The ameliorative effects of spermidine and calcium chloride on chilling injury in pomegranate fruits after long-term storage

Asghar Ramezanian[1]*, Majid Rahemi[1], Manoochehr Maftoun[2], Bahman Kholdebarin[3], Saeid Eshghi[1], Mohammad Reza Safizadeh[4], Vahid Tavallali[1]

[1] Shiraz Univ., Coll. Agric., Dep. Hortic. Sci., Shiraz, I.R. Iran
ramezanian@shirazu.ac.ir

[2] Shiraz Univ., Coll. Agric., Dep. Soil Sci., Shiraz, I.R. Iran

[3] Shiraz Univ., Coll. Agric., Dep. Biol., Shiraz, I.R. Iran

[4] Shiraz Univ., Darab Coll. Agric., Dep. Plant Prod., Darab, I.R. Iran

* *Correspondence and reprints*

Received 28 August 2009
Accepted 21 October 2009

Fruits, 2010, vol. 65, p. 169–178

DOI: 10.1051/fruits/2010011
www.fruits-journal.org

***Resumen Español**, p. 178*

The ameliorative effects of spermidine and calcium chloride on chilling injury in pomegranate fruits after long-term storage.

Abstract — Introduction. Pomegranate fruits (*Punica granatum* L.) are chilling-sensitive crops. **Materials and methods**. Pomegranate fruits were treated with calcium chloride and spermidine, alone or in combination, by normal dip and vacuum infiltration methods. The treated fruits were stored at 2 °C for 4 months. At the end of the storage period, samples were held for 3 days at 20 °C, then the qualitative constituents were evaluated. **Results and discussion**. Treated fruits exhibited less weight loss and higher juice content than control fruits. Non-treated fruits developed chilling injury manifested as an increase in K^+ leakage and polyphenol oxidase activity. Calcium chloride and spermidine treatments resulted in lower soluble solid content, but some fruits showed higher titratable acidity. All treatments significantly increased ascorbic acid content. The pH of aril juice in treated fruits was lower than that of non-treated fruits, probably due to higher titratable acidity. Total antioxidant activity and total phenolic content increased in treated fruits. In our study, a correlation was observed between total phenolic content and total antioxidant activity. **Conclusion**. The treatments applied in our experiments maintained overall quality of pomegranate fruits during long-term storage. Postharvest application of calcium and spermidine either alone or in combination could ameliorate adverse effects of low temperature on pomegranate fruit quality during cold storage. Vacuum infiltration was as effective as the normal dip method. However, normal dip is a simpler and faster treatment method.

Iran Islamic Republic / *Punica granatum* / fruits / chemicophysical properties / phenolic content / antioxidants / catechol oxidase / cold tolerance

La spermidine et le chlorure de calcium atténuent les dommages dus au froid pour des grenades stockées à long terme.

Résumé — Introduction. Les fruits du grenadier (*Punica granatum* L.) sont sensibles au froid. **Matériel et méthodes**. Des grenades ont été traitées avec du chlorure de calcium et de la spermidine, seuls ou en combinaison, par simple immersion et par une méthode d'infiltration sous vide. Les fruits traités ont été stockés à 2 °C pendant 4 mois. À la fin de la période de stockage, les échantillons ont été maintenus pendant 3 jours à 20 °C, puis certains caractères qualitatifs ont été évalués. **Résultats et discussion**. Les fruits traités ont perdu moins de poids et ont présenté une meilleure teneur en jus que les fruits témoins. Les fruits non traités ont présenté des dommages dus au froid qui se sont manifestés par une augmentation de la perte de K^+ et de l'activité de la polyphénol oxydase. Les traitements de chlorure de calcium et de spermidine ont entrainé de plus faibles teneurs en solides solubles, mais certains fruits ont montré une augmentation de l'acidité titrable. Tous les traitements ont significativement augmenté la teneur en acide ascorbique des fruits. Le pH du jus de l'arille dans les fruits traités a été plus faible que celui des fruits non traités, probablement à cause de l'augmentation de l'acidité titrable. L'activité antioxydante totale et la teneur totale en phénol ont augmenté dans les fruits traités. Dans notre étude, une corrélation a été observée entre la teneur totale en phénol et l'activité antioxydante totale. **Conclusion**. Les traitements appliqués dans nos expérimentations ont maintenu la qualité globale des fruits du grenadier tout au long de l'entreposage à long terme. L'application après récolte de calcium et de spermidine, seuls ou en combinaison, pourrait atténuer les effets néfastes des basses températures sur la qualité des grenades au cours de leur stockage au froid. La méthode d'infiltration sous vide a été aussi efficace que la simple immersion. Toutefois, l'immersion est une méthode de traitement plus simple et plus rapide.

