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# Quality attributes and antioxidant properties of Serrano chili peppers (*Capsicum annuum* L.) affected by thermal conditions postharvest

<sup>1</sup>Miranda-Molina, F. D., <sup>1\*</sup>Valle-Guadarrama, S., <sup>1</sup>Guerra-Ramírez, D., <sup>2</sup>Arévalo-Galarza, M. D. L., <sup>1</sup>Pérez-Grajales, M. and <sup>3</sup>Artés-Hernández, F.

<sup>1</sup>Universidad Autónoma Chapingo, Mexico-Texcoco km 38.5, Chapingo, 56230, Texcoco de Mora, Mexico

<sup>2</sup>Colegio de Posgraduados, Mexico-Texcoco km 36.0, Montecillo, 56230, Texcoco de Mora, Mexico

<sup>3</sup>Postharvest and Refrigeration Group, Department of Agricultural Engineering, Universidad Politécnica de Cartagena, Cartagena, Murcia, 30203, Spain

#### Article history

<u>Abstract</u>

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**Keywords** 

Capsicum annuum Postharvest Refrigeration Serrano chili pepper Fruits of the genus *Capsicum* are common food ingredients in many countries; therefore it is important to preserve their nutritional properties. The objective of the present work was to evaluate the modification of the quality attributes and antioxidant properties of Serrano chili peppers (*Capsicum annuum* L.) during the postharvest period under different thermal conditions. A storage period of 33 d was implemented at 4, 8, and 25°C. Serrano chilli peppers stored at 4 and 8°C showed the smallest changes in weight loss, texture, and hue angle; but darkening was identified in the seeds and tissues of those stored at 4°C, which was associated with chilling injury. Total soluble phenols and capsaicin increased with time at 25°C but remained unchanged in Serrano chilli peppers stored at 4 and 8°C. The antioxidant capacity was not affected by ripening or by low temperature. Storage at 8°C allowed for the maintenance of Serrano chilli peppers at optimum conditions throughout the postharvest period.

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#### Introduction

The genus Capsicum (Solanaceae), whose origin and domestication have been located in the regions of Latin America (DeWitt and Bosland, 2014), produces fruits that are consumed in many countries either fresh, as a spice, in combination with other vegetables, in the form of salads or sauces (Kantar et al., 2016), or in processed foods (Toledo-Aguilar et al., 2016). The fruits of Capsicum spp. constitute an important source of vitamin C (Li et al., 2017), vitamin E, several minerals (Kantar et al., 2016), and phenolic compounds that confer high antioxidant potential (Ghasemnezhad et al., 2011; Loizzo et al., 2015; Sricharoen et al., 2017). Depending on maturity, these fruits may also have high carotenoid content (Avalos-Llano et al., 2018), and pungent species are rich in capsaicinoids (Sricharoen et al., 2017), which further increase the antioxidant potential.

Among the different species, the cultivars of Capsicum annuum L. exhibit the highest antioxidant potential (Loizzo et al., 2015). The Serrano chili pepper is one of the most preferred cultivars among those that are of the pungent type (Castellón-Martínez et al., 2012), and is also among those with the highest antioxidant potential due to its high content of capsaicinoids (González-Zamora et al., 2013), phenolic compounds (Alvarez-Parrilla et al., 2011), and ascorbic acid (Medina-Juárez et al., 2012). Serrano chili peppers are consumed mainly in their fresh and green stages (Vázquez García et al., 2010), so their postharvest life has been defined as the time required for them to change from a green to a red or orange tonality or the time required to lose their turgid consistency due to transpiration (Martínez Zambrano et al., 2005). Refrigeration in the range between 4 and 8°C has been used to maintain quality attributes in several types of pepper fruit (Martínez et al., 2005;



Lim *et al.*, 2007). Refrigeration has been evaluated, even in combination with other technologies, such as biopolymer coatings (Xing *et al.*, 2011) or the application of substances such as methyl jasmonate (Wang *et al.*, 2019), to prolong shelf life. However, it is necessary to know the specific resistance of a fruit to low temperatures because the sensitivity to chilling injury is unique to each cultivar (Smith *et al.*, 2006), and this has not been studied in Serrano chili peppers. The objective of the present work was therefore to evaluate the modification of the quality attributes and antioxidant properties of Serrano chili peppers under different thermal conditions postharvest to provide information that allows for the improvement of their commercialisation potential.

## Materials and methods

## Plant material

The experimental material consisted of 40 kg of Serrano chili peppers (*Capsicum annuum* L. cv. Camino Real). Their physiological conditions corresponded to commercial maturity. Selected Serrano chilli peppers were free of damage and had a length of 10 cm and a dark green colour.

