AcademicPres

Available online: www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not Bot Horti Agrobo, 2016, 44(2):508-517. DOI:10.15835/nbha44210534

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Original Article

Effect of Salicylic Acid, Calcium Chloride and Calcium Lactate Applications on Quality Attributes of Minimally-Processed 'Wonderful' Pomegranate Arils

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Abstract

'Wonderful' pomegranate arils were treated with 0.5% and 1% of calcium chloride, 0.5% and 1% of calcium lactate, and salicylic acid (1 and 2 mM), then treated and untreated (control) arils packaged in clean (sterilized) plastic containers. Fresh produce was then stored at 5±1 °C. Assessment of arils quality was carried out at 3-day intervals by evaluating the following quality parameters: appearance, decay, off odor, flavor, total soluble solids, acidity, anthocyanin and vitamin C content, firmness, colour development (L* value and hue angle), and microbial load. Pomegranate arils treated with salicylic acid, calcium chloride, and calcium lactate maintained a general good quality and appearance up to 12 days of storage at 5±1 °C, with no visible decay and off odor development. The total microbial population was lower in arils treated with salicylic acid, in comparison to treatments with calcium chloride, calcium lactate and control arils. All treatments scored above the limit of marketability, maintaining good quality of fresh-cut produce during storage. However, the use of salicylic acid and calcium chloride helped to keep a better overall quality of arils at the end of the 12-day storage at 5±1 °C.

Keywords: calcium chloride, calcium lactate, fresh-cut, pomegranate, *Punica granatum* L., quality attributes

Abbreviations: Ca-lactate, calcium lactate; SA, salicylic acid; TA, titratable acidity; TSS, total soluble solids

Introduction

Fresh-cut fruits and vegetables are a relatively new and rapidly developing segment of the fresh produce industry, and the demand is continuously increasing, being the convenience factor and health promoting benefits, associated with their consumption, the main reasons for such an increment (Sanchís *et al.*, 2015). During the last decade, in particular, an interest in the consumption of pomegranates (*Punica granatum* L.) has been recorded, due to the health benefits produced by the very high content of bioactive phytochemicals of the fruit (Holland *et al.*, 2009; Viuda-Martos *et al.*, 2010). Despite these health benefits, pomegranate consumption is still limited due to the difficulty of extracting the arils, and the inconvenience due to phenolic metabolites which stain the hands during preparation of seeds (Opara *et al.,* 2009). Packaged ready-to-eat arils is a more appealing product to consumers and increases the prospect of both production and consumption (Caleb *et al.*, 2012).

Improperly preparing, packaging and handling fresh-cut fruit may compromise overall quality and decrease consumer acceptability (Beaulieu and Gorny, 2002). Physiological changes may be accompanied by flavor loss, cut surface discoloration, color loss, decay, increased rate of vitamin loss, rapid softening, shrinkage and a shorter storage-life. These attributes reflect visual acceptance and physicochemical properties associated with the product quality. Therefore, proper treatments of the product are essential for the maintenance of such quality (Gorny *et al.*, 2002). Among them, the application of calcium compounds is effective in maintaining the flesh firmness of fresh-cut fruit products. The dipping of fresh-cut products in solutions of 0.5 to 1.0% calcium chloride is the common method of application (Ponting *et al.*, 1971; Ponting *et al.*, 1972). Calcium is used from long time as an agent for maintaining firmness of whole produce (Poovaiah, 1986), and its subsequent use in fresh-cut production has been an obvious consequence. According to Elyatem and Kader (1984), chilling injury, decay, and weight loss are the most important

Received: 18 Aug 2016. Received in revised form: 25 Nov 2016. Accepted: 01 Dec 2016. Published online: 14 Dec 2016.

problems, limiting pomegranate storability. Treatments like calcium dipping can help to maintain firmness and visual quality, resulting in a longer shelf life of the fresh-cut products as melon (Aguayo *et al.,* 2008) and apple slices (Aguayo *et al.,* 2010).