Iran République islamique / *Punica granatum* / fruits / propriété physicochimique / teneur en phénols / antioxydant / catéchol oxydase / tolérance au froid

1. Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest known edible fruits and is widely grown in many tropical and subtropical countries, especially in the moderate climate of the Mediterranean region [1]. The tree is grown extensively in Iran, India, Pakistan, Afghanistan, Saudi Arabia and in the subtropical areas of South America [2].

Low temperature has been widely used to store fruits and vegetables, with its beneficial effects for delaying senescence and maintaining quality. Chilling injury (CI) is a physiological disorder induced by low, but not freezing, temperatures, which seriously affects fruit quality [3].

Pomegranate is a highly perishable commodity and cold storage of this crop has received little attention, although refrigeration is the only method for extending its shelf life for up to 3 months [4]. However, this crop shows high sensitivity to chilling injury when stored at temperatures below 5 °C. The most common symptoms are surface pitting, husk scald and skin browning [2].

Calcium is applied before and after harvest to prevent physiological disorders, delay ripening and improve quality of various fruit crops [5–7]. Calcium, as a constituent of the cell wall matrix, has an important function in interacting with pectic acid polymers to form cross-bridges that influence cell wall strength, and is regarded as the last barrier before total cell separation [8]. Furthermore, the use of the polyamines putrescine (Put), spermine (Spm) and especially spermidine (Spd) can maintain functional properties of cold-stored pomegranate arils [9].

The browning reaction, which results from either mechanical or chilling injury during postharvest storage or processing of fruits and vegetables, is a widespread phenomenon. Polyphenol oxidase (PPO) is the main enzyme that causes browning. This enzyme catalyzes the oxidation of o-dihydroxyphenols into o-quinones. The quinones then condense to form dark pigments [10].

Despite the commercial importance of pomegranate in Iran, there is little information about the effects of spermidine and calcium, alone or in combination, on chilling injury control and quality attribution in pomegranate fruits after long-term storage. In this case, the aim of our work was to study the effect of spermidine and calcium chloride used in normal dip and vacuum infiltration on some of the qualitative constituents, weight loss (WL), juice content, titratable acidity (TA), total phenolic content (TPC), ascorbic acid (AA), total antioxidant activity (TAA), soluble solid content (SSC) and juice pH of pomegranate fruits and chilling injury-related characteristics such as browning, K^+ leakage and polyphenol oxidase after long-term storage.

2. Materials and methods

Commercially ripe fresh fruits were picked in September 2006 and 2007 in the Agricultural Research Center of Yazd province (Iran). Fruits were picked manually and immediately transferred to the Department of Horticultural Science, Shiraz University laboratory. After being sorted by uniform size and absence of visual defects, fruits were divided into eighteen lots of sixteen fruits. Nine lots were treated by normal dip and the remaining nine lots were treated by the vacuum infiltration method. The two groups were then subjected to the following treatments in quadruplicate for 4 min: Control (distilled water at 25 °C), S1 (1 mM spermidine), S2 (2 mM spermidine), C2 (2% calcium chloride), C4 (4% calcium chloride), C2S1 (2% calcium chloride + 1 mM spermidine), C2S2 (2% calcium chloride + 2 mM spermidine), C4S1 (4% calcium chloride + 1 mM spermidine) and C4S2 (4% calcium chloride + 2 mM spermidine). For each treatment, fruits were placed in 20 L of solution containing Tween-20 (2 $g \cdot L^{-1}$) as a non-ionic polysorbate surfactant. For vacuum infiltration, a reduced pressure of 247.52 mm Hg was used in a 15-L capacity vacuum desiccator (Model B-1834-Gallenkamp, England). The treated fruits were air-dried at (24 ± 1) °C and stored at 2 °C with (85 ± 5)% RH for 4 months. At the end of the storage period, fruits were transferred to shelf life (SL) conditions (20 °C, 30% RH) for 3 days.

2.1. Evaluation of weight loss and certain chemical characteristics in treated fruits after storage

Fruit weight loss was evaluated by weighing the fruit before and after storage. Soluble solid content was measured using an ATC-1E ATAGO hand-held refractometer. Titratable acidity was determined by titrating an aliquot of juice against 0.1 N NaOH and expressing the result as percentage of anhydrous citric acid. Ascorbic acid was measured by the indophenol titration method. The rate of potassium ion leakage was evaluated with a flame photometer after incubation in 0.4 M mannitol.

