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Effect of Water Treatment and Immersion in Calcium Salt Solutions on the Quality of Fruits of Peumo Pink Tomato (*Solanum lycopersicum* L.) Stored under Cold Conditions

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Tomato is one of the most consumed vegetable crops worldwide. In the break stage, it is perishable, and it has a postharvest life that does not exceed three weeks at 12 to 15°C. The aim of this study was to evaluate the effect of immersion in water and in calcium salt solutions on the metabolism and quality of tomato of traditional Peumo Pink cultivar stored for 28 days at 10°C plus a simulated trading period of 2 days at 20°C. Fruits were treated in water at 5, 45 and 60°C at two immersion times of 1 and 4 min or in 2% solutions of calcium chloride, lactate and propionate at 10 and 45°C for 4 min. The respiration rate, ethylene production and heat shock protein gene expression as well as firmness, total phenolic content and antioxidant capacity of tomato were determined. Dipping in water at 60°C reduced the loss of firmness and respiratory rate of tomatoes up to 21 days at 10°C + 2 days at 20°C. Treatment in water at 45°C for 4 min and at 60°C for 1 or 4 min stimulated heat shock protein gene expression. However, fruit treated at 60°C for 1 or 4 min showed uneven ripening; hence, the immersion in water at 45°C for 4 min was the most recommended to extend the postharvest life of tomatoes. In turn, the immersion in calcium lactate and propionate solutions at 45°C increased total calcium content and maintained firmness after 28 days at 10°C + 2 days at 20°C. The use of high temperature and calcium salts for dipping would be beneficial to provide the vegetal tissues with calcium and reduce the softening of the tomato after prolonged cold storage.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a vegetable of Western South and Central America origin. In 2018, its global production was 182 million tons, and it is grown annually on 4.76 million hectares. The main world producers are: China, the European Union, India, the United States, and Turkey [FAOSTAT, 2020]. One of the ancestral tomato cultivars still grown by small farmers in the central area of Chile is the Peumo Pink tomato (Spanish: Rosado de Peumo). It is a non-genetically modified cultivar, characterized by fruits with a bright pink color, juicy flesh and large size (500 to 600 g per fruit).

In general, tomato is classified as a climacteric fruit and shows a rise in the respiratory rate (40 mg CO_2/kg×h at 25°C) and ethylene production (10 μL C_2H_4/kg×h at 25°C) during ripening [Li *et al.,* 2020]. These metabolic processes cause physicochemical effects after harvesting and affect color, texture and taste, generating significant losses associated mainly with deteriorated sensory and microbiological quality [Sun *et al.,* 2015]. The recommendation for a tomato is to store it for two to four weeks at temperatures between 12 and 15°C and 90% relative humidity (RH) to avoid chilling injury symptoms, particularly for immature fruits [Polenta *et al.,* 2015]. In the case of Peumo Pink tomatoes, no formal postharvest studies have been reported to date. However, preliminary experiments carried out at our laboratory demonstrated a very short shelf life of 5 to 7 days at 18°C and sensitivity to chilling injury at temperatures below 10°C [Escalona, data unpublished].

There are many reports pointing to heat treatment as an effective way to reduce decay and chilling injury of sensitive fruits like tomatoes [Aguayo *et al.*, 2008; 2015]. However, the optimal combination of temperature and exposure time to heating for each species must be defined in order to ensure

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the benefits of the method [Wang, 1998]. Hot water treatment is cheap and easily applied on a small commercial scale, and is a good solution for small growers lacking access to advanced technology. Postharvest calcium salt solution dips are another way to extend the shelf-life of the sensitive fruits. Immersion in different calcium salts, such as chloride, lactate, citrate, ascorbate, propionate and silicate, has been used before fruit storage at low temperatures [Aguayo *et al.,* 2008]. The effects of such treatments included a reduction of chilling injury or mechanical damage during cold storage [Serrano *et al*., 2004], a reduction of firmness loss and resistance to attack by rotting [Lara *et al.,* 2004; Silveira *et al.,* 2011b]. Short-time calcium dips (1–3 min) were recommended for application in combination with high temperature (40–60°C) treatments [Valero & Serrano, 2010]. On the other hand, it is not clear whether thermal shock and calcium salt treatments affect the total phenolic content and the antioxidant capacity of tomatoes, although they seem to decrease with storage time [Aguayo *et al.*, 2015].

Heat causes changes in gene expression and protein synthesis during fruit ripening. These changes cause the inhibition of ethylene synthesis and the action of the cell wall-degrading enzymes [Paull & Chen, 2000]. Heat shock proteins (HSPs) are a group of conserved proteins that function as molecular chaperones and accumulate under heat shock. Previous studies have suggested that HSPs play a pivotal role in heat shock response [Xu *et al*., 2020]. Specifically, accumulation of heat shock proteins has been found in chilling-injured pepper, cucumber, orange, lime and lemon [Valero & Serrano, 2000].

Therefore, the objective of this study was to apply different calcium salts at different temperatures for the treatment of Peumo Pink tomatoes in order to extend their storability at chilling temperature by reducing the metabolic activity, softening and decay of the fruit for more than three weeks. This study was divided into two experiments: in the first, water treatments at different temperatures were carried out on the tomatoes to verify which temperature and exposure time ensured the best fruit quality, which were then applied in calcium salt treatments in the second experiment. The novelty of this study was the combination of heat treatment with calcium salts being able to extend the shelf life of traditional and perishable Chilean 'Peumo Pink' tomatoes.

MATERIALS AND METHODS

Plant material, treatments and experimental design

The Peumo Pink tomato plants were grown in an open field in Pichidegua at 34°S 37' and 71°W 34' in the Libertador Bernardo O'Higgins Region, Chile. In February, 6 to 8 kg of fruit were harvested per plant and placed in clean plastic boxes. Those 11–14 cm in size and whose skin was green breaking to pink were selected (30% in color pink). The fruits were transported immediately to the laboratory (within 2 h) in the Centro de Estudios Postcosecha (CEPOC), Facultad de Ciencias Agronómicas, Universidad de Chile in Santiago de Chile, Chile. Each fruit was cleaned with a damp cloth to remove field dust and dirt. Damaged tomatoes were disposed of and those with a green-pink external color (lightness: 51.6±5.1; chroma: 31.0±3.7; hue angle: 93.9±6.0) and with firmness of 16.9±1.1 N were selected and stored in a cold room at 15°C until the application of treatments the next day.

The fruits were sanitized by immersing them for 2 min in a solution of 150 mg/L of sodium hypochlorite at 10°C and pH 6.5 to eliminate dirt and possible pathogenic microorganisms. About 10 L of sanitized solution was used for every 3 or 4 fruits. The fruits were placed on stainless steel meshes to remove excess water. For water treatments (Experiment 1), fruits were immersed in water at 5, 45 and 60°C for 1 and 4 min. To this end, 60 L of water at the different temperatures were used for every 5 fruits. The water was placed in an 80 L stainless container and heated on an industrial stove. The water temperatures were monitored permanently at different levels by digital thermometers and only a few fruits were treated at the same time to avoid changes in this parameter during the treatments. After the immersion, fruits were placed on stainless steel meshes and each fruit was dried individually with blotting paper. Then, the fruits were weighed on a precision electronic balance (EMB3000–1, Kern, Balingen, Germany) and individually packaged in 20×25 cm polyethylene (PE) bags with a thickness of 0.04 mm and 10 perforations of 1 mm^2 to get a high humidity and air atmosphere. The bags were heat sealed with a manual sealer (FR400, Plastic Film Sealer, Zhejiang, China) and placed on trays in a cold room at 10°C and 85 to 90% RH.