#### Experimental design

Batches with 250 g of Serrano chilli peppers were prepared. Three groups of 33 batches were formed and placed in storage rooms at 4 ( $\pm$  2), 8 ( $\pm$  2), and 25 ( $\pm$  2)°C, labelled as T4, T8, and T25, with an average relative humidity (RH) of 88, 87, and 76%, respectively. Samples from each condition were taken every 72 h for 33 d to evaluate weight loss, colour, firmness, titratable acidity, total soluble solids, content of total soluble phenols, antioxidant activity, and capsaicin content. Additionally, Serrano chilli peppers were visually inspected to identify symptoms of chilling injury in tissue. All measurements were made in triplicate, and the experimental unit was a batch of 250 g of Serrano chilli peppers.

### Cumulative weight loss

Batches of Serrano chilli peppers were weighed on day one and when they were removed from the storage. A scale (Ohaus, USA) with a precision of 0.01 g was used. The cumulative weight loss was evaluated based on the initial condition (AOAC, 1994).

### Colour

Colour was measured with a MiniScan XE Plus 45/OL colorimeter (Hunter Associates Laboratory Inc., USA) and was expressed as lightness (L\*), hue angle (H\*), and chroma (C\*).

#### **Firmness**

The pericarp firmness was measured with a texture analyser (TA-TX2i, Stable Micro Systems, UK) using a 5 mm spherical probe with a compression routine based on a test speed of 2 mm/s and a deformation distance of 2 mm. Measurements were performed in the equatorial zone, and firmness was expressed in Newton (N).

# Total soluble solids and titratable acidity

The total soluble solids (TSS), expressed as °Brix, were determined with a manual refractometer (Alla France ATC, France) with a 0 - 32% scale (AOAC, 1994). Acidity was evaluated with 20 g of fruit tissue that was macerated with 100 mL of deionised water and a titration using 0.1 N NaOH and phenolphthalein as the indicator (AOAC, 1994). The results were reported as the percentage of citric acid (Avalos-Llano *et al.*, 2018).

#### Total soluble phenols

To evaluate the content of total soluble phenols (TSP), 20 g sample of fruit was mixed with 125 mL of 80% methanol. The mixture was sonicated in a Cole-Parmer sonicator (Cole-Parmer Instrument Co., USA) for 30 min in the dark. Centrifugation was applied at 2,000 g, and the supernatant was recovered. The solid residue was subjected to a similar second extraction, and the supernatants were pooled (Alvarez-Parrilla et al., 2011). The method of Singleton and Rossi (1965) was used for soluble phenol analysis. Samples of 25 µL of extract were mixed with 20 µL of the Folin-Ciocalteu reagent, which was diluted at a 1:1 ratio from the commercial 2 N solution (Sigma-Aldrich, Co., Germany). The mixture was neutralised with 30  $\mu$ L of 20% Na<sub>2</sub>CO<sub>3</sub> (w/v) and 125  $\mu$ L of distilled water. The treatments were placed in the dark for 30 min, and absorbance was read at 760 nm in a spectrophotometer (Synergy<sup>™</sup> HTX, BioTek Instruments, Inc., USA). TSP quantification was based on a standard curve of gallic acid, and the results were expressed in mg equivalents of gallic acid per 100 g of fresh material (mg GAE/100 g).

#### Capsaicin content

The capsaicin content was evaluated in 1 g of tissue that was mixed with 12 mL of acetonitrile and placed in a water bath at 80°C for 4 h, and agitation was applied every 30 min. The extract was cooled and passed through 0.45  $\mu$ m membrane filters. Next, analysis with HPLC (PerkinElmer Series 200 HPLC Systems) was carried out using a Zorbax SB-C18 column (4.6 × 250 mm) with a particle size of 5  $\mu$ m. The mobile phase consisted of a 73:27

methanol:water solution, and an isocratic separation was used with a flow rate of 1 mL/min (Contreras-Padilla *et al.*, 1998). The quantification was based on a standard curve of capsaicin of 99% purity (Sigma-Aldrich, Co., Germany).

## Antioxidant activity

The antioxidant activity was determined by the FRAP (ferric reducing antioxidant power) method (Benzie and Strain, 1996) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) method (Re et al., 1999). In the former method, a 300 mM sodium acetate trihydrate buffer solution, pH 3.6, was prepared by mixing 20 mM ferric chloride hexahydrate with 10 mM 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) dissolved in 40 mM HCl. The solution was added to the samples, and the absorbance was read at 530 nm in a spectrophotometer (Synergy<sup>™</sup> HTX, BioTek Instruments, Inc., USA). The results were expressed as µmol equivalents of Trolox per 100 g of fresh tissue ( $\mu$ mol TE/100 g). In the latter method, a solution of ABTS was prepared at a concentration of 7.4 mM, which was dissolved in a 2.6 mM sodium persulfate solution. The mixture was allowed to react for 16 h in the dark. Next, 20 µL of the extract were mixed with 230 µL of the ABTS solution. The absorbance reduction was measured in a spectrophotometer (Synergy<sup>™</sup> HTX, BioTek Instruments, Inc., USA) at 734 nm after 10 min. The results were expressed as

 $\mu$ mol equivalent of Trolox per 100 g of fresh chili pepper ( $\mu$ mol TE/100 g).