2.2. Determination of total antioxidant activity

Total antioxidant activity was determined by the 1,1-diphenyl-2-picrylhydrasyl (DPPH) method. Fresh pomegranate juice (0.1 mL) was mixed with 0.9 mL of 100 mM Tris-HCl buffer (pH 7.4) to which 1 mL of DPPH was added. The mixture was shaken vigorously and left to stand for 30 min. Absorbance (A) of the resulting solution was measured at 517 nm by a UV-Visible spectrophotometer (UV-160 A, Shimadzu Co., Japan). The reaction mixture without DPPH was used for the background correction. The antioxidant activity was calculated using the following equation: Antioxidant activity (%) = $\{1-[(A_{sample\ (517\ nm)} / A_{control\ (517\ nm)})]\} \times 100$.

2.3. Evaluation of external browning, pH and total phenolics

The amount of external browning of pomegranate fruits was rated on a hedonic scale of 1 = no browning, 2 = slight (up to 20%), 3 = moderate (21–40%), 4 = moderately severe (61–80%) and 5 = severe (81–100%). The pH of fruit juice was measured using a Jenway 3320 pH meter calibrated by buffer solutions of pH 4 and pH 7. Total phenolics was determined by the Folin-Ciocalteu method [11].

2.4. Polyphenol oxidase activity

The activity of polyphenol oxidase was conducted following the method of Cheng *et al.* [12]. Skin powder (300 mg) was homogenized in 15 mL chilled 0.2 M phosphate buffer (pH 6.2). Immediately after homogenization, 5% PVPP (w/v), 2% wet Amberlite XAD-4 (w/v) and 2% Triton-X-100 (by volume) were added and vortexed. After 5 min in an ice bath, the homogenate was filtered and centrifuged at 20,000 × *g* for 20 min at 4 °C. The polyphenol oxidase activity was measured by the change in A_{420nm} of the assay mixture (30 °C) that contained 0.5 mL supernatant (enzyme extract), 2.3 mL 0.1 M phosphate buffer (pH 6.2) and 0.2 mL 0.2 M catechol (in buffer), which was added after a 5-min preincubation. The polyphenol oxidase activity was presented as the change in units of OD (optical density) at 420 nm·g^{-1} dry weight·min^{-1}.

2.5. Statistical analysis

The data of both harvest years were pooled as the year interaction was not significant, and subjected to analysis. The results of both years were similar and we analyzed the mean of both years' results. Data for the analytical determinations were subjected to analysis of variance (ANOVA). A mean comparison using Duncan's Multiple Range Test (DNMRT) at the 5% level was performed using SAS.

3. Results

3.1. Weight loss, soluble solid content and titratable acidity

The effect of spermidine and calcium chloride on reducing weight loss was evident in both application methods (*table I*). Vacuum infiltration significantly caused more weight loss in 2% $CaCl_2$-treated and untreated fruits. The most weight loss (10.48%) was observed in control fruits dipped in distilled water under vacuum conditions, whereas fruits treated with 2% $CaCl_2$ by the normal dip method showed the smallest weight loss (6.97%). There was no significant difference between $CaCl_2$ and spermidine in reducing weight loss. In both normal dip and vacuum infiltration application methods, soluble

Table I.
Effect of pre-storage application of different concentrations of spermidine in combination with calcium chloride on weight loss (%), soluble solid concentration (%) and titratable acidity (%) of pomegranate fruits after 4 months of cold storage (2 °C).

Treatment	Weight loss		Soluble solid concentration		Titratable acidity	
	Normal dip	Vacuum infiltration	Normal dip	Vacuum infiltration	Normal dip	Vacuum infiltration
Control (distilled water at 25 °C)	9.22 b	10.48 a	16.63 b	17.25 a	1.22 e	1.33 cde
2% calcium chloride	6.97 e	8.00 cd	15.00 fg	15.57 def	1.23 de	1.33 cde
4% calcium chloride	7.19 e	7.66 d	15.13 efg	15.8 cde	1.29 cde	1.49 bc
1 mM spermidine	7.07 e	7.92 cd	15.73 cdef	16.3 bc	1.26 cde	1.26 cde
2 mM spermidine	7.77 d	7.96 cd	15.63 bcde	15.9 cd	1.20 e	1.27 cde
2% calcium chloride + 1 mM spermidine	7.15 e	7.14 e	15.53 def	15.9 cd	1.67 ab	1.68 ab
2% calcium chloride + 2 mM spermidine	7.10 e	7.20 e	15.70 cdef	15.47 bc	1.71 a	1.70 a
4% calcium chloride + 1 mM spermidine	8.02 cd	8.25 c	14.57 gh	14.13 h	1.34 cde	1.43 cde
4% calcium chloride + 2 mM spermidine	7.71 d	7.83 d	15.10 efg	14.77 gh	1.34 cde	1.45 cd