In Experiment 2, the fruits were immersed in calcium salt solutions at 10 and 45°C for 4 min. Calcium chloride, calcium lactate and calcium propionate were dissolved in tap water in concertation of 2% (*w/v*). Tomatoes immersed in tap water at 10°C for 4 min (without calcium salt) were used as a control. The fruits were placed on stainless steel meshes as described for Experiment 1 and packed in the same perforated bags.

The storage time for both experiments was 28 days at 10°C and 85–90% RH plus 2 days at 20°C and 70% RH (simulated commercial conditions). Fruits were taken for analyses after each period of 7, 14, 21 and 28 days (Experiment 1) or 14, 21 and 28 days (Experiment 2) at 10°C followed by 2 days at 20°C (storage period codes: 7+2; 14+2; 21+2; 28+2, respectively). Fifteen bagged tomatoes for both experiments were stored for each period and treatment.

Determinations made in Experiment 1

Respiratory rate and ethylene production

Determinations of respiratory rate and ethylene production were carried out for the treated tomatoes stored at 10°C and for the treated and non-stored fruits. Each fruit was placed in a 500 mL hermetically-sealed plastic jar for 1 h at 10°C. Gas samples were taken from the headspace by a portable gas analyzer (PBI Dansensor, Check Point, Ringsted, Denmark) to determine the concentration of CO_2 and O_2. The values were expressed in mg of CO_2/kg×h [Silveira *et al*., 2017]. An aliquot of 1 mL of a gas sample was also injected into a gas chromatograph (6890 N GC System, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector with a Porapak QN 80/100 column (1.2 m × 3.18 mm) (Supelco, Norwalk, CT, USA). The injector, oven and detector temperatures were 200, 50 and 200°C,

respectively. Helium gas was used as the carrier gas with a flow rate of 55 mL/min. The GC was calibrated daily with a standard gas of 0.5 μL/L (Indura, Santiago, Chile). The ethylene production was expressed in μL C_2H_4/kg×h. Five fruits were used as repetitions for each treatment.

Firmness measurement

A texture analyzer was used to measure fruit firmness (TA. XT express, Stable Micro Systems, Godalming, UK) by a 50 N load cell and a 1.27 cm diameter probe. The penetration test was performed at 3 mm/s speed and 15 mm penetration on three equidistant points of the equatorial zone (after the peel was removed). The results were expressed in Newtons (N). At the beginning of the storage, five individual untreated fruits were used to obtain an initial value. After the storage, five fruits after each treatment were analyzed and three analytical repetitions were performed for each sample (n=15).

Heat shock protein gene expression

Five whole tomatoes after treatments at 5, 45 and 60°C for 1 and 4 min and another five tomatoes not immersed in water as a control were taken for analysis. The fruits were cut into wedges, then frozen in liquid nitrogen and stored in an ultra--low temperature freezer (MDF-U33V, Sanyo, Osaka, Japan) at -80°C until analysis.

The method proposed by Polenta *et al*. [2015] was used with slight modifications for RNA extraction. A piece of frozen tomato with peel was ground in a mortar. Next, 0.1 g of ground sample was weighed and placed in a 1.5 mL Eppendorf tube, then 1 mL of a TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was added. The solution was left for 10 min at room temperature, then 200 μL of chloroform was added, and the mixture was stirred manually for 20 s and left for 15 min at room temperature. Subsequently, the sample was centrifuged at 12,000×g for 15 min at 4°C (Heraeus Megafuge 16R, Thermo Fisher Scientific, Waltham, MA, USA). The sample was separated into a red lower phase with phenol-chloroform, an interface and an aqueous upper phase containing RNA. The aqueous phase was transferred to a new 1.5 mL Eppendorf tube, to which 500 μL of 100% isopropanol was added, and the sample was left for 15 min at room temperature. Subsequently, it was centrifuged at 12,000×g for 10 min at 4°C and then the supernatant was removed. The RNA pellet was washed in 1 mL of 75% (v/v) ethanol and stirred for 15 s. Then, it was centrifuged at 12,000×g for 5 min at 4°C, the supernatant was discarded, and the RNA pellet was dried for 5 min. Afterward, 30 μL of RNase-free water (DEPC water) was added and finally incubated at 55°C for 10 min in a thermal water bath. The quality of the extracted RNA was determined according to the method proposed by Meisel *et al.* [2005]. Two μg were first loaded on an agarose gel in an electrophoresis chamber and the presence of the band was verified, and then 1 μg was loaded and the A_{260}/A_{280} ratio was calculated using a microplate spectrophotometer (Epoch, BioTek Instruments Inc., Winooski, VT, USA).

After RNA extraction, genomic DNA was removed from the samples to perform reverse transcription. To this end, 1 μg of the sample was taken and mixed with 1 μL of 10× reaction buffer $MgCl_2$, 1 μL of DNase I free of RNase and 7 μL of DEPC water. Samples were incubated for 30 min at 37°C in a thermal cycler (Multigene Gradient Labnet, Edison, NY, USA). After a completed cycle, 1 μL of 50 mM ethylenediaminetetraacetic acid (EDTA) was added, and the sample was incubated for 10 min at 65°C. Subsequently, 1 μL of first oligo(dT), 1 μL of random first, 4 μL of 5× reaction buffer, 1 μL of RiboLock RNase inhibitor (20 U/μL), 2 μL of 10 mM dNTP Mix and 1 μL of RevertAid M-MuLVRT (200 U/μL) were added. The sample was immediately incubated for 5 min at 25°C, followed by a cycle of 60 min at 42°C and ended at 70°C for 5 min cycle.

After reverse transcription, the gene expression was assayed for different heat stress proteins. Primers that amplify the actin gene were used to check the effectiveness of reverse transcription and the presence of cDNA. For the polymerase chain reaction (PCR) amplification, 1 μg of cDNA was mixed with 1.5 μL of 50 mM $MgCl_2$; 1 μL of 10 mM dNTP Mix; 5 μL of 10X PCR buffer; 1.5 μL of first forward; 1.5 μL of first reverse; 14 μL of DEPC water and 0.5 μL of Platinum Taq DNA Polymerase (Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA). The PCR was used to amplify the gene encoding for HSP21 and HSP70 proteins (Table 1). Finally, 6 μL of the PCR reaction product were loaded on 1% agarose gel to verify the presence or absence of the expressed gene.

Determinations made in Experiment 2

Each analysis was performed for the treated and stored tomatoes and, for five individual untreated fruits (at the beginning of the storage) to obtain an initial value.

Firmness measurement

The firmness of fruits was measured using a texture analyzer as described before. The results were expressed in N. Five fruits for each treatment were analyzed in triplicate (n=15).

Total calcium content

The method described by Carvajal *et al.* [1999] was used to determine the total calcium content with slight modifications. The fruits were lyophilized in a freeze-dryer (FD5508, IlShin BioBase, South Korea). A portion of 1 g of lyophilized tomato from three fruits was placed in a 200 mL glass flask, then acid digestion was performed by adding 6 mL of nitric acid (65%) and 4 mL of hydrogen peroxide (33%). Samples were placed in an orbital shaker (KS Model 125, IKA, Staufen im Breisgau, Germany) for 15 h. Then, they were autoclaved at 125°C and 147.1 kPa for 70 min, cooled and filtered

TABLE 1. PCR steps used to amplify gene expression for heat shock HSP21 and HSP70 proteins.