#### Statistical analysis

The experimental design considered two variation sources: the temperature, with three levels (4, 8, and 25°C) and the storage time, with 11 evaluation points within 33 d. Thus, data were submitted to analysis of variance ( $3 \times 11$  factorial) in a completely randomized design. Tukey's Test was employed to compare the means at a significance level of 0.05. All analyses were performed with the SAS program (SAS Institute, 1999).

## **Results and discussion**

#### *Cumulative weight loss*

Weight loss was affected by temperature and storage time, although the interaction between such factors was not significant (Table 1). The greatest weight loss occurred at 25°C (T25), followed by 4°C (T4), and, finally, by 8°C (T8), with a significant difference ( $p \le 0.05$ ) between them (Figure 1A). The cumulative weight loss was practically linear in all cases, with rates of 1.77, 0.30, and 0.51%/d at 25, 8, and 4°C, respectively. The weight loss during storage was significant ( $p \le 0.05$ ) under the three conditions, but only at 25°C it was accompanied by an appearance of wilt (data not shown). The loss of

Variable	Variation factor			Error	VC (%)
	Temperature	Time	Temp. × Time		
df	2	10	20	66	
F <sub>0.05</sub>	3.14	1.98	1.74		
Physiological vari	ables				
Weight loss	24.26*	3.13*	1.28 <sup>ns</sup>		15.76
Lightness	18.25*	0.91*	2.95*		6.06
Chroma	$0.60^{*}$	$1.08^{*}$	6.33*		8.03
Hue angle	129.29*	24.42*	24.35*		7.03
Firmness	204.44*	17.56*	0.92 <sup>ns</sup>		26.03
Chemical variable	25				
TSS	1.1 <sup>ns</sup>	2.22*	8.64*		7.47
TA	1.51 <sup>ns</sup>	0.61 <sup>ns</sup>	$10.00^{*}$		10.9
TSP	6.81*	13.09*	1.84*		12.31
Capsaicin	1.15 <sup>ns</sup>	0.6 <sup>ns</sup>	$2.92^{*}$		41.97
AA (FRAP)	2.05 <sup>ns</sup>	3.72*	1.18 <sup>ns</sup>		36.52
AA (ABTS)	1.80 <sup>ns</sup>	3.06*	1.14 <sup>ns</sup>		20.63

Table 1. Percentile values of the Fisher distribution (F<sub>0.05</sub>) and F-values corresponding to the analysis of variance of the postharvest behaviour of Serrano chili peppers stored at 4, 8, and 25°C for 33 d.

Temperature = variation factor given by the storage temperature (4, 8, and  $25^{\circ}$ C); time = variation factor given by the storage time (11 sampling points); temp. × time = interaction between variation factors; df = degrees of freedom; VC = variation coefficient; L\* = lightness; C\* = chroma; hue = hue angle; TSS = total soluble solids; TA = titratable acidity; TSP = total soluble phenols; AA = antioxidant activity; \* = indicates that at least one level within the variation factor produced a different effect in relation to the rest (Tukey, 0.05); ns = indicates a non-significant effect (Tukey, 0.05) between levels within the variation factor.



Figure 1. Modification of cumulative weight loss (A), firmness (B), total soluble solids (C), and acidity (D) in Serrano chili peppers stored at 4, 8, and 25°C for 33 d. Symbols correspond to the average of three repetitions (n = 3). Error bars indicate standard error. HSD<sub>gen</sub> is a general honest significant difference (Tukey, 0.05) and applies to the comparison of means between temperatures. HSD<sub>4</sub>, HSD<sub>8</sub>, and HSD<sub>25</sub> are honest significant differences, which apply to the comparison of means inside each temperature, respectively. Different letters indicate significant difference between temperatures. ns and \* indicate non-significant and significant variation, respectively, of parameters inside each temperature.

mass of horticultural products is mainly caused by transpiration induced by a vapour pressure deficit between the internal tissue and the environment, and it is a common characteristic of these produce postharvest (Hübert and Lang, 2012). In the case of *Capsicum* spp., water loss, in addition to chilling injury, is a factor that limits their shelf life (Avalos-Llano et al., 2018). The higher transpiration rate at 25°C can be explained, both by the higher thermal condition and by the lower RH of the storage room (76%) than those of the other storage conditions. In contrast, the relatively low weight losses of T4 and T8 were caused by the higher RH (87 - 88%) than that of T25 and because a low temperature reduces the rate of transpiration (Hübert and Lang, 2012). During the first two weeks, the tendency of weight loss was similar between T4 and T8; however, after day 15, Serrano chilli peppers of T4 showed an increase in the rate of weight loss (Figure 1A), which suggested a modification in the physical structure of plant material that might be associated with low temperatures. The exposure of sweet peppers to very low temperatures can cause an increase in weight loss

by transpiration due to the leakage of electrolytes in the tissue caused by chilling injury (Lim *et al.*, 2007; Xing *et al.*, 2011), and a similar situation could occur with Serrano chili peppers. The most common visible symptom associated with low-temperature stress is the loss of water associated with an alteration in the structure of cell membranes, which is indicative of chilling injury (Korkmaz *et al.*, 2010; Wang *et al.*, 2019).