Means within the two columns for normal dip and vacuum infiltration application methods with the same letters are not significantly different using Duncan's Multiple Range Test at $P \leq 0.05$.

solid content of treated fruits decreased. The most soluble solid content was detected in non-treated fruits under vacuum conditions (17.25%). There was no significant difference between different concentrations of $CaCl_2$ and spermidine used alone. The smallest soluble solid content was detected in fruits treated with 4% $CaCl_2$ in combination with 1 mM spermidine by vacuum infiltration (14.13%). For titratable acidity, there was no significant difference between the two application methods. The most titratable acidity was detected in fruits treated with 2% calcium chloride in combination with 2 mM spermidine by normal dip and vacuum infiltration methods (1.71% and 1.70%, respectively).

3.2. Juice content, ascorbic acid and K^+ leakage

The juice content of non-treated pomegranate fruits decreased significantly compared with treated fruits (*table II*). There was no significant difference between the two methods regarding juice loss. $CaCl_2$ at 2% in combination with 1 mM and 2 mM of spermidine resulted in the highest juice content (80.71 g and 82.39 g for the normal dip method and 80.61 g and 83.55 g for the vacuum infiltration method, respectively). The applied treatments significantly increased ascorbic acid content. The combination of $CaCl_2$ and spermidine had an additive effect on ascorbic acid content. All treatments significantly decreased K^+ leakage. However, the rate of K^+ leakage in fruits treated with 2% $CaCl_2$ in combination with 2 mM spermidine was significantly lower than the other treatments. In our work, a significant difference was observed between the two treatment methods.

3.3. pH, total antioxidant activity and total phenolic content

All solutions significantly decreased pH (*table III*). Furthermore, there was no significant difference between the two application methods. The lowest pH was noted in fruits treated with 2% $CaCl_2$ in combination with 2 mM spermidine. All treatments

Table II.
Effect of pre-storage application of different concentrations of spermidine in combination with calcium chloride on juice content, ascorbic acid and K^+ leakage of pomegranate fruits after 4 months of cold storage (2 °C) (final leakage = leakage after 90-min autoclave at 120 °C).

Treatment	Juice content (g·100 g aril^{-1})		Ascorbic acid (mg·100 mL^{-1} juice)		[Final K^+ leakage / initial K^+ leakage] × 100	
	Normal dip	Vacuum infiltration	Normal dip	Vacuum infiltration	Normal dip	Vacuum infiltration
Control (distilled water at 25 °C)	67.23 f	60.91 g	8.75 e	8.49 f	63.33 a	63.89 a
2% calcium chloride	77.85 c	77.66 c	9.23 d	9.23 d	15.50 cd	16.07 cd
4% calcium chloride	78.66 c	79.62 bc	9.55 c	9.67 c	18.00 c	17.40 c
1 mM spermidine	71.49 d	70.66 de	8.95 de	9.16 d	13.67 d	16.40 cd
2 mM spermidine	68.36 ef	67.82 ef	9.23 d	9.21 d	14.90 cd	16.17 cd
2% calcium chloride + 1 mM spermidine	80.71 abc	80.61 abc	11.07 a	11.24 a	15.00 cd	15.00 cd
2% calcium chloride + 2 mM spermidine	82.39 ab	83.55 a	11.25 a	11.29 a	9.33 e	13.31 d
4% calcium chloride + 1 mM spermidine	78.53 c	78.04 c	10.35 b	10.42 b	24.67 b	25.25 b
4% calcium chloride + 2 mM spermidine	78.13 c	78.73 c	10.44 b	10.53 b	24.33 b	24.55 b

Means within the two columns for normal dip and vacuum infiltration application methods with the same letters are not significantly different using Duncan's Multiple Range Test at $P \leq 0.05$.

maintained total antioxidant activity at a high level. The highest total antioxidant activity was detected in fruits treated with 2 mM spermidine, regardless of application method. The highest total antioxidant activity was detected in fruits treated with 4% $CaCl_2$ in combination with 1 mM spermidine by the vacuum infiltration application method. The lowest total antioxidant activity was observed in non-treated fruits. There was no significant difference between the two application methods for maintaining total antioxidant activity during long-term storage. However, fruits treated by the vacuum infiltration method had higher total antioxidant activity compared with fruits treated by the normal dip method. Total phenolic content of treated fruits was significantly greater than total phenolic content of non-treated fruits. The highest total phenolic content was detected in fruits treated with 4% $CaCl_2$ in combination with 1 mM spermidine by the vacuum infiltration method (36.98 mg·L^{-1}). Moreover, there was no significant difference between the two application methods.