Steps	Temperature (°C)	Time (s)	Number of cycles
Initial denaturation	94	120	1
Denaturation	94	30	
Hybridization	54	30	40
Elongation	72	45	

through a 0.22 μm nylon syringe. The calcium content was determined by using microwave plasma atomic emission spectroscopy (MP-AES 4200, Agilent Technologies, USA). Calcium calibration standards were prepared in concentrations of 5, 10, 25, 50 and 75 mg/L in 1% HNO_3 (*v/v*) medium. Calcium content was quantified by measuring peak area at 396.847 mm emission wavelength. The total calcium content was expressed in mg Ca/100 g fresh weight (FW) of fruits.

Extract preparation for determination of total phenolic content and antioxidant capacity

The extracts were obtained from lyophilized (as mentioned above) tomatoes. An aliquot of 10 mL of 70% (*v/v*) methanol was added to 2 g of the lyophilizate following the adapted method of Swain & Hills [1959], and the resulting suspension was homogenized in an Ultra-Turrax disperser (T18 basic, IKA, USA) for approximately 30 s until a uniform consistency was obtained. Then, the sample was centrifuged (Z326K centrifuge, Hermle Labortechnik, Wehingen, Germany) for 15 min at 6,037×*g*. The supernatant was filtered through a 0.45 μm polyvinylidene fluoride (PVDF) filter and the extract was stored for one week at –20°C until the analysis.

Determination of total phenolic content

Total phenolic content (TPC) was measured using the method proposed by Singleton & Rossi [1965]. In a 2 mL Eppendorf tube, 200 μL of the Folin-Ciocalteu reagent were added to 100 μL of the extract, which was left for 5 min. Then, 800 μL of Na_2CO_3 solution were added and after 1 h of reaction time, the tube was centrifuged (Z326K centrifuge, Hermle Labortechnik, Germany) for 2 min at 6,037×*g*. The absorbance of supernatant was read at 765 nm using a UVM340 micro-plate reader (Biochrom Asys, Cambridge, United Kingdom). The total phenolic content was calculated through a calibration curve with gallic acid. The results were expressed as μg of gallic acid equivalents (GAE) per g FW of tomatoes.

Determination of antioxidant capacity

DPPH radical scavenging activity was determined using the method proposed by Brand–Williams *et al*. [1995]. In an Eppendorf tube, 250 μL of the extract and 1 mL of the DPPH radical solution (0.2·mM) were mixed. After 30 min, 200 μL of the sample was transferred to a 96-well plate to measure the absorbance at 517 nm in a spectrophotometer micro-plate reader (UVM340, Biochrom Asys). The results were calculated based on a Trolox calibration curve [Silveira *et al*., 2017] and expressed as μg Trolox equivalents (TE) per g FW of tomatoes. Ferric reducing antioxidant power (FRAP) was determined following the method proposed by Benzie & Strain [1996]. The FRAP reagent was prepared by adding 300 mM acetate buffer (pH 3.5), 200 mM ferric chloride hexahydrate solution to 10 mM 2,4,6-tripyridyl-*s*-triazine in 40 mM HCl. Subsequently, 20 μL of the extract and 600 μL of the FRAP reagent were added in a 96-well plate and measured every 30 min for 120 min at 593 nm in a microplate reader (UVM340, Biochrom Asys). The FRAP was calculated by a Trolox calibration curve. The results were expressed as μg Trolox equivalents (TE) per g FW of fruits.

Statistical analysis

The results were expressed as mean ± standard error. After each storage evaluation period, one-way analysis of variance (one-way ANOVA) was performed for data of both experiments with InfoStat software [Di Rienzo *et al*., 2013]. In the case of finding statistically significant differences between treatments, Tukey's multiple comparisons test was used. All analyses were performed at 5% significance.

RESULTS AND DISCUSSION

Experiment 1

Respiratory rate and ethylene production

Water temperature (5, 45 and 60°C) and immersion time (1 and 4 min) were observed to elicit significant effects on the respiratory rate of tomatoes before storage (Figure 1). The highest values were reached for the treatment at 60°C for 4 min (31.6 mg CO_2/kg×h). At the same time, fruits treated at 5°C for 1 or 4 min had the lowest values of 18.9 to 19.1 mg CO_2/kg×h, respectively. Compared to the immersion at 5°C, at temperatures of 45 and 60°C the respiratory rates

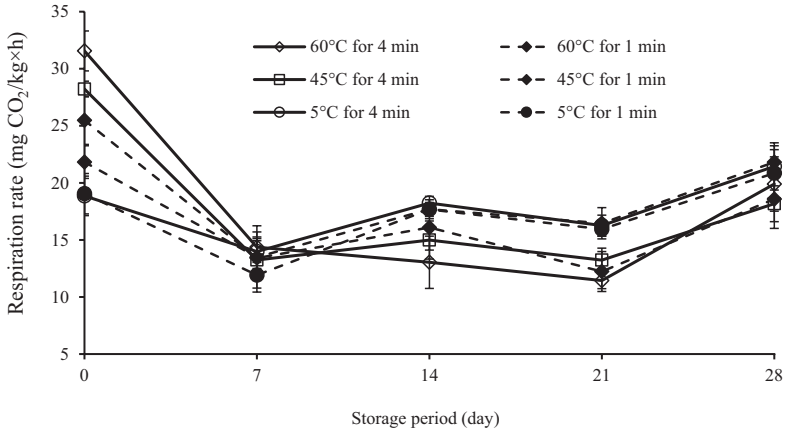

FIGURE 1. Respiratory rate of Peumo Pink tomato fruits treated in water at different temperatures (5, 45 and 60°C) and immersion times (1 and 4 min), and stored for 28 days at 10°C. The values are the mean ± standard error (n=5).

were higher, particularly when exposure time increased from 1 to 4 min. These increased values for the treatments at high temperatures and longer exposure times could be due to heat stress affecting the fruit. This behavior has also been observed in other heat-treated fruits such as mango, melon and pears immersed at 46°C for 75 min, 60°C for 1 min and 45°C for 6 min, respectively [Dea *et al.,* 2010; Silveira *et al.,* 2011a].

After 7 days of cold storage at 10°C, respiratory rates of tomatoes decreased for all treatments (Figure 1) because low temperatures retarded metabolism, as evidenced by reduced respiratory rate and ethylene production values [Oms-Oliu *et al.,* 2008; Silveira *et al.,* 2011a]. After 14 and 21 days of storage at 10°C, a lower respiration rate was found for tomatoes treated in water at 45°C and 60°C than those treated at 5°C (Figure 1); however, no clear differences were found between 1 and 4 min of exposure time. These results are consistent with those obtained by Silveira *et al*. [2011b], who stored cut melon at 5°C for 10 days after dipping in 2% peracetic acid solutions for 1.0, 1.5 and 2.0 min at 5 and 60°C, and found that the respiration rates of all samples treated at 60°C and stored for 3 or more days were lower than those of the samples dipped at 5°C.