Calcium (Ca^{2+}) has been extensively reviewed both as an essential element, and for its potential role in maintaining postharvest quality of fruit and vegetable crops by contributing to the linkage between pectic substances within the cell wall (Kirkby and Pilbeaam, 1984; Demarty *et al.,* 1984; Arhtar *et al.,* 2010). The presence of Ca^{2+} ions increases the cohesion of cell walls (Lara *et al*., 2004; Kazemi *et al*., 2011). It is also involved in reducing the rate of senescence and fruit ripening (Ferguson, 1984; White and Broadley, 2003; Mahajan and Dhatt, 2004). A 1% solution of $CaCl_2$ delayed fruit ripening, improved resistance to fungal attack and maintained structural integrity of cell walls of strawberry during a 10-day storage period at 3 °C (Lara *et al.,* 2004).

Calcium lactate (Ca-lactate) has recently been shown to be as effective as the chloride form (at higher concentrations) without imparting a bitter flavor (Luna-Guzmán and Barrett, 2000). A 1% Ca-lactate dip was an effective alternative to ascorbate in fresh-cut 'Bartlett' pears stored 1 to 2 days at 20 °C (68 °F), while 1% Ca-lactate plus 2% ascorbate was the most effective (Gorny *et al.,* 1998). On the other hand, a combination dip with 0.01% 4-hexylresorcinol, 0.5% ascorbate and 1% Ca-lactate extended shelf-life of 'Anjou,' 'Bartlett,' and 'Bosc' pear slices for 15 to 30 days (Dong *et al.,* 2000), while kiwifruit slices treated with 2% Ca-lactate had a shelf-life of 9-12 days (Agar *et al.,* 1999; Massantini and Kader, 1995). In addition, Ca-lactate showed an anti-browning effect, as browning was also reduced in fresh-cut 'Carnival' peaches treated with 1% Ca-lactate plus 2% ascorbate (Gorny *et al.,* 1999). Gorny *et al.* (2002) reported that a post-cutting dip (pH 7.0) of 2% ascorbate, 1% Ca-lactate and 0.5% (w/v) cysteine significantly extended shelf-life of 'Bartlett' pear slices by inhibiting loss of firmness and preventing browning.

Salicylic acid (SA) is a natural phenolic compound involved in the regulation of many processes in plant growth and development. Among them, it is noteworthy that SA exhibits a high potential in controlling post-harvest losses of horticultural crops (Asghari and Aghdam, 2010). Moreover, dietary salicylates from fruit and vegetables are described as bioactive compounds with health care potential (Hooper and Cassidy, 2006), and considered as "Generally Recognized As Safe" (GRAS). For its action at minimal concentrations, SA is considered a plant hormone (Raskin, 1992), inhibiting ethylene biosynthesis and delaying fruit senescence (Khademi *et al*., 2012). Exogenous SA treatment may also induce the expression of pathogenesis-related proteins (Malamy *et al.,* 1990), and establish systemic acquired resistance (Gaffney *et al.,* 1993, Beckers and Spoel, 2006). The application of acetylsalicylic acid (a derivative of SA) slowed down the softening rate of kiwifruit by inhibiting ethylene production and maintaining higher endogenous SA levels (Zhang *et al.,* 2003). SA also prevented the softening of banana and kiwifruit during ripening (Srivastava and Dwivedi, 2000; Zhang *et al*., 2003). It has been also reported that SA application, either in pre- or post-harvest, reduced fungal decay in sweet cherry (Yao and Tian, 2005; Xu and Tian, 2008), strawberry (Babalar *et al.,* 2007; Shafiee *et al.,* 2010) and peach fruits (Wang *et al.,* 2006). SA can extend the shelf life of the harvested fruit by delaying the development of disease incidence (Terry and Joyce, 2004), through induction of the defense resistance system and stimulation of antioxidant enzymes (Khademi and Ershadi, 2013).

With attention to the risk of improper use of chemicals in postharvest technology and consumer's demand for healthy products, studies on the application of postharvest treatments, along with cold storage, are today considered of strategic importance (Khademi and Ershadi, 2013). In this context, the aim of this study was to determine the effects of calcium chloride ($CaCl_2$), SA, and Ca-lactate on qualitative characteristics of minimally processed 'Wonderful' pomegranate arils during cold storage at 5±1 °C.