3.4. Chilling injury and polyphenol oxidase

All treatments significantly decreased chilling injury (*table IV*). The fruits treated with spermidine maintained better general appearance after the storage period. The least chilling injury occurred in fruits treated with 2% $CaCl_2$ in combination with 2 mM spermidine. There was no significant difference between the two methods of application. All treatments significantly decreased polyphenol oxidase activity. However, treatment with 2% $CaCl_2$ by the normal dip method had greater impact on decreasing polyphenol oxidase activity compared with the vacuum infiltration method, and there was no significant difference between the two application methods for other treatments. Generally, combination of 2% $CaCl_2$

Table III.
Effect of pre-storage application of different concentrations of spermidine in combination with calcium chloride on juice pH, total antioxidant activity and total phenolic content of pomegranate fruits after 4 months of cold storage (2 °C).

Treatment	Juice pH		Total antioxidant activity (%)		Total phenolic content ($mg \cdot L^{-1}$ of juice)	
	Normal dip	Vacuum infiltration	Normal dip	Vacuum infiltration	Normal dip	Vacuum infiltration
Control (Distilled water at 25 °C)	3.89 a	3.95 a	15.33 e	15.67 e	19.95 f	19.24 f
2% calcium chloride	3.44 b	3.45 b	22.67 abc	23.00 abc	22.35 e	23.54 de
4% calcium chloride	3.22 bc	3.48 b	21.00 bc	25.00 abc	24.79 de	25.50 d
1 mM spermidine	3.26 bc	3.37 bc	21.00 d	23.33 bcd	24.83 de	24.35 de
2 mM spermidine	3.29 bc	3.32 bc	20.50 a	24.00 ab	24.79 de	25.12 d
2% calcium chloride + 1 mM spermidine	3.21 bc	3.27 bc	24.00 bc	25.33 abc	32.78 c	33.27 bc
2% calcium chloride + 2 mM spermidine	3.16 c	3.33 bc	24.98 bcd	25.80 bcd	35.67 a	36.51 a
4% calcium chloride + 1 mM spermidine	3.22 bc	3.41 bc	26.2 cd	28.30 bc	36.06 a	36.98 a
4% calcium chloride + 2 mM spermidine	3.3 bc	3.31 bc	24.67 abc	25.67 abc	35.45 ab	35.76 a

Means within the two columns for normal dip and vacuum infiltration application methods with the same letters are not significantly different using Duncan's Multiple Range Test at $P \leq 0.05$.

with 1 mM and 2 mM spermidine were the best treatments to reduce polyphenol oxidase activity.

4. Discussion

The vast reports on the health benefits of pomegranate fruits [13–17] have created an increased interest in preharvest and postharvest factors that influence pomegranate fruit quality [7, 18]. While the health-related quality, *i.e.*, antioxidative capacity, is mainly dependent on the phenolic content, fruit attractiveness is primarily related to visual appearance and taste parameters of the arils and juice [18]. Postharvest treatment of pomegranate fruits with calcium and spermidine could reduce weight loss by 24.4% (2% calcium chloride applied by the normal dip method) and 31.87% (2% calcium chloride + 1 mM spermidine applied by the vacuum infiltration method) after 4 months of cold storage. Weight loss in fruits during storage is caused by water exchange between the internal and external atmosphere, the transpiration rate being accelerated by cellular breakdown [19]. Thus, the use of appropriate treatments such as those used in the present study might maintain membrane integrity and delay the removal of epicuticular waxes, which play an important role in water exchange through the skin, as has been reported in mandarin [20] and plums [21]. The results of this experiment also indicate that the solutions used lead to more juice in fruits.

In pomegranate, loss of ascorbic acid (vitamin C) also occurred either during cold storage or at ambient temperatures [22]. Increased ascorbic acid content in fruits treated with calcium and spermidine may be ascribed to the suppression of ascorbate oxidase activity. The effects of calcium and spermidine on activities of ascorbate oxidase in pomegranate fruit are yet to be investigated. Higher concentration of polyamines in bell pepper compared with tomato fruits has been associated with higher ascorbic acid content [23].