In the same storage conditions, ethylene production showed no significant differences among treatments on days 0 and 7 with values between 1.0 to 1.5 μL C_2H_4/kg×h (Figure 2). On day 14, the ethylene production by tomatoes treated at 5°C for 1 min was higher (2.0 μL C_2H_4/kg×h) compared to those immersed at 45°C for 4 min (1.2 μL C_2H_4/kg×h). On day 21, the values for all treatments increased, and ethylene production by fruits after heat treatments, particularly at 60°C for 4 min, was lower compared to the tomatoes treated at 5°C for 1 min. After 28 days no differences were found among treatments (3.4 to 4.5 μL C_2H_4/kg×h).

Firmness

Firmness of tomatoes after immersion in water at different temperatures (5, 45 and 60°C) and times (1 and 4 min) and cold-storage are shown in Figure 3. At the beginning of the storage, the firmness of untreated tomatoes was 16.9±1.09 N. Fruits treated at 45 and 60°C for 4 min maintained the highest firmness on days 14+2 and 21+2, which could be a direct effect of heat stress from hot water immersion. In general, firmness of fruits treated at 5°C for 1 or 4 min was similar to those immersed at 45 and 60°C for 1 min.

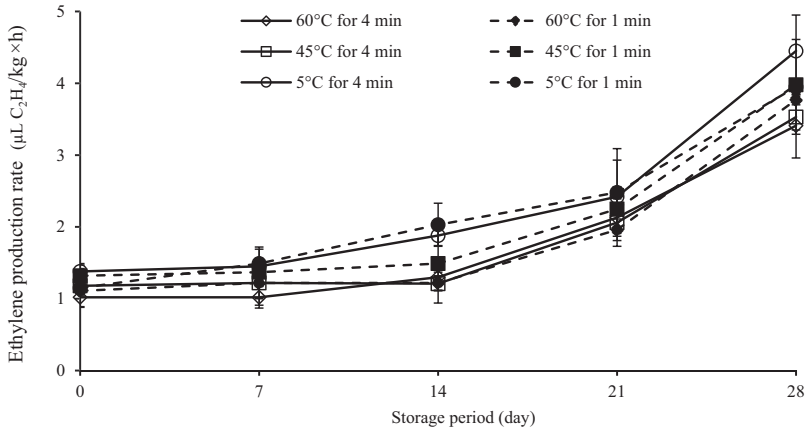

FIGURE 2. Ethylene production rate of Peumo Pink tomato fruits treated in water at different temperatures (5, 45 and 60°C) and immersion times (1 and 4 min), and stored for 28 days at 10°C. The values are the mean ± standard error (n=5).

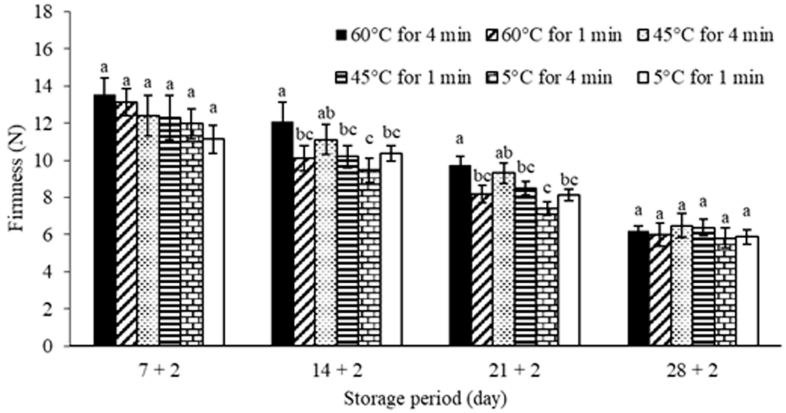

FIGURE 3. Firmness of Peumo Pink tomato fruits treated in water at different temperatures (5, 45 and 60°C) and immersion times (1 and 4 min), and stored for 28 days at 10°C + 2 days at 20°C. The values are the mean ± standard error (n=15). Different letters above bars separately for each storage period indicate significant differences (p<0.05).

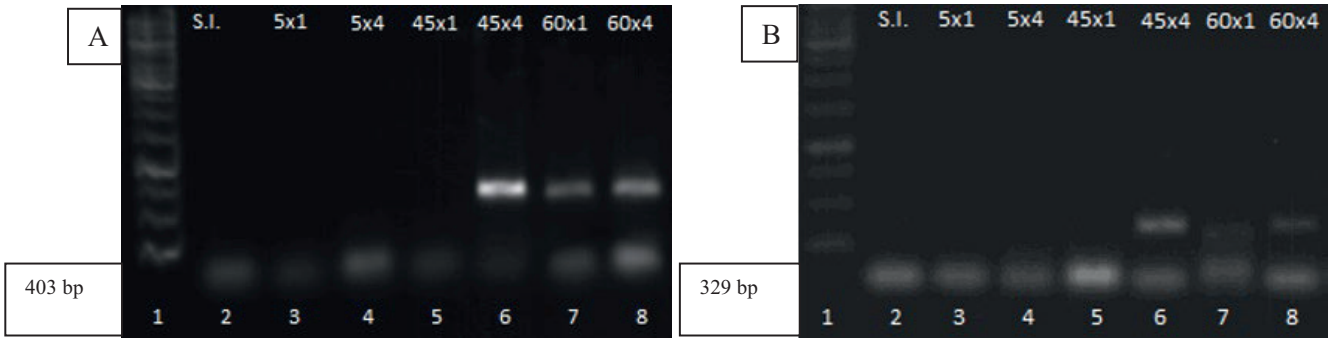

FIGURE 4. PCR for gene expression of heat shock proteins HSP21 (A) and HSP70 (B) in Peumo Pink tomato fruits treated in water at different temperatures (5, 45 and 60°C) and immersion times (1 and 4 min). Lane 1 corresponds to a molecular weight standard GeneRuler 1kb DNA Ladder; lane 2: no immersion (SI); lane 3: 5°C for 1 min (5×1); lane 4: 5°C for 4 min (5×4); lane 5: 45°C for 1 min (45×1); lane 6: 45°C for 4 min (45×4); lane 7: 60°C for 1 min (60×1) and lane 8: 60°C for 4 min (60×4). Samples were run in 1% agarose gel. bp: base pairs.

These responses could indicate that more than 1 min of exposure time is required at temperatures between 45 to 60°C to delay firmness losses. The decrease in fruit firmness as a result of heat treatment could be due to the fact that the high temperature caused a direct inhibition of the activity of pectin methylesterase and polygalacturonase [Akbudak *et al*., 2007; Pinheiro *et al.*, 2015].

Other authors have also demonstrated the effect of applying hot water immersion to firmness retention. Pinheiro *et al.* [2015] treated whole tomatoes in water at 40°C for 30 min and during 14 days of storage at 10°C the firmness of treated and untreated fruits decreased by 2.1 N and 4.3 N, respectively. Meanwhile, Lurie *et al*. [1997] used hot air at 48°C for 2 min on whole tomato and after 1 day of storage at 5°C, firmness of heat-treated fruits decreased by 2 N. On the other hand, Akbudak *et al.* [2007] found that whole tomatoes immersed in water at 54°C for 5 min had firmness of 6.87 N compared to the control with 1.67 N after 28 days of storage.