Materials and Methods

Plant material, treatments and evaluations

The current study was carried out throughout two successive seasons, 2013 and 2014. 'Wonderful' pomegranate (*Punica granatum* L.) fruits were harvested at maturity stage from a private farm located in the desert road, Giza governorate, Egypt. Fruits were immediately transported to the laboratory, and then were selected in uniformity of weight, size, maturity stage and absence of physical injuries. All preparation steps were applied in clean room conditions. Fruits were washed in sterilized water with 200 µl l^{-1} of sodium hypochlorite (NaOCl) solution. Fruit husks were processed for aril extraction, the arils were gently removed by hand and then collected and mixed together for uniformity in a clean container. After removing the damaged ones, arils were divided into six portions and subjected to the following treatments in triplicate: (1) for calcium dip treatments, 0.5% (5 g l^{-1} of $CaCl_2$) and 1% (10 g l^{-1} of $CaCl_2$) solutions were prepared and arils were dipped in one of the two for 5 min; (2) for Ca-lactate treatments, the same concentrations of 0.5% and 1% were applied with 5 min dipping; (3) for SA treatments, arils were dipped in 1 mM or 2 mM solutions for 5 min. Arils dipped in sterile water for 5 min served as control. After dipping, arils of each treatment were drained in a colander and left for drying using clean cloth absorbent paper. Finally, 110 g of pomegranate arils from each treatment were packaged in previously sterilized rigid plastic container (PET, diameter: 110 mm, height: 55 mm), and then kept in cold storage at 5±1 °C and 95±2% RH for up to 12 days. Samples were taken for assessment at day 0, and then at 3-day intervals during storage. The quality characteristics of arils were evaluated in terms of: (i) total soluble solids (TSS) using digital pocket refractometer (model PAL 1, ATAGO™, Tokyo Tech.) and expressed as percentage, (ii) titratable acidity (TA) percentage, according to A.O.A.C. (1990), (iii) ascorbic acid content (mg/100 ml), according to Lucas (1944), (iv) total anthocyanin content (mg/100 ml) was calorimetrically determined in fruit juice as described by Hsia *et al.* (1965), (v) texture (firmness) of arils was measured using lefra texture analyzer (Mehteric Stevens, model TA/000), a test speed of 2 mm and 1 mm depth. Aril color was determined and expressed as: L* value, i.e. a measure of lightness, ranging from 0 (black) to +100 (white), and hue angle (h°), using a Minolta colorimeter CR-40 (Konica Minolta Sensing Inc, Sakai, Japan).

Sensorial overall quality attributes

Sensory evaluation was performed during storage at 3-day intervals. Panel members were requested to assess fresh quality measurement as follows:

1. Visual quality: based on overall visual appearance, it was evaluated following a 9-point rating scale where 9, excellent; 7, very good; 5, good (limit of consumer acceptability); 3, fair (limit of usability), and 1, extremely poor (Gorny *et al.,* 2002; Medina *et al.,* 2012).

2. Decay: estimated visually using scores, as described by Kader *et al.* (1973) on 5–1 scale, with reference points of: 5, severe; 4, moderately severe; 3, moderate; 2, slight; 1, none. The score attribution depends on morphological effects such as color change, microorganism effects, smell and decay percentage on arils.

3. Off odors: determined just after opening the package by using a 5-point scale where: 5, severe; 4, moderately severe; 3, moderate; 2, slight; 1, none. This scale depends on unlike or bad smell (El-Bassiouny, 2003).

4. Flavor: estimated using a 5-point scale where: 5, fully typical flavor; 4, moderately full; 3, moderate; 2, slight; 1, poor.

Microbial load studies

For media preparation, Nutrient glucose agar (Dowson, 1957) and Rose-bengal agar media (Johnson *et al.*, 1960) were prepared and sterilized by autoclaving at 121 °C for 20 min, after which the media were allowed to cool to 45-50 °C before inoculating them with sample material. The serial dilution method was used for the recording of the micro-organism load count. The inoculated plates were placed in two different incubators that had been pre-set at a suitable temperature for both fungi and bacteria.