# Colour

The hue angle (H\*) of the Serrano chili peppers was also affected by the thermal conditions and storage time ( $p \le 0.05$ ), and in this case, the interaction between these factors was significant (Table 1). H\* was approximately 178.9° during the first 21 d, without a significant difference between temperatures (p > 0.05, Figure 2A), which was characteristic of the green colour of immature Serrano chili peppers (Martínez Zambrano *et al.*, 2005). However, after 21 d, Serrano chilli peppers of the T25 group experienced a significant change ( $p \le 0.05$ ) in H\*, reaching 37.3° ( $\pm 1.9^{\circ}$ ) at the end of 33 d of storage,



Figure 2. Variation of lightness (A), chroma (B), and hue angle (C) in Serrano chili peppers stored at 4, 8, and 25°C for 33 d. Notation is as described in Figure 1.

which characterised the transition to the typical redorange tones of mature fruit (Martínez Zambrano et al., 2005). Moreover, at T4 and T8, the hue angle of Serrano chilli peppers remained approximately constant, with values of  $178.82^{\circ}$  (± 0.05°) during the whole period, with no difference between both conditions (p > 0.05). Similarly, lightness (L\*) was affected by the temperature modification (Table 1), with observed significant differences between the three thermal conditions ( $p \le 0.05$ , Figure 2B). The highest values were observed at 25°C, which varied between 39.80 and 48.05%, followed by those stored at 4°C, with variation between 35.51 and 43.27%, and, finally, by those stored at 8°C, whose lightness varied between 33.83 and 39.07%, which indicated that refrigeration caused a reduction in such attribute. The storage duration caused different effects under different thermal conditions, and the temperature  $\times$ 

time interaction was significant ( $p \le 0.05$ , Table 1). Only Serrano chilli peppers stored at 25°C showed significant fluctuations of L\*, which occurred in the last stage of storage, coinciding with the modification of H\*, although a clear trend was not confirmed. Chroma  $(C^*)$  presented a similar behaviour since the highest values were observed at 25°C ( $p \le 0.05$ ), with fluctuations between 32.64 and 52.08, followed by that of Serrano chilli peppers stored at 8°C, which varied between 33.50 and 43.47, and, finally, by that of Serrano chilli peppers stored at 4°C, whose variation was between 31.88 and 40.93. Although there were fluctuations in C\* throughout storage, the change at the end was only significant at 25°C  $(p \le 0.05)$  (Figure 2C), coinciding again with the transition from green to orange-red tonality. Serrano chili pepper is consumed more frequently in the green state (Vázquez García et al., 2010), and its shelf life is determined by the time required to modify the hue angle towards a red-orange appearance (Martínez Zambrano et al., 2005). The observed behaviour indicated that refrigeration at 4 or 8°C constitutes an adequate alternative to maintain the colour attributes without major modifications during 33 d postharvest. However, the condition of 25°C favoured ripening, causing the modification of hue angle from day 21 and the increase of lightness and chroma.

The storage at 4°C caused darkening in the seeds and internal tissue of Serrano chilli peppers in the later stage of storage, which did not occur with the other two thermal conditions and suggested the possible presence of chilling injury at the lowest temperature (Figure 3). The use of low temperatures can affect the fruit quality of Capsicum spp., and the darkening of seeds is a symptom associated with chilling injury (Boonsiri et al., 2007; Valenzuela et al., 2017). Xing et al. (2011) showed that storage at 8°C caused considerable decay in sweet peppers. However, Lim et al. (2007) did not observe adverse symptoms from low-temperature storage, even with exposure to 5°C. Smith et al. (2006) showed that the sensitivity to chilling injury depends on the cultivar, and the data of the present work showed that exposure to 8°C did not cause chilling injury in Serrano chili peppers, but the use of 4°C induced symptoms that might be related to such a physiological disorder. In general, chilling injury affects the commercialisation ability of fruits and must be avoided. Xing et al. (2011) reported losses of 40 and 60% sensory acceptability in sweet peppers after 14 and 21 d of storage at 8°C, respectively. In addition, colour is a quality indicator in seed production (Tu et al., 2018); so, darkening caused by chilling injury directly affects this industry.