Gene expression for heat shock proteins

In Figure 4, the presence of bands shows the gene expression for the HSP21 protein in fruits treated at 45°C for 4 min and 60°C for 1 or 4 min. However, in the case of the fruit treated at 5°C for 1 or 4 min, 45°C for 1 min and untreated fruit (no immersion, SI), there was no expression, likely due to the exposure time or the temperature not being sufficient to provoke a gene response. A similar trend was found for HSP70, where the gene was expressed in fruits treated at 45 or 60°C for 4 min, while the treatment at 60°C for 1 min contributed to a very low expression compared to the other heat treatments. Finally, no expression was observed after tomato treatments at 5°C for 1 or 4 min and 45°C for 1 min.

According to Lurie *et al.* [1997] and Zhang *et al*. [2005], temperatures above 30°C produce a rapid induction and increase of the gene expression that encodes heat shock proteins (HSPs), which exert a protective role against heat stress in the tissues.

Cruz-Mendívil *et al*. [2015] found that the most important heat shock proteins of tomato fruits are HSP21 and HSP70, which have an effect of thermal tolerance to chilling injury. This effect was linked to the chaperone action of these proteins in which it aided the correct folding of other partially denatured proteins, preventing them from irreversibly aggregating with other cell proteins. Other authors, such as Zou *et al*. [2012], mentioned the important role of these proteins as cell membrane stabilizers, since they help maintain their fluidity and integrity, as well as reduce the leakage of electrolytes in the membrane at the time of stress. The same authors observed a significant decrease in cell membrane electrolyte loss in transgenic rice plants that overexpress genes encoding for HSP23 and HSP17. In addition, HSP70 induced the activity of antioxidant enzymes, which would protect against oxidative stress. According to Zhang *et al.* [2011], there is a synergistic relationship between HSP70 and the antioxidant capacity in the plant cell that could mitigate the effect of chilling injury on grape berries by applying forced air heat stress at 39°C for 10 h. This response was achieved due to the decrease in electrolyte leakage in the cell membrane and increased activity of catalase (CAT) and superoxide dismutase (SOD). The same authors suggested that exposure to forced air induced an expression of HSP70 in these berries, which enhanced the activity of antioxidant enzymes, maintained greater membrane integrity and, subsequently, caused resistance to chilling injury.

After high-temperature treatment, an increase in gene expression for HSP was reported by Polenta *et al.* [2015], who, by applying forced air at 39°C for 24 h to tomato fruit, observed a higher expression of HSP21 and HSP70. By contrast, Guidi *et al.* [2008] obtained a higher expression of HSP21, HSP23 and HSP70 in tomatoes treated in water at 42°C for 60 min or forced air at 39°C for 24 h compared to the untreated fruits.

Summarizing, the tomatoes treated at 45°C showed less respiration, the same firmness and a slightly higher expression of the HSP21 and HSP70 proteins than the tomatoes treated at 60°C. In addition, tomatoes immersed in water at 60°C presented uneven ripening that did not occur in tomatoes treated at 45°C (data not shown). Therefore, 45°C was chosen as the treatment temperature in the second experiment.

Experiment 2

Firmness

The initial firmness of untreated tomatoes before calcium application was 18.2±2.1 N. However, the firmness of Peumo Pink tomatoes treated in solutions of calcium salts (calcium

TABLE 2. Firmness (N) and total calcium content (mg Ca/100 g fresh weight) of Peumo Pink tomato fruits treated in solutions of calcium salts at two immersion temperatures (10 and 45°C), and stored for 28 days at 10°C + 2 days at 20°C.

Treatment	Firmness	Ca content	Firmness	Ca content	Firmness	Ca content
	14+2 days		21+2 days		28+2 days	
10°C + NSA	13.6±0.9^{c}	6.2±0.4^{b}	7.4±0.5^{de}	6.4±0.4^{NS}	5.9±0.5^{b}	6.4±0.4^{cd}
10°C + CaCh	8.0±0.8^{e}	5.5±0.7^{b}	9.4±0.3^{cd}	6.6±0.2	5.7±0.3^{b}	7.1±0.5^{b}
10°C + CaLa	11.5±0.7^{d}	5.3±0.7^{b}	8.2±0.4^{de}	6.0±0.2	7.0±0.3^{b}	7.5±0.2^{ab}
10°C + CaPr	17.5±0.5^{a}	6.2±0.5^{b}	7.5±0.8^{de}	7.7±0.3	6.2±0.4^{b}	6.3±0.2^{d}
45°C + NSA	16.0±0.7^{ab}	6.0±0.2^{b}	6.5±0.8^{e}	7.1±0.2	6.3±0.3^{b}	5.5±0.4^{e}
45°C + CaCh	17.5±0.7^{a}	8.9±0.5^{a}	14.7±0.8^{a}	8.4±0.3	6.8±0.3^{b}	7.6±0.4^{ab}
45°C + CaLa	15.1±1.0^{bc}	8.4±0.6^{a}	12.6±0.8^{ab}	7.7±0.3	9.4±0.4^{a}	7.5±0.1^{ab}
45°C + CaPr	13.8±0.8^{c}	7.5±0.5^{a}	10.4±0.8^{bc}	8.1±0.6	9.4±0.4^{a}	8.3±0.3^{a}

The values are the mean ± standard error (n=15 for firmness and n=5 for total calcium content). Different letters vertically indicate significant differences (p<0.05). NS, not significant. CaCh, calcium chloride; CaLa, calcium lactate; CaPr, calcium propionate; NSA, no salt application.

chloride, propionate and lactate) and two immersion temperatures (10 and 45°C) changed significantly during storage for 28 days at 10°C + 2 days at 20°C (Table 2). In general, after day 14+2, the heat-treated fruit showed higher firmness than those dipped in corresponding cold calcium solution or water, except the treatment with calcium propionate. On day 21+2, firmness of tomatoes treated in all heat calcium solutions was higher and the highest values were reached for calcium chloride (14.7 N) and calcium lactate (12.6 N). At the end of storage, after day 28+2, the fruits treated in propionate and lactate calcium at 45°C had the lowest firmness losses. According to Magee *et al.* [2003], the treatment of tomatoes with a 2% calcium chloride solution at 50°C maintained their firmness for 20 days as opposite to dipping in water at 10°C. On the other hand, Mahmud *et al.* [2008] reported that firmness of papaya dipped in 2% and 2.5% calcium chloride was maintained after storage for 24 days at 12°C. A previous report by Luna-Guzman & Barrett [2000] showed a higher firmness in fresh-cut melon immersed in a 2.5% calcium lactate solution at 60°C after 12 days at 5°C compared to controls without salt.

An increase in temperatures caused the activation of the pectin methylesterase. This enzyme catalyzes the demethoxylation of pectin of the cell wall favoring adhesion of free carboxyl groups by calcium ions known as calcium pectates. The calcium pectates provide firmness to the tissue, reducing softening and water loss [Aguayo *et al*., 2008; Luna-Guzman & Barrett, 2000; Ni *et al*., 2005].