K^+ leakage is a good indicator of chilling injury severity in pomegranate fruits. In our

Table IV.
Effect of pre-storage application of different concentrations of spermidine in combination with calcium chloride on the chilling injury index and polyphenol oxidase of pomegranate fruits after 4 months of cold storage (2 °C).

Treatment	Chilling injury index		Polyphenol oxidase ($\Delta OD \cdot g^{-1} \cdot$dry weight$\cdot min^{-1}$)	
	Normal dip	Vacuum infiltration	Normal dip	Vacuum infiltration
Control (distilled water at 25 °C)	2.75 a	2.73 a	12.40 a	12.16 a
2% calcium chloride	2.03 bc	2.07 bc	5.34 c	4.83 d
4% calcium chloride	2.00 bc	2.20 b	3.60 ef	3.60 ef
1 mM spermidine	2.01 bc	1.90 bc	10.42 b	10.25 b
2 mM spermidine	2.13 b	2.03 bc	9.97 b	10.36 b
2% calcium chloride + 1 mM spermidine	1.87 bc	2.00 bc	2.60 g	2.54 g
2% calcium chloride + 2 mM spermidine	1.50 cd	1.25 d	2.30 g	2.35 g
4% calcium chloride + 1 mM spermidine	1.98 bc	2.10 b	4.03 e	3.60 ef
4% calcium chloride + 2 mM spermidine	2.20 b	1.88 bc	3.92 ef	3.47 f

Means within the two columns for normal dip and vacuum infiltration application methods with the same letters are not significantly different using Duncan's Multiple Range Test at $P \leq 0.05$.

experiment, we found that calcium and spermidine have a unique ability to decrease chilling injury. Considering the fact that polyamines and calcium are both associated with membrane lipids [24], it is suggested that membrane integrity and stability could explain the action of the exogenously added calcium and spermidine. This might account for the decrease in the membrane permeability and K^+ leakage.

Calcium and spermidine treatments decreased polyphenol oxidase activity. Combination of 2% $CaCl_2$ with 1 mM and 2 mM spermidine were the most effective treatments. Calcium and polyamines are able to bind negatively charged molecules like pectic polysaccharides [25].

Total antioxidant activity derives from endogenous bioactive compounds [26]. In pomegranate arils, anthocyanin, ascorbic acid and phenolics are responsible for the total antioxidant activity [21], and such findings have been observed in several fruits [27].

Among the phenolic compounds, punicalagin, as well as derivatives of ellagic acid, has been described as the major compound in pomegranate arils contributing to total antioxidant activity [28, 29]. A high correlation was observed between total phenolic content and total antioxidant activity (R^2 = 0.65 in the vacuum infiltration method and R^2 = 0.57 in the normal dip method) (*figures 1, 2*). This is in

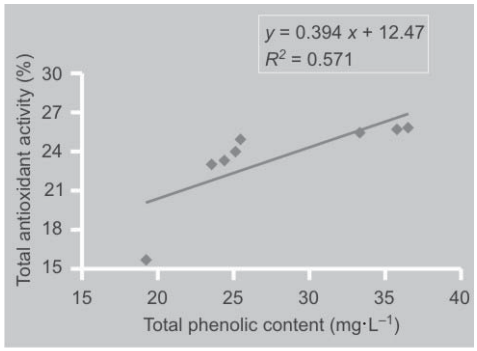

Figure 1.
Correlation of total phenolic content and total antioxidant activity in pomegranate fruits pretreated with spermidine in combination with calcium chloride by the normal dip application method.

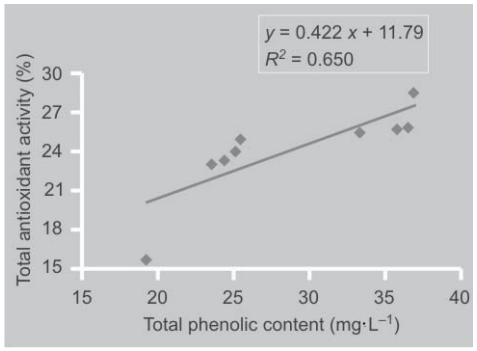

Figure 2.
Correlation of total phenolic content and total antioxidant activity in pomegranate fruits pretreated with spermidine in combination with calcium chloride by the vacuum infiltration method.

accordance with results from experiments on other cultivars [22].

The lower soluble solid concentration in treated pomegranate after storage may be due to the higher juice content. The data for titratable acidity were different for the various treatments. However, treatments generally increased titratable acidity, especially the combination of 2% $CaCl_2$ with 1 mM and 2 mM of spermidine. The lower pH measured in fruits treated with the solutions might be due to the higher titratable acidity.