Figure 3. (A) Serrano chili peppers without the presence of chilling injury, (B) Serrano chili peppers stored at 4°C with symptoms of chilling injury (darkening of seeds and surrounding tissue).

#### Firmness

The firmness of Serrano chilli peppers was affected both by the variation of the thermal condition and by the storage time (Table 1). Firmness was approximately 6.1 N initially, and decreased significantly in Serrano chilli peppers under all three thermal conditions ( $p \le 0.05$ ). The greatest change occurred in those stored at 25°C, followed by those stored at 8°C, and, finally, by those stored at 4°C (Figure 1B). Loss of firmness is a common feature in horticultural products that are handled fresh in postharvest and can be caused by the loss of water by transpiration, which induces reduction of cellular turgor, or by degradation of the cell wall polymers (Smith et al., 2003). Tsegay et al. (2013) showed that the firmness of sweet peppers decreased by 26.7% throughout a ripening process under refrigeration. Likewise, Rao et al. (2011) showed that the modification of firmness is related to enzymes such as polygalacturonase (PG), pectin-methyl esterase (PME), cellulase, and β-galactosidase. However, based on the observed cumulative weight loss under the three thermal conditions, it was accepted that the loss of firmness was a combined effect of both the degradation of polymeric compounds in cell walls and the loss of turgor caused by transpiration, which manifested to a greater extent in Serrano chilli peppers stored at 25°C than in those stored at 8°C. This behaviour was expected since the higher the temperature, the higher the metabolic process rates (Nunes and Emond, 2003). According to Rao et al. (2011), PG and PME activities increase with temperature.

#### Total soluble solids and titratable acidity

The total soluble solids (TSS) remained unaffected by temperature modification during the first 25 d of storage, but from that point, the Serrano chilli peppers stored at 25°C experienced a considerable increase in this variable and became the group with the highest TSS value ( $p \le 0.05$ , Figure 1C). Significant variation was identified in all the cases, but no clear trend was observed in the groups stored at 4 and 8°C, where TSS varied between 6.50 and 8.97 and between 6.67 and 9.00 °Brix, respectively. These values were similar to those reported by Rao et al. (2011) for sweet peppers. In the case of 25°C, the increasing trend from 5.40 to 11.50 °Brix could be associated with the continuous weight loss that caused a concentration of soluble compounds; additionally, based on the fact that the Serrano chilli peppers went through a ripening process, as evidenced by a change in colour, the increase in TSS may be a characteristic feature of ripening, similar to that which occurs with other fruits of the genus Capsicum (Castro et al., 2008; Ghasemnezhad et al., 2011).

The acidity content showed a similar behaviour, without a significant difference between Serrano chilli peppers stored at all three temperatures during the first 25 d, with values between 0.04 and 0.06%, without a clear trend; thereafter, Serrano chilli peppers stored at 25°C experienced a significant increase in acidity, reaching values of 0.09% (Figure 1D), which could also be a consequence of both ripening (Ghasemnezhad et al., 2011) and weight loss. Castro et al. (2008) reported an increase in acidity of 2.5 times during the ripening of sweet peppers due to an increase in the concentration of organic acids. Ghasemnezhad et al. (2011) explained that as fruits ripen, the concentrations of organic acids involved in the Krebs cycle increase. The acidity found in Serrano chili peppers was similar to those reported in habanero chili peppers, according to Kantar et al. (2016).



Figure 4. Modification of the content of total soluble phenols (A), capsaicin content (B), antioxidant activity evaluated by the ABTS method (C), and antioxidant activity evaluated by the FRAP method (D) in Serrano chili peppers stored at 4, 8, and 25°C for 33 d. Notation is as described in Figure 1.

#### Total soluble phenols

The total soluble phenols (TSP) in the Serrano chilli peppers varied in the ranges of 84.81 - 150.72, 74.84 - 136.17, and 80.33 - 136.53 mg GAE/100 g when stored at 4, 8, and 25°C, respectively, with significant influence from temperature and storage time ( $p \le 0.05$ ; Table 1), despite exhibiting differences that did not have practical importance nor a clear trend of change during storage (Figure 4A). These results coincided with those reported in the range of 90 - 120 mg GAE/100 g for sweet peppers by Ghasemnezhad et al. (2011), but Alvarez-Parrilla et al. (2011) reported a TSP content of 1032 mg GAE/100 g in Serrano chili peppers, but the difference is that these authors used a dry basis to express results, while the data of the present work are reported on a wet basis. According to Ghasemnezhad et al. (2011), the TSP content decreased with ripening of sweet pepper; however, this behaviour was not observed in the present work, where Serrano chili peppers were used, which suggested that the compositional behaviour may be affected by the cultivar. Serrano chilli peppers stored at 4°C showed darkening in the seeds and in the tissue around them. This was interpreted as a chilling injury symptom since it is a behaviour previously reported for fruits of the genus Capsicum (Valenzuela *et al.*, 2017). It is common that in fruits where this physiological disorder causes darkening, the content of phenolic compounds decreases (Gao *et al.*, 2016), but this did not occur in the present work. Boonsiri *et al.* (2007) found that severe darkening in the pungent fruit seeds of *C. annuum* correlated with visible cell damage, electrolyte leakage, high activity of the polyphenol oxidase enzyme, and the initial levels of free phenols. The absence of a clear variation trend in TSP in Serrano chilli peppers assessed in the present work suggested that the damage was slight and only the beginning of chilling injury.