Total calcium content

The total calcium content of untreated Peumo Pink tomatoes at the beginning of storage was 6.6±0.4 mg Ca/100 g FW. In tomatoes treated in solutions of calcium salts (calcium chloride, propionate and lactate) and two immersion temperatures (10 and 45°C), it changed significantly during storage for 28 days at 10°C + 2 days at 20°C. The total calcium content was similar for fruits treated in water and calcium salt solutions at 10°C. However, the fruit treated with calcium chloride, lactate and propionate at 45°C had higher total calcium contents, although significant differences were noted only at 14+2 days (Table 2). According to Silveira *et al.* [2011a], the application of calcium salts at high temperatures increased the diffusion of calcium in porous apoplasts and increased calcium retention in the cell wall. The increased fruit calcium contents upon the treatment with heat and calcium salts were also reported by Aguayo *et al.* [2015] in fresh-cut apples. Naser *et al*. [2018] increased total calcium in persimmon by applying 2% and 4% calcium lactate at 45°C. According to Manganaris *et al.* [2005], applications of Ca in the form of calcium chloride, calcium propionate and calcium lactate to canned peaches caused a 2.5-fold increase in Ca content in the treated fruit, saturating the Ca content of the cell wall, leading to increased firmness and crispiness of canned peaches.

Total phenolic content

Before calcium treatments, TPC of tomatoes was 214±58 μg GAE/g FW. The TPC of tomatoes after immersion in calcium solutions and in water (control) at two immersion temperatures and during cold-storage is shown in Table 3. Peumo Pink tomatoes immersed in 2% calcium chloride at 45°C and control at 10°C had the highest total phenolic content on days 21+2; however, on days 28+2, the highest TPC was found in tomatoes treated in a calcium lactate solution at 45°C and calcium propionate at 10°C followed by calcium chloride at 45°C. However, no clear trend was observed in each evaluated storage period. Nevertheless, higher total phenolic contents along with cold storage time (Table 3). The increasing TPC during storage may be due to the activity of the phenylalanine ammonium lyase (PAL), a key enzyme in the synthesis of phenylpropanoid, and some secondary metabolites [Son *et al.,* 2012], including phenolic compounds. According to Aghdam *et al.* [2013] and Nisar *et al*. [2015], calcium salts can increase PAL activity, and Nasef [2018] found that immersion in water at 45°C raised TPC in pumpkin samples compared to those treated at 10°C. At the same time, the increased TPC in our study found in Peumo Pink tomatoes during storage at 10°C can be caused by a stress

TABLE 3. Total phenolic content (TPC, μg GAE/g fresh weight, FW) and antioxidant capacity determined as ferric-reducing antioxidant power (FRAP) and DPPH radical scavenging activity (both as μg TE/g FW) of Peumo Pink tomato fruits treated in solutions of calcium salts at two immersion temperatures (10 and 45°C), and stored for 28 days at 10°C + 2 days at 20°C

Treatments	TPC	FRAP	DPPH assay	TPC	FRAP	DPPH assay	TPC	FRAP	DPPH assay
	14+2 days			21+2 days			28+2 days		
10°C + NSA	288±10^{NS}	248±22^{ab}	286±10^{NS}	680±26^{a}	448±30^{ab}	456±10^{a}	650±15^{b}	520±1de	563±20^{cd}
10°C + CaCh	277±23	151±23^{c}	278±10	493±26^{b}	247±41^{cd}	417±15^{b}	668±30^{b}	505±30^{e}	547±19^{d}
10°C + CaLa	226±8	196±28^{bc}	282±9	588±18^{ab}	347±33^{bc}	414±18^{b}	577±11^{c}	363±26^{f}	521±29^{e}
10°C + CaPr	222±16	207±16^{ab}	278±12	427±30^{c}	217±39^{d}	401±6^{c}	747±20^{a}	631±31^{b}	593±24^{b}
45°C + NSA	228±9	148±26^{c}	277±12	474±15^{bc}	331±35^{c}	411±12^{bc}	679±41^{b}	569±20^{cd}	580±13^{bc}
45°C + CaCh	217±12	148±30^{c}	276±11	705±22^{a}	509±36^{a}	455±18^{a}	708±23^{ab}	600±46^{bc}	582±20^{bc}
45°C + CaLa	266±21	268±11^{a}	286±9	552±25^{b}	347±20^{bc}	416±15^{bc}	746±33^{a}	696±26^{a}	615±13^{a}
45°C + CaPr	218±26	156±21^{c}	279±6	551±30^{b}	342±15^{bc}	429±13^{ab}	680±35^{b}	592±16^{bc}	574±9^{bc}

The values are the mean ± standard error (n=5). Different letters vertically indicate significant differences (p<0.05). NS, not significant. CaCh, calcium chloride; CaLa, calcium lactate; CaPr, calcium propionate; NSA, no salt application; GAE, gallic acid equivalent; TE, Trolox equivalent.

affecting the fruit at this low temperature. Many authors recommended a minimum temperature of 12 to 15°C to avoid chilling injury of the tomatoes [Polenta *et al.,* 2015; Zhang *et al.,* 2016]; thus, after a prolonged storage, increased activity of the PAL enzyme could occur as a response to chilling stress [Silveira & Escalona, 2014].

Antioxidant capacity

The antioxidant capacity of Peumo Pink tomatoes before treatments with calcium salt solutions were 71.8±7.7 and 247±15 μg TE/g FW determined as FRAP and DPPH radical scavenging activity, respectively. After treatments with calcium salt solutions. the FRAP of tomatoes at both immersion temperatures and cold-stored for 28+2 days ranged from 363 to 696 μg TE/g FW (Table 3). No clear trend was found between treatments for each storage period; however, like TPC, the FRAP of tomatoes after individual treatments increased as their storage time increased. A similar trend was found for DPPH radical scavenging activity (Table 3). The differences between the treatments, as for the FRAP, did not show clear trends. On days 14+2, 21+2 and 28+2, the values were between 276 to 286, 401 to 456 and 521 to 615 μg TE/g FW, respectively.

As previously mentioned, a clear trend was observed for all treatments during cold storage at 10°C where higher values were found at a longer storage period. This increased antioxidant capacity, which as related to total phenolic content, may be as a response to chilling injury at temperatures below 10°C. Several studies have shown that fruit treated in calcium salts, such as peach [Zhi *et al.,* 2017] or cherry [Aghdam *et al.,* 2013], reached a higher antioxidant capacity. According to Naser *et al*. [2018], persimmon fruits dipped in 2% and 4% calcium lactate at 45°C and stored for 40 days had a higher antioxidant capacity compared to untreated fruit stored at 10°C. Use of a calcium salt solution at elevated temperatures activated the ionic calcium channels in the plasma membrane allowing calcium to enter the cytosol. Calcium could bind to the calmodulin protein, causing intracellular signaling and enhancing PAL enzyme activity, which would increase the antioxidant capacity [De Freitas & Mitcham, 2012; Wang *et al*., 2014]. Additionally, the increase in antioxidant capacity in all treatments throughout cold storage could also be due to a natural ripening evolution of the tomato fruit, where antioxidant compounds, such as lycopene, β-carotene and flavonoids, are synthesized [Dorais *et al*., 2008].

CONCLUSIONS

The immersion in water at 45 and 60°C for 4 min caused a delay in the ripening of the Peumo Pink tomato, keeping firmness for 21 days at 10°C plus 2 days at 20°C without symptoms of rotting. Additionally, the gene expression for the heat shock proteins found for these combinations of temperatures and exposure times could be an interesting alternative to predict the effectiveness of heat treatment in such fruit as Peumo Pink tomatoes.