In conclusion, we found that postharvest application of calcium and spermidine either alone or in combination could ameliorate adverse effects of low temperature on pomegranate fruit quality during cold storage. Vacuum infiltration was as effective as the normal dip method. However, normal dip was a simpler and faster treatment method.

Acknowledgement

The authors would like to express their sincere thanks to Shiraz University Research Council for financial support and to the Agricultural Research Center of Yazd for providing pomegranate fruits.

References

[1] Artes F., Tudela J. A., Villaescusa R., Thermal postharvest treatments for improving pomegranate quality and shelf life, Postharv. Biol. Technol. 18 (2000) 245–251.

[2] Elyatem S.M., Kader A., Post-harvest physiology and storage behaviour of pomegranate fruits, Sci. Hortic. 24 (1984) 287–298.

[3] Meng X., Han J., Wang Q., Tian S., Changes in physiology and quality of peach fruits by methyl jasmonate under low temperature stress, Food Chem. 114 (2009) 1028–1035.

[4] Artes F., Tudela J.A., Gil M., Improving the keeping quality of pomegranate fruit by intermittent warming, Eur. Food Res. Technol. 207 (1998) 316–321.

[5] Cheour F., Willemot C., Arul J., Desjardins Y., Makhlouf J., Charest P.M., Gosselin A., Foliar application of calcium chloride delays postharvest ripening of strawberry, J. Am. Soc. Hortic. Sci. 115 (1990) 789–792.

[6] Pooviah B.W., Role of calcium in ripening and senescence, Commun. Soil Sci. Plant Anal. 10 (1979) 83–88.

[7] Ramezanian A., Rahemi M., Vazifehshenas M.R., Effects of foliar application of calcium chloride and urea on quantitative and qualitative characteristics of pomegranate fruits, Sci. Hortic. 121 (2009) 171–175.

[8] Fry S.C., Primary cell wall metabolism: tracking the careers of wall polymers in living plant cells, New Phytol. 161 (2004) 641–675.

[9] Mirdehghan S.H., Rahemi M., Serano M., Guillen F., Martinez-Romero D., Valero D., The application of polyamines by pressure or immersion as a tool to maintain functional properties in stored pomegranate arils, J. Agric. Food Chem. 55 (2007) 755–760.

[10] Segovia-Bravo K.A., Jarén-Galán M., García-García P., Garrido-Fernández A., Browning reactions in olives: Mechanism and polyphenols involved, Food Chem. 114 (2009) 1380–1385.

[11] Malik C.P., Singh M.B., Plant enzymology and histoenzymology, Kalyani Publ., New Delhi, India, 1980.

[12] Cheng G.W., Crisosto H., Browning potential, phenolic composition, and polyphenoloxidase activity of buffer extracts of peach and nectarine skin tissue, J. Am. Soc. Hortic. Sci. 120 (1995) 835-838.

[13] Aviram M., Dornfeld L., Pomegranate juice consumption inhibits serum angiotensin converting enzyme activity and reduces systolic blood pressure, Atheroscler. 158 (2001) 195–198.

[14] Aviram M., Dornfeld L., Rosenblat M., Volkova N., Kaplan M., Coleman R., Hayek T., Presser D., Fuhrman B., Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: Studies in humans and in atherosclerotic apolipoprotein E-deficient mice, Am. J. Clin. Nutr. 71 (2000) 1062–1076.

[15] De Nigris F., William-Ignarro S., Lerman L.O., Crimi E., Botti C., Mansueto G., D'Armiento F.P., De Rosa G., Sica V., Ignarro L.J., Napoli C., Beneficial effects of pomegranate juice on oxidation-sensitive genes and endothelial nitric oxide synthase activity at sites of perturbed shear stress, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 4896–4901.

[16] Kaplan M., Hayek T., Raz A., Coleman R., Dornfeld L., Vaya J., Aviram M., Pomegranate juice supplementation to atherosclerotic mice reduces macrophage lipid peroxidation, cellular cholesterol accumulation and development of atherosclerosis, J. Nutr. 131 (2001) 2082–2089.

[17] Lee J., Watson R.R., Pomegranate: a role in health promotion and AIDS?, in: Watson R.R. (Ed.), Nutrition, food and AIDS, CRC Press, Boca Raton, FL, USA, 1998.

[18] Brochov-Neori H., Judeinstein S., Tripler E., Harari M., Greenberg A., Shomer I., Holland D., Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum* L.) fruit, J. Food Comp. Anal. 22 (2009) 189–195.

[19] Woods J.L., Moisture loss from fruits and vegetables, Postharv. News Inf. 1 (1990) 195–199.