# Capsaicin

The pungency of hot peppers is attributed to capsaicinoids, whose major components are capsaicin and dihydrocapsaicin (Domínguez-Cañedo *et al.*, 2014). The capsaicin content was not affected by the temperature variation (Table 1). With regard to time, large fluctuations occurred between 4.2 and 15.5 mg/100 g during storage, with similar ranges under the three conditions, which was similar to the report of Alvarez-Parrilla *et al.* (2011) for Serrano chili peppers. The capsaicin content can remain constant (Li *et al.*, 2017) or can experience modification with development (Ornelas-Paz *et al.*, 2010). In the present

work, the capsaicin content increased only in Serrano chilli peppers stored at 25°C (Figure 4B). However, since Ornelas-Paz et al. (2010) reported that in green-stage Serrano chili peppers, the compound concentration was higher than that found in the red stage, it is believed that the observed behaviour was due to a concentration phenomenon derived from weight loss. Serrano chilli peppers stored at 4°C showed no tendency in capsaicin variation. However, Patel et al. (2019) found a reduction in capsaicin concentration during the storage of green peppers stored at 4°C for 40 d, which suggests that the effect of low temperature may depend on the cultivar. Alvarez-Parrilla et al. (2011) mentioned that the variability of capsaicin content may be due to maturity state, cultivar, and the pre- and postharvest conditions to which fruits are subjected.

## Antioxidant activity

The antioxidant activity (AA) was evaluated using the ABTS and FRAP methods. Both assays indicated that the temperature did not affect this property in Serrano chili peppers. Only at 8°C there were significant differences between some days during storage, but without a clear trend (Table 1, Figure 4C and 4D), which suggests that there was natural variability between samples. The ABTS method reported AA between 318.7 and 653.5 µmol TE/100 g, while values of the FRAP method were between 267.9 and 1148.7  $\mu$ mol TE/100 g. The ABTS method is based on evaluation of electron transfer, while the FRAP method is based on hydrogen atom transfer (Apak et al., 2016), which explains the differences in the observed results. Alvarez-Parrilla et al. (2011) reported an antioxidant activity value of 4103  $\mu$ mol TE/100 g, and this difference is again due to the dry weight basis used by these authors. On the other hand, data of Serrano chilli peppers stored at 4 and 25°C showed that there was no significant effect of the storage time. In the first case, there could have been chilling injury; in the second case, Serrano chilli peppers underwent ripening, but the results suggest that neither of these phenomena affected the AA in Serrano chili peppers. According to Sricharoen et al. (2017), the AA of Capsicum spp. is determined mainly by TSP since they constitute the majority of substances that contribute to this characteristic, which is consistent with data from the present work for Serrano chili peppers since a TSP / capsaicin ratio of 11.3 / 1.0 was found. However, the presence of capsaicin allows for pungent peppers to have greater antioxidant activity than non-pungent ones.

## Conclusion

The storage temperature significantly affected the quality attributes and the antioxidant properties of Serrano chili peppers. Temperature of 25°C favoured weight loss, softening, and transition throughout the ripening process. Temperature of 4°C caused symptoms associated with chilling injury, manifested as seed and tissue darkening. Storage at 8°C maintained the best characteristics of Serrano chilli peppers postharvest without the presence of chilling injury symptoms. The contents of total soluble phenols and capsaicin, and antioxidant capacity were not affected by the thermal condition or storage time.