Microbiological assay

One gram of pomegranate arils from each treatment were homogenized to obtain the microbial count. Ten-fold serial dilutions were carried out and pour-plate method was used for enumeration of bacteria and fungi from tested samples, since 1.0 ml of each dilution was pour-plated in triplicate onto appropriate media. Total bacterial counts were enumerated on nutrient glucose agar medium (3 g beef extract, 5 g peptone, 8 g glucose, and 20 g agar per liter) after incubation at 37 °C for 24-48 h. Total molds and yeasts were enumerated on Rose-bengal agar medium (10 g glucose, 5 g peptone, 1 g KH_2PO_4, 0.03 g Rose-bengal, 0.5 g $MgSO_4$, and 20 g agar per liter). Incubation for total mold and yeast counts was performed at 28 °C for 2-3 days. The evaluation of the microbial load was performed 3 times during the storage experiment, and results were determined as colony-forming units per gram (CFU g^{-1}) (Sogvar *et al.,* 2016). As for the enumeration of colonies, following incubation, all colonies on dishes containing 30-300 colonies were counted and the results per dilution were calculated. Microbial data were then transformed into logarithms of the number colony forming units (log CFU g^{-1}).

Statistical analysis

For experiments, complete factorial randomized designs were applied. Treatments were always replicated three times, and the obtained data were statistically analyzed by ANOVA. Mean comparisons were performed by Duncan's Multiple range test at 5% level (Snedecor and Cochran, 1982).

Results and Discussions

Total soluble solids

Data reported in Table 1 show that TSS percentages increased, often significantly, by the increasing of the storage period, and this observation was true for both the studied seasons. These results are in accordance with Khademi and Ershadi (2013) who reported that, irrespectively to treatments, soluble sugar content (SSC) of peach fruits increased slightly during storage. Moreover, Arendse *et al.* (2014) reported that TSS of 'Wonderful' pomegranate fruits increased significantly during storage at the investigated temperature regimes, i.e. after one-month storage TSS increased from 13 to 16.22, 15.36, 14.84 and 14.35 °Brix at 5, 7.5, 10 and 21 °C, respectively.

As for the different treatments, arils treated with 1% $CaCl_2$ in the first season and 2 mM SA in both seasons showed the highest significant TSS as compared to control. Control arils and 0.5% $CaCl_2$ treated ones had the lowest values of TSS after 3 days of storage. In the first season, at 12 days of storage, arils treated with 1% $CaCl_2$ scored the highest TSS percentages as compared to control ones, while, in the second season, $CaCl_2$ at both tested concentrations recorded the highest TSS respecting to the control. Mirdehghan and Ghotbi (2014) indicated that SA applied at 1 mM significantly increased TSS compared to untreated pomegranate fruits. Results are also in accordance with Srivastava and Dwivedi (2000) who reported that SA treatments increased TSS in banana fruits. They hypothesized that cell walls contain large amounts of polysaccharides, mainly pectin and cellulose, which are digested due to the activity of the cell wall-degrading enzymes, leading to a significant increase in TSS content. In this regard Amith *et al.* (2015) found that the highest TSS was for pomegranate arils treated with 1% calcium chloride. On the other side, other reports showed that TSS was not greatly influenced by SA treatment in pomegranate (Sayyari *et al.,* 2009) and mango (Ding *et al.,* 2007). Similarly, Mirdehghan *et al.* (2012) affirmed that TSS of pomegranate fruit juice was not influenced when fruits were treated with SA, methyl jasmonate, and potassium sulfate.

The effects of SA treatments on the sugar content of fruits and vegetables are controversial. SA treatment had no effect on SSC on fruits of grape (Ranjbaran *et al.,* 2011) and persimmon (Khademi *et al.,* 2012). Asghari and Aghdam (2010) reported that the lower contents of TSS were obtained in kiwifruit treated with 32 µl l^{-1} of methyl salicylic acid (MeSA) at the end of cold storage. According with this observation, Mirdehghan and Ghotbi (2014) proposed that MeSA reduces ethylene production, resulting in the decrease of sucrose-phosphate synthase enzyme activity that, in turn, leads to the decrease of sucrose synthesis.