Applications of calcium salts at 45°C for 4 min caused changes in the physicochemical properties of Peumo Pink tomatoes. Immersion in calcium lactate and propionate at 45°C for 4 min proved best to maintain the firmness of the fruit for 28 days at 10°C plus 2 days at 20°C. The TPC and antioxidant capacity increased with the storage time of tomatoes. Although calcium applications showed an increase in TPC, no clear trend was observed on each evaluation day. The use of high temperatures with calcium salts for dipping would be beneficial to provide the vegetal tissues with calcium and reduce the softening of the tomato after prolonged cold storage.

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CONFLICT OF INTEREST

The authors declare that the study was conducted in absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REFERENCES

1. Aghdam, S.M., Dokhanieh, A.Y., Hassanpour, H., Fard, J.R. (2013). Enhancement of antioxidant capacity of cornelian cherry (*Cornus mas*) fruit by postharvest calcium treatment. *Scientia Horticulturae, 161*, 160–164. https://doi.org/10.1016/j.scienta.2013.07.006
2. Aguayo, E., Escalona, V.H., Artés, F. (2008). Effect of hot water treatment and various calcium salts on quality of fresh-cut 'Amarillo' melon. *Postharvest Biology and Technology, 47*(3), 397–406. https://doi.org/10.1016/j.postharvbio.2007.08.001
3. Aguayo, E., Requejo-Jackman, C., Stanley, R., Woolf, A. (2015). Hot water treatment in combination with calcium ascorbate dips increases bioactive compounds and helps to maintain fresh-cut apple quality. *Postharvest Biology and Technology, 110*, 158–165. https://doi.org/10.1016/j.postharvbio.2015.07.001
4. Akbudak, B., Akbudak, N., Seniz, V., Eris, A. (2007). Sequential treatments of hot water and modified atmosphere packaging in cherry tomatoes. *Journal of Food Quality, 30*(6), 896–910. https://doi.org/10.1111/j.1745-4557.2007.00168.x
5. Benzie, I.F., 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. https://doi.org/10.1006/abio.1996.0292
6. Brand–Williams, W., Cuvelier, M.E., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – – Food Science and Technology, 28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
7. Carvajal, M., Martínez, V., Cerda, A. (1999). Influence of magnesium and salinity on tomato plants grown in hydroponic culture. *Journal Plant Nutrition, 22*(1), 177–190. https://doi.org/10.1080/01904169909365616
8. Cruz-Mendívil, A., López-Valenzuela, J., Calderón-Vázquez, C., Vega-García, M., Reyes-Moreno, C., Valdez-Ortiz, A. (2015). Early transcriptional responses to chilling stress in tomato fruit with hot water pre-treatment. *Postharvest Biology and Technology, 109*, 137–144. https://doi.org/10.1016/j.postharvbio.2015.06.015
9. De Freitas, S.T., Mitcham, E.J. (2012). Factors involved in fruit calcium deficiency disorders. *Horticultural Revision, 40*, 107–146.
10. Dea, S., Bretch, J.K., Nunes, M.C.N., Baldwin, E.A. (2010). Quality of fresh-cut "Kent" mango slices prepared from hot water or non-hot water-treated fruit. *Postharvest Biology and Technology, 56*(2), 171–180. https://doi.org/10.1016/j.postharvbio.2010.01.007
11. Di Rienzo J.A., Casanoves, F., Balzarini, M.G., González, L., Tablada, M., Robledo, W. (2013). InfoStat versión 2013. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.
12. Dorais, M., Ehret, D.L., Papadopoulos, A.P. (2008). Tomato (*Solanum lycopersicum* L.) health components: from the seed to the consumer. *Phytochemistry Reviews, 7*, 23–50. https://doi.org/10.1007/s11101-007-9085-x
13. FAOSTAT. 2020. Available at: [http://www.fao.org/faostat/en/#home].
14. Guidi, S., Nanni, M., Polenta, G. (2008). Evaluation of heat stress proteins in fruits subjected to postharvest heat treatments (English). Evaluación de proteínas de estrés térmico en frutos sometidos a tratamientos térmicos en postcosecha (Spanish original title). Instituto Nacional de Tecnología Agropecuaria, p. 5. [https://inta.gob.ar/sites/default/files/script-tmp-inta__evaluacin_de_protenas_de_estrs_trmico_en_frutos.pdf].
15. Lara, I., García, P., Vendrell, M. (2004). Modifications in cell wall composition after cold storage of calcium-treated strawberry (*Fragaria* × *ananassa* Duch.) fruit. *Postharvest Biology and Technology, 34*(3), 331–339. https://doi.org/10.1016/j.postharvbio.2004.05.018
16. Li, S., Zhu, B., Pirrello, J., Xu, C., Zhang, B., Bouzayen, M., Chen, K., Grierson, D. (2020). Roles of RiN and ethylene in tomato fruit ripening and ripening-associated traits. *New Phytologist, 226*(2), 460–475. https://doi.org/10.1111/nph.16362
17. Luna-Guzmán, I., Barrett, D.M. (2000). Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupes. *Postharvest Biology and Technology, 19*(1), 61–72. https://doi.org/10.1016/S0925-5214(00)00079-X
18. Lurie, S., Laamin, M., Lapsker, Z., Fallik, E. (1997). Heat treatments to decrease chilling injury in tomato fruit. Effects in lipids, pericarp lesions and fungal growth. *Physiology Plantarum, 100*(2), 297–302. https://doi.org/10.1111/j.1399-3054.1997.tb04786.x
19. Magee, R.L., Caporaso, F., Prakash, A. (2003). Effects of exogenous calcium salt treatments on inhibiting irradiation–induced softening in diced Roma tomatoes. *Journal Food Science, 68*(8), 2430–2435. https://doi.org/10.1111/j.1365-2621.2003.tb07041.x
20. Mahmud, T.M.M., Al Eryani-Raqeeb, A., Syed Omar, S.R., Mohamed, Z., Abdul-Rahman, A.E. (2008). Effects of different concentrations and applications of calcium on storage life and physicochemical characteristics of papaya (*Carica papaya* L.). *American Journal of Agricultural and Biological Sciences, 3*(3), 526–533. https://doi.org/10.3844/ajabssp.2008.526.533
21. Manganaris, G.A., Vasilakakis, M., Diamantidis, G., Mignani, I. (2005). Effect of calcium additives on physicochemical aspects of cell wall pectin and sensory attributes of canned peach (*Prunus persica* L. Batsch cv. Andross). *Journal of the Science of Food and Agriculture, 85*(10), 1773–1778. https://doi.org/10.1002/jsfa.2182
22. Meisel, L., Fonseca, B., González, S., Baeza-Yates, R., Cambiazo, V., Campos, R., Gonzalez, M., Orellana, A., Retamales, J., Silva, H. (2005). A rapid and efficient method for purifying high quality total RNA from peaches (*Prunus persica*) for functional genomics analyses. *Biological Research, 38*(1), 83–88. https://doi.org/10.4067/s0716-97602005000100010.