#### References

- Alvarez-Parrilla, E., de la Rosa, L. A., Amarowicz, R. and Shahidi, F. 2011. Antioxidant activity of fresh and processed Jalapeño and Serrano peppers. Journal of Agricultural and Food Chemistry 59(1): 163–173.
- Apak, R., Özyürek, M., Güçlü, K. and Çapanoğlu, E.
  2016. Antioxidant activity/capacity measurement.
  1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays.
  Journal of Agricultural and Food Chemistry 64(5): 997–1027.
- Association of Official Analytical Chemists (AOAC). 1994. Official methods of analysis of AOAC International. (16th ed). United States: AOAC.
- Avalos-Llano, K. R., Sgroppo, S. C. and Chaves, A. R. 2018. Changes in quality parameters, antioxidant compounds and enzymes of phenolic metabolism of hot water-treated organic 'cherry' peppers. International Food Research Journal 25(4): 1633–1641.
- Benzie, I. F. and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry 239(1): 70–76.
- Boonsiri, K., Ketsa, S. and van Doorn, W. G. 2007. Seed browning of hot peppers during low temperature storage. Postharvest Biology and Technology 45(3): 358–365.
- Castellón-Martínez, E., Chávez-Servia, J. L., Carrillo-Rodríguez, J. C. and Vera-Guzman, A. M. 2012.
  Consumption preferences of pepper (*Capsicum annuum* L.) landraces in the central valleys of Oaxaca, México. Revista Fitotecnia Mexicana 35: 27–35.
- Castro, S. M., Saraiva, J. A., Lopes-da-Silva, J. A., Delgadillo, I., Loey, A. V., Smout, C. and Hendrickx, M. 2008. Effect of thermal blanching and of high pressure treatments on sweet green and red bell pepper fruits (*Capsicum annuum* L.). Food Chemistry 107(4): 1436–1449.
- Contreras-Padilla, M. and Yahia, E. M. 1998. Changes in capsaicinoids during development, maturation, and senescence of chili peppers and relation with peroxidase activity. Journal of Agricultural and Food Chemistry 46(6): 2075–2079.

- DeWitt, D. and Bosland, P. W. 2014. The complete chili pepper book: a gardener's guide to choosing, growing, preserving, and cooking. United States: Timber Press.
- Domínguez-Cañedo, I. L., Beristain-Guevara, C. I., Díaz-Sobac, R. and Vázquez-Luna, A. 2014. Degradation of carotenoids and capsaicin in the molecular inclusion complex of habanero chili oleoresin (*Capsicum* chinense) in β-cyclodextrin. CyTA - Journal of Food 13(1): 151–158.
- Gao, H., Zhang, Z., Lv, X., Cheng, N., Peng, B. and Cao, W. 2016. Effect of 24-epibrassinolide on chilling injury of peach fruit in relation to phenolic and proline metabolisms. Postharvest Biology and Technology 111: 390–397.
- Ghasemnezhad, M., Sherafati, M. and Payvast G. A. 2011. Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum* annum) fruits at two different harvest times. Journal of Functional Foods 3(1): 44–49.
- González-Zamora, A., Sierra-Campos, E., Luna-Ortega, J. G., Pérez-Morales, R., Rodríguez Ortiz, J. C. and García-Hernández, J. L. 2013. Characterization of different *Capsicum* varieties by evaluation of their capsaicinoids content by high performance liquid chromatography, determination of pungency and effect of high temperature. Molecules 18(11): 13471–13486.
- Hübert, T. and Lang, C. 2012. Artificial fruit: postharvest online monitoring of agricultural food by measuring humidity and temperature. International Journal of Thermophysics 33(8-9): 1606–1615.
- Kantar, M. B., Anderson, J. E., Lucht, S. A., Mercer, K., Bernau, V., Case, K. A., ... and Baumler, D. J. 2016. Vitamin variation in *Capsicum* spp. provides opportunities to improve nutritional value of human diets. PLoS ONE 11(8): article ID e0161464.
- Korkmaz, A., Korkmaz, Y. and Demirkıran, A. R. 2010. Enhancing chilling stress tolerance of pepper seedlings by exogenous application of 5-aminolevulinic acid. Environmental and Experimental Botany 67(3): 495– 501.
- Li, D., Long, H.-Y., Zhang, S.-X., Wu, X.-P., Shao, H.-Y. and Wang, P. 2017. Effect of continuous negative pressure water supply on the growth, development and physiological mechanism of *Capsicum annuum* L. Journal of Integrative Agriculture 16(9): 1978–1989.
- Lim, C. S., Kang, S. M., Cho, J. L., Gross, K. C. and Woolf, A. B. 2007. Bell pepper (*Capsicum annuum* L.) fruits are susceptible to chilling injury at the breaker stage of ripeness. HortScience 42(7): 1659–1664.
- Loizzo, M. R., Pugliese, A., Bonesi, M., Menichini, F. and Tundis, R. 2015. Evaluation of chemical profile and antioxidant activity of twenty cultivars from *Capsicum annuum*, *Capsicum* baccatum, *Capsicum* chacoense and *Capsicum* chinense: a comparison between fresh and processed peppers. LWT - Food Science and Technology 64(2): 623–631.
- Martínez Zambrano, G., Dorantes González, J. R. A., Ramírez Meraz, M., de la Rosa Loera, A. and Pozo Campodónico, O. 2005. Genetic effects and heterosis of shelf life in serrano hot pepper. Revista Fitotecnia Mexicana 28(4): 327–332.