Titratable acidity

In this study, acidity percentages markedly decreased as storage period increased (Table 2). The reduction in acidity was significant up to 6 days of storage, then continued to decrease but with no significant differences up to 9 days (in the first season), and to the end of storage period (in the second season).

In both the seasons, $CaCl_2$ at 0.5%, Ca-lactate at 0.5%, and SA at 1 mM showed the lowest acidity percentages in comparison to untreated (control) arils, while 2 mM SA and 1% $CaCl_2$ had the highest mean values.

These results are in accordance with those obtained by Khademi and Ershadi (2013) who showed that TA reduced slightly with SA treatments during storage, although the reduction was never significant. Moreover, these results are in agreement with those reported by Sayyari *et al.* (2009) and Ranjbaran *et al.* (2011). The observed decrease in acidity could be related to initial response and metabolic activities of the arils during the storage, as suggested by Caleb *et al.* (2013). On the other hand, other authors reported that TA was not affected by SA on mango

Table 1. TSS (%) of minimally-processed pomegranate arils, cv. Wonderful, as affected by $CaCl_2$, Ca-lactate and salicylic acid treatments during storage at 5±1 °C (2013 and 2014 seasons)

TSS	Season 2013						Season 2014					
	Storage period (days)						Storage period (days)					
Treatments	0	3	6	9	12	Mean	0	3	6	9	12	Mean
Control	13.63 jk	13.73 jk	14.37 g-k	14.60 g-k	15.17 b-i	14.30 B	13.83 h	13.77 h	14.90 f	15.13 c-f	15.33 b-f	14.59 B
$CaCl_2$ 0.5%	13.63 jk	13.73 jk	15.13 c-i	16.03 a-e	16.20 a-d	14.95 AB	13.83 h	13.83 h	15.07 ef	16.07 abc	16.33 a	15.03 AB
$CaCl_2$ 1%	13.63 jk	14.13 h-k	14.17 h-k	16.37 abc	16.77 a	15.01 A	13.83 h	13.93 gh	14.80 fg	16.03 a-d	16.37 a	14.99 AB
Ca-lactate, 0.5%	13.63 jk	14.07 ijk	14.07 ijk	15.17 b-i	15.97 a-f	14.54 AB	13.83 h	13.90 gh	14.53 fgh	15.03 f	16.00 a-e	14.66 AB
Ca-lactate, 1%	13.63 jk	14.70 f-j	14.83 e-j	15.37 b-h	16.40 ab	14.99 AB	13.83 h	14.67 fgh	14.97 f	14.97 f	16.30 a	14.95 AB
Salicylic acid, 1 mM	13.63 jk	14.67 g-k	15.10 d-i	15.10 d-i	15.93 a-f	14.89 AB	13.83 h	14.53 fgh	14.50 fgh	15.10 def	16.27 ab	14.85 AB
Salicylic acid, 2 mM	13.63 jk	14.17 h-k	15.50 b-g	16.07 a-e	16.13 a-d	15.10 A	13.83 h	13.93 gh	15.43 a-f	16.00 a-e	16.30 a	15.10 A
Mean	13.63 C	14.18 B	14.74 B	15.53 A	16.08 A		13.83 D	14.08 D	14.89 C	15.48 B	16.13 A	

Note: Different letters indicate significantly different values by ANOVA followed by Duncan test at P≤0.05 (small letters refer to values recorded in each season, different capital letters refer to mean values)

Table 2. Acidity (%) of minimally-processed pomegranate arils, cv Wonderful, as affected by $CaCl_2$, Ca-lactate and salicylic acid treatments during storage at 5±1 °C (2013 and 2014 seasons)

