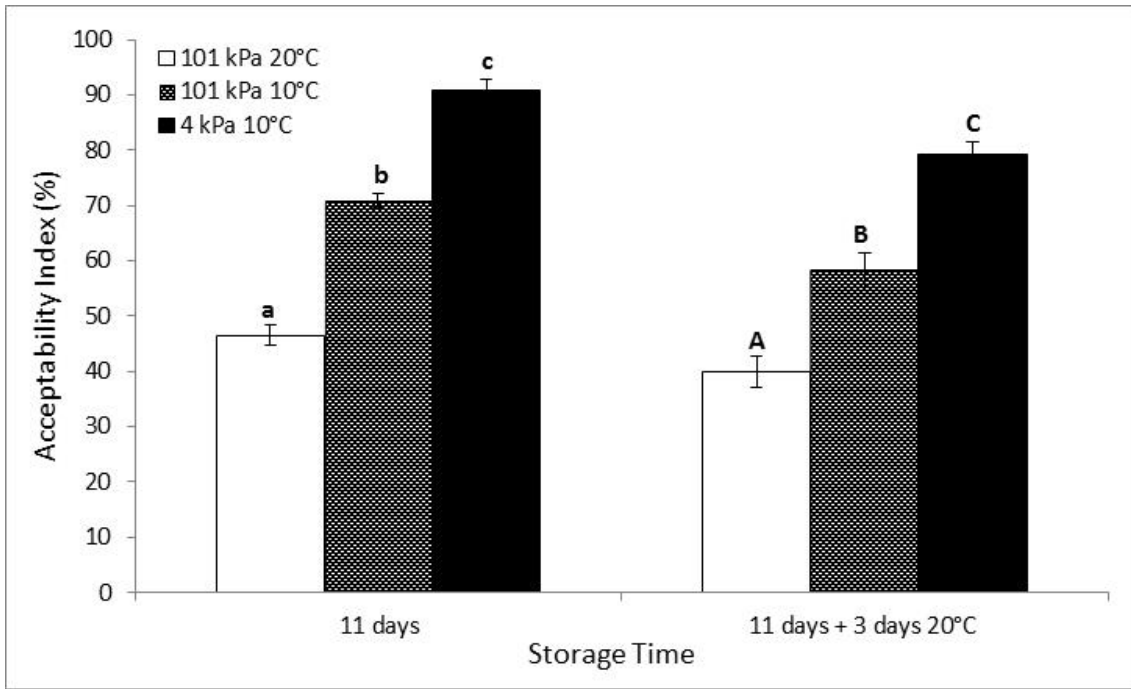


Figure 3. The acceptability index of zucchinis exposed to different treatments. The values are the mean of three replicates. The different letters indicate significant differences between treatments for each storage time ($p < 0.05$).