- Martínez, S., López, M., González-Raurich, M. and Bernardo Álvarez, A. 2005. The effects of ripening stage and processing systems on vitamin C content in sweet peppers (*Capsicum annuum* L.). International Journal of Food Sciences and Nutrition 56(1): 45–51.
- Medina-Juárez, L. A., Molina-Quijada, D. M. A., Del Toro-Sánchez, C. L., González-Aguilar, G. A. and Gámez-Meza, N. 2012. Antioxidant activity of peppers (*Capsicum annuum* L.) extracts and characterization of their phenolic constituents. Interciencia 37(8): 588–593.
- Nunes, M. C. N. and Emond, J. P. 2003. Storage temperature. In Bartz, J. A. and Brecht, J. K. (eds). Postharvest Physiology and Pathology of Vegetables, p. 209-228. New York: Marcel Dekker.
- Ornelas-Paz, J. J., Martínez-Burrola, J. M., Ruiz-Cruz, S., Santana-Rodríguez, V., Ibarra-Junquera, V., Olivas, G. I. and Pérez-Martínez, J. D. 2010. Effect of cooking on the capsaicinoids and phenolic contents of Mexican peppers. Food Chemistry 119(4): 1619–1625.
- Patel, N., Gantait, S. and Panigrahi, J. 2019. Extension of postharvest shelf-life in green bell pepper (*Capsicum annuum* L.) using exogenous application of polyamines (spermidine and putrescine). Food Chemistry 275: 681–687.
- Rao, T. V. R., Gol, N. B. and Shah, K. K. 2011. Effect of postharvest treatments and storage temperatures on the quality and shelf life of sweet pepper (*Capsicum* annum L.). Scientia Horticulturae 132: 18–26.
- Re, R., Pellegrini N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26(9–10): 1231–1237.
- SAS Institute. 1999. SAS/STAT® user's guide, version 8. United States: SAS Institute.
- Singleton, V. L. and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16: 144–158.
- Smith, A. C., Waldron, K. W., Maness, N. and Perkins-Veazie, P. 2003. Vegetable texture: measurement and structural implications. In Bartz, J. A. and Brecht, J. K. (eds). Postharvest Physiology and Pathology of Vegetables, p. 297-329. New York: Marcel Dekker.
- Smith, D. L., Stommel, J. R., Fung, R. W. M., Wang, C. Y. and Whitaker, B. D. 2006. Influence of cultivar and harvest method on postharvest storage quality of pepper (*Capsicum annuum* L.) fruit. Postharvest Biology and Technology 42(3): 243–247.
- Sricharoen, P., Lamaiphan, N., Patthawaro, P., Limchoowong, N., Techawongstien, S. and Chanthai S. 2017. Phytochemicals in *Capsicum* oleoresin from different varieties of hot chilli peppers with their antidiabetic and antioxidant activities due to some phenolic compounds. Ultrasonics Sonochemistry 38: 629–639.
- Toledo-Aguilar, R., López-Sánchez, H., López, P. A., Guerrero-Rodríguez, J. D., Santacruz-Varela, A. and Peña, A. H. 2016. Morphological diversity of native

population's poblano pepper. Revista Mexicana de Ciencias Agrícolas 7(5): 1005-1015.

- Tsegay, D., Tesfaye, B., Mohammed, A., Yirga, H. and Bayleyegn, A. 2013. Effects of harvesting stage and storage duration on postharvest quality and shelf life of sweet bell pepper (*Capsicum annuum* L.) varieties under passive refrigeration system. International Journal for Biotechnology and Molecular Biology Research 4(7): 98–104.
- Tu, K.-L., Li, L.-J., Yang, L.-M., Wang, J.-H. and Sun, Q. 2018. Selection for high quality pepper seeds by machine vision and classifiers. Journal of Integrative Agriculture 17(9): 1999–2006.
- Valenzuela, J. L., Manzano, S., Palma, F., Carvajal, F., Garrido, D. and Jamilena, M. 2017. Oxidative stress associated with chilling injury in immature fruit: postharvest technological and biotechnological solutions. International Journal of Molecular Sciences 18(7): article ID 1467.
- Vázquez García, E., Ramírez Meraz, M., Mata Vázquez, H., Ariza Flores, R. and Alia Tejacal, I. 2010. Fruit quality attributes and shelf life of Serrano pepper cultivars in México. Revista Fitotecnia Mexicana 33: 79–82.
- Wang, Y., Gao, L., Wang, Q. and Zuo, J. 2019. Low temperature conditioning combined with methyl jasmonate can reduce chilling injury in bell pepper. Scientia Horticulturae 243: 434–439.
- Xing, Y., Li, X., Xu, Q., Yun, J., Lu, Y. and Tang, Y. 2011. Effects of chitosan coating enriched with cinnamon oil on qualitative properties of sweet pepper (*Capsicum annuum* L.). Food Chemistry 124(4): 1443–1450.