[20] Schirra M., D'Hallewin G., Storage performance of 'Fortune' mandarins following hot water dips, Postharv. Biol. Technol. 10 (1997) 229–238.

[21] Serrano M., Martinez-Romero D., Guillén F., Valero D., Effects of exogenous putrescine on improving shelf life of four plum cultivars, Postharv. Biol. Technol. 30 (2003) 259–271.

[22] Mirdehghan S.H., Rahemi M., Serano M., Guillen F., Martinez-Romero D., Valero D., Prestorage heat treatment to maintain nutritive and functional properties during postharvest cold storage of pomegranate, J. Agric. Food Chem. 54 (2006) 8495–8500.

[23] Yahia E.M., Contreras Padilla M., Gonzalez Aguilar G., Ascorbic acid content in relation to ascorbic acid oxidase activity and polyamine content in tomato and bell pepper fruits during development, maturation and senescence, Lebensm. Wiss. Technol. 34 (2001) 452–457.

[24] Galston A.W., Kaur-Sawhney R.K., Polyamines in plant physiology, Plant Physiol. 94 (1990) 406–410.

[25] Martínez-Téllez M.A., Ramos-Clamont M.G., Gardea A.A., Vargas-Arispuro I., Effect of infiltrated polyamines on polygalacturonase activity and chilling injury responses in zucchini squash (*Cucurbita pepo* L.), Biochem. Biophys. Commun. 295 (2002) 98–101.

[26] Imahori Y., Takemura M., Bai J., Chilling-induced oxidative stress and antioxidant responses in mume (*Prunus mume*) fruit during low temperature storage, Postharv. Biol. Technol. 49 (2008) 54–60.

[27] Kalt W., Forney C.F., Martin A., Prior R.L., Antioxidant capacity, vitamin C, Phenolics, and anthocyanins after fresh storage of small fruits, J. Agric. Food Chem. 50 (1999) 2308–2312.

[28] Gil M.I., Tomás-Barberán F.A., Hess-Pierce B., Holcroft D.M., Kader A.A., Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing, J. Agric. Food Chem. 48 (2000) 4581–4589.

[29] Kulkarni A.P., Aradhya S.M., Divakar S., Isolation and identification of a radical scavenging antioxidant – punicalagin from pith and carpellary membrane of pomegranate fruit, Food Chem. 87 (2004) 551–557.

La espermidina y el cloruro de calcio atenúan los daños causados por el frío de granadas almacenadas a largo plazo.

Resumen — Introducción. Los frutos del granadero (*Punica granatum* L.) son sensibles al frío. **Material y métodos**. Se trataron granadas con cloruro de calcio y con espermidina, individual o combinadamente, mediante simple inmersión y mediante un método de infiltración al vacío. Los frutos tratados se almacenaron a 2 °C durante 4 meses. Al finalizar el periodo de almacenaje, las muestras se mantuvieron durante 3 días a 20 °C, a continuación se evaluaron ciertos rasgos cualitativos. **Resultados y discusión**. Los frutos tratados perdieron menos peso y presentaron un mejor contenido en jugo que los frutos testigo. Los frutos no tratados presentaron daños causados por el frío manifestados por un aumento de la pérdida de K^+ y de la actividad de la polifenol oxidasa. Los tratamientos de cloruro de calcio y de espermidina conllevaron unos contenidos más bajos en sólidos solubles, pero ciertos frutos mostraron un aumento de la acidez valorable. Todos los tratamientos aumentaron significantemente el contenido de ácido ascórbico de los frutos. El pH del jugo del arilo en los frutos tratados fue más flojo que aquél de los frutos no tratados, probablemente a causa del aumento de la acidez valorable. La actividad antioxidante total así como el contenido total de fenol, aumentaron en los frutos tratados. En nuestro estudio, se observó una correlación entre el contenido total de fenol y la actividad antioxidante total. **Conclusión**. Los tratamientos aplicados en nuestros experimentos mantuvieron la calidad general de los frutos del granadero en el trascurso del almacenamiento a largo plazo. La aplicación post cosecha de calcio y de esperdimidina, individual o combinadamente, podría atenuar los efectos nefastos de las bajas temperaturas en la calidad de las granadas durante su almacenamiento en frío. El método de infiltración al vacío fue tan eficaz como la simple inmersión. No obstante, la inmersión es un método de tratamiento más simple y más rápido.

Iran República Islámica / *Púnica granatum* / frutas / propiedades fisicoquímicas / contenido fenólico / antioxidantes / catecol oxidasa / tolerancia al frío