23. Nasef, I. (2018). Short hot water as safe treatment induces chilling tolerance and antioxidant enzymes, prevents decay and maintains quality of cold-stored cucumbers. *Postharvest Biology and Technology, 138*, 1–10. https://doi.org/10.1016/j.postharvbio.2017.12.005
24. Naser, F., Rabiei, V., Razavi, F., Khademi, O. (2018). Effect of calcium lactate in combination with hot water treatment on the nutritional quality of persimmon fruit during cold storage. *Scientia Horticulturae, 233,* 114–123. https://doi.org/10.1016/j.scienta.2018.01.036
25. Ni, L., Lin, D., Barret, M.D. (2005). Pectin methylesterase catalyzed firming effects of low temperature blanched vegetables. *Journal of Food Engineering, 70*(4), 546–556. https://doi.org/10.1016/j.jfoodeng.2004.10.009
26. Nisar, R., Baba, W.N., Masoodi, F.A. (2015). Effect of chemical and thermal treatments on quality parameters and antioxidant activity of apple (pulp) grown in high Himalayan regions. *Cogent Food & Agriculture, 1*(1), 10–16. https://doi.org/10.1080/23311932.2015.1063797
27. Oms-Oliu, G., Aguiló-Aguayo, I., Soliva-Fortuny, R., Martin-Belloso, O. (2008). Effect of ripeness at processing on fresh-cut "Flor de invierno" pears packaged under modified atmospheres conditions. International *Journal of Food Science and Technology, 44*(5), 900–909. https://doi.org/10.1111/j.1365-2621.2007.01635.x
28. Paull, R.E., Chen, N.J. (2000). Heat treatment and fruit ripening. *Postharvest Biology and Technology, 21*(1), 21–37. https://doi.org/10.1016/S0925-5214(00)00162-9
29. Pinheiro, J., Alegria, C., Abreu, M., Sol, M., Goncalves, E.M., Silva, C.L.M. (2015). Postharvest quality of refrigerated tomato fruit (*Solanum Lycopersicum*, cv. Zinac) at two maturity stages following heat treatment. *Journal of Food Processing and Preservation, 39*(6), 697–709. https://doi.org/10.1111/jfpp.12279
30. Polenta, G., Budde, C., Sivakumar, D., Nanni, M., Guidi, S. (2015). Evaluation of biochemical and quality attributes to monitor the application of heat and cold treatments in tomato fruit (*Licopersicum esculentum* Mill.). *Journal of Food Quality, 38*(3), 153–163. https://doi.org/10.1111/jfq.12139
31. Serrano, M., Martínez-Romero, D., Castillo, S., Guillén, F., Valero, D. (2004). Role of calcium and heat treatments in alleviating physiological changes induced by mechanical damage in plum. *Postharvest Biology and Technology, 34*(2), 155–167. https://doi.org/10.1016/j.postharvbio.2004.05.004
32. Silveira, A.C., Aguayo, E., Chisari, M., Artés, F. (2011a). Calcium salts and heat treatment for quality retention of fresh-cut 'Galia' melón. *Postharvest Biology and Technology, 62*(1), 77–84. https://doi.org/10.1016/j.postharvbio.2011.04.009
33. Silveira, A.C., Aguayo, E., Escalona, V.H., Artés, F. (2011b). Hot water treatment and peracetic acid to maintain fresh-cut Galia melon quality. *Innovative Food Science and Emerging Technologies, 12*(4), 569–576. https://doi.org/10.1016/j.ifset.2011.02.010
34. Silveira, A., Escalona, V.H. (2014). The use of the physical treatment on fresh-cut produce. *Stewart Postharvest Review, 10*(3), 1–5.
35. Silveira, A.C., Oyarzún, D., Sepúlveda, A., Escalona, V. (2017). Effect of genotype, raw-material storage time and cut type on native potato suitability for fresh-cut elaboration. *Postharvest Biology and Technology, 128*, 1–10. https://dx.doi.org/10.1016/j.postharvbio.2017.01.011
36. Singleton, V.L., Rossi, A.J. (1965). Colorimetry of total phenolics with phosphomolybdic – phosphotungstic and reagents. *American Journal of Enology and Viticulture, 16*, 144–157.
37. Son, K-H., Park, J-H., Kim, D., Oh, M.-M. (2012). Leaf shape index, growth, and phytochemicals in two leaf lettuce cultivars grown under monochromatic light-emitting diodes. *Korean Journal Horticultural Science & Technology, 30*(6), 664–672. https://doi.org/10.7235/hort.2012.12063
38. Sun, Q., Zhang, N., Wang, J., Zhang, H., Li, D., Shi, J., Li, R., Weeda, S., Zhao, B., Ren, S., Guo, Y.-D. (2015). Melatonin promotes ripening and improves quality of tomato fruit during postharvest life. *Journal of Experimental Botany, 66*(3), 657–668. https://doi.org/10.1093/jxb/eru332
39. Swain, T., Hills, W.E. (1959). The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture, 10*(1), 63–68. https://doi.org/10.1002/jsfa.2740100110
40. Valero, D., Serrano, M. (2010). *Postharvest Biology and Technology for Preserving Fruit Quality*. 1st edition, CRC Press. Taylor & Francis Group, pp. 109–124.
41. Wang, C. (1998). Heat treatment affects postharvest quality of kale and collard, but not of brussels sprouts. *HortScience, 33*, 881–883. https://doi.org/10.21273/HORTSCI.33.5.881
42. Wang, Y., Xie, X., Long, L. (2014). The effect of postharvest calcium application in hydro cooling water on tissue calcium content, biochemical changes, and quality attributes of sweet cherry fruit. *Food Chemistry, 160*, 22–30. https://doi.org/10.1016/j.foodchem.2014.03.073
43. Xu, L., Gao, J., Guo, L., Yu, H. (2020). Heat shock protein 70 (HmHsp70) from *Hypsizygus marmoreus* confers thermotolerance to tobacco. *AMB Express, 10*, art. no. 12. https://dx.doi.org/10.1186%2Fs13568-020-0947-6
44. Zhang, J., Huang, W., Pan, Q., Liu, Y. (2005). Improvement of chilling tolerance and accumulation of heat shock proteins in grape berries (*Vitis vinifera* cv. Jingxiu) by heat pretreatment. *Postharvest Biology and Technology, 38*(1), 80–90. https://doi.org/10.1016/j.postharvbio.2005.05.008
45. Zhang, B., Tieman, D.M., Jiao, C., Xu, Y.M., Chan, K.S., Fe, Z.J., Giovannoni, J.J., Klee, H.J. (2016). Chilling-induced tomato flavour is associated with altered volatile synthesis and transient changes in DNA methylation. *Proceedings of the National Academy of Sciences of the United States of America, 113*(44), 12580–12585. https://doi.org/10.1073/pnas.1613910113
46. Zhang, L., Yu, Z., Jiang, L., Jiang, J., Luo, H., Fu, L. (2011). Effect of post-harvest heat treatment on proteome change of peach fruit during ripening. *Journal Proteomics, 74*(7), 1135–1149. https://doi.org/10.1016/j.jprot.2011.04.012
47. Zhi, H.H., Liu, Q., Dong, Y., Liu, M., Zong, W. (2017). Effect of calcium dissolved in slightly acidic electrolyzed water on antioxidant system, calcium distribution, and cell wall metabolism of peach in relation to fruit browning. *Journal Horticultural Science and Biotechnology, 92*(6), 621–629. https://doi.org/10.1080/14620316.2017.1309994
48. Zou, J., Liu, C., Liu, A., Zou, D., Chen, X. (2012). Overexpression of OsHsp17.0 and OsHsp23.7 enhances drought and salt tolerance in rice. *Journal Plant Physiology, 169*(6), 628–635. https://doi.org/10.1016/j.jplph.2011.12.014