Acidity	Season 2013						Season 2014					
	Storage period (days)						Storage period (days)					
Treatments	0	3	6	9	12	Mean	0	3	6	9	12	Mean
Control	1.20 a	0.93 b	0.60 d-g	0.60 d-g	0.50 gh	0.77 ABC	1.33 a	1.1 b	0.63 def	0.60 ef	0.50 efg	0.83 A
$CaCl_2$ 0.5%	1.20 a	0.73 c	0.60 d-g	0.50 gh	0.43 h	0.69 D	1.33 a	0.67 de	0.63 def	0.47 fg	0.47 fg	0.71 C
$CaCl_2$ 1%	1.20 a	0.87 b	0.67 cde	0.60 d-g	0.53 fgh	0.77 AB	1.33 a	0.60 ef	0.60 ef	0.57 efg	0.53 efg	0.73 BC
Ca-lactate, 0.5%	1.20 a	0.63 c-f	0.60 d-g	0.60 d-g	0.53 fgh	0.71 CD	1.33 a	0.63def	0.57 efg	0.47 fg	0.40 g	0.68 C
Ca-lactate, 1%	1.20 a	0.70 cd	0.67 cde	0.66 cde	0.53 fgh	0.75 ABC	1.33 a	0.87 c	0.67 de	0.60 ef	0.57 efg	0.81 AB
Salicylic acid, 1 mM	1.20 a	0.70 cd	0.60 d-g	0.57 efg	0.53 fgh	0.72 BCD	1.33 a	0.63def	0.57 efg	0.53 efg	0.47 fg	0.71 C
Salicylic acid, 2 mM	1.20 a	0.87 b	0.70 cd	0.60 d-g	0.57 efg	0.79 A	1.33 a	0.80 cd	0.63 def	0.53 efg	0.47 fg	0.75 ABC
Mean	1.20 A	0.78 B	0.63 C	0.59 C	0.52 D		1.33 A	0.75 B	0.61 C	0.54 CD	0.49 D	

Note: Different letters indicate significantly different values by ANOVA followed by Duncan test at P≤0.05 (small letters refer to values recorded in each season, different capital letters refer to mean values)

Table 3. Ascorbic acid (Vitamin C) content (mg/100 ml) of minimally-processed pomegranate arils, cv Wonderful, as affected by $CaCl_2$, Ca-lactate and salicylic acid treatments during storage at 5±1 °C (2013 and 2014 seasons)

Treatments	Season 2013						Season 2014					
	Storage period (days)						Storage period (days)					
	0	3	6	9	12	Mean	0	3	6	9	12	Mean
Control	4.80 a	4.57 ab	3.67 d-h	2.40 mno	2.23 o	3.53 BC	5.03 a	4.467 b	3.70 cd	2.57 ghi	2.03 j	3.56 B
CaCl2, 0.5%	4.80a	4.23 bc	3.63 e-i	2.77 k-n	2.27 no	3.54 BC	5.03 a	4.33 b	3.70 cd	2.70 f-i	2.50 hi	3.65 AB
CaCl2, 1%	4.80 a	4.33 ab	3.77 c-f	3.13 ijk	2.87 j-m	3.78 AB	5.03 a	4.40 b	3.50 de	2.87 fgh	2.67 f-i	3.69 AB
Ca-lactate, 0.5%	4.80a	3.63 e-i	3.37 f-j	2.87 j-m	2.80 klm	3.49 C	5.03 a	4.20 b	3.67 cd	2.80 fgh	2.67 f-i	3.67 AB
Ca-lactate, 1%	4.80 a	4.16 bcd	4.10 b-e	3.17 h-k	2.57 l-o	3.76 ABC	5.03 a	4.30 b	3.73 cd	3.07 ef	2.57 ghi	3.74 AB
Salicylic acid, 1 mM	4.80 a	4.50 ab	3.73 c-g	3.17 h-k	2.93 jkl	3.82 A	5.03 a	4.47 b	4.07 bc	3.10 ef	2.33 ij	3.80 A
Salicylic acid, 2 mM	4.80 a	4.33 ab	4.07 b-e	3.23 g-k	2.57 l-o	3.80 AB	5.03 a	4.27 b	4.07 bc	2.97 fg	2.50 hi	3.77 AB
Mean	4.80 A	4.25 B	3.76 C	2.96 D	2.61 E		5.03 A	4.35 B	3.78 C	2.87 D	2.47 E	

Note: Different letters indicate significantly different values by ANOVA followed by Duncan test at P≤0.05 (small letters refer to values recorded in each season, different capital letters refer to mean values)

Table 4. Anthocyanin content (mg/100 ml) of minimally-processed pomegranate arils, cv Wonderful, as affected by $CaCl_2$, Ca-lactate and salicylic acid treatments during storage at 5±1 °C (2013 and 2014 seasons)

Treatments	Season 2013						Season 2014					
	Storage period (days)						Storage period (days)					
	0	3	6	9	12	Mean	0	3	6	9	12	Mean
Control	12.59 a	10.68 bcd	9.85 cde	6.49 lmn	5.19 op	8.96 A	13.20 a	9.46 c-h	8.77 f-i	7.19 jkl	6.26 lmn	8.98 BC
$CaCl_2$, 0.5%	12.59 a	9.18 e-h	7.98 h-k	7.00 klm	5.45 nop	8.44 A	13.20 a	10.20 b-f	9.84 c-g	8.99 e-h	5.06 no	9.45 ABC
$CaCl_2$, 1%	12.59 a	9.64 d-g	8.48 ghi	6.84 klm	5.26 op	8.56 A	13.20 a	10.32 b-e	9.48 c-h	8.71 f-j	5.47 mno	9.44 ABC
Ca-lactate, 0.5%	12.59 a	11.41 ab	8.29 hij	7.14 jkl	5.53 nop	8.99 A	13.20 a	9.37 d-h	7.97 h-k	7.36 i-l	5.32 mno	8.64 C
Ca-lactate, 1%	12.59 a	10.93 bc	8.33 hij	6.91 klm	6.23 l-o	8.99 A	13.20 a	11.55 b	9.15 d-h	8.84 e-i	6.55 k-n	9.89 A
Salicylic acid, 1 mM	12.59 a	9.72 def	8.61 f-i	6.29 l-o	4.86 p	8.42 A	13.20 a	10.98 bc	8.34 g-j	6.33 lmn	4.71 o	8.71 BC
Salicylic acid, 2 mM	12.59 a	9.73 c-f	8.27 hij	7.83 ijk	5.83 m-p	8.85 A	13.20 a	10.67 bcd	9.19 d-h	7.96 h-k	6.63 klm	9.53 AB
Mean	12.59 A	10.18 B	8.54 C	6.93 D	5.48 E		13.20 A	10.36 B	8.96 C	7.91 D	5.71 E	

Note: Different letters indicate significantly different values by ANOVA followed by Duncan test at P≤0.05 (small letters refer to values recorded in each season, different capital letters refer to mean values)

Table 5. Firmness ($g\,cm^{-2}$) of minimally-processed pomegranate arils, cv Wonderful, as affected by $CaCl_2$, Ca-lactate and salicylic acid treatments during storage at 5±1 °C (2013 and 2014 seasons)

Treatments	Season 2013						Season 2014					
	Storage period (days)						Storage period (days)					
	0	3	6	9	12	Mean	0	3	6	9	12	Mean
Control	15.67 a	11.67 c-f	8.34 h-l	7.00 l-o	5.33 o	9.60 D	13.67 a	11.00 c-g	8.33 i-l	7.00 lmn	4.67 o	8.93 C
$CaCl_2$ 0.5%	15.67 a	13.33 bc	11.00 d-g	9.00 g-l	7.33 k-o	11.27 AB	13.67 a	12.00 a-d	10.33 d-h	8.67 h-l	7.67 j-m	10.47 B
$CaCl_2$ 1%	15.67 a	14.00 ab	11.67 c-f	9.00 g-l	7.33 k-o	11.53 A	13.67 a	13.00 ab	12.33 abc	11.33 b-f	9.67 f-i	12.00 A
Ca-lactate, 0.5%	15.67 a	12.67 bcd	10.00 e-i	8.33 h-l	7.00 l-o	10.73 ABC	13.67 a	11.67 b-e	10.00 ei	9.00 h-k	7.33 k-n	10.33 B
Ca-lactate, 1%	15.67 a	12.67 bcd	10.33 e-h	8.00 i-m	6.00 mno	10.53 A-D	13.67 a	12.00 a-d	10.33 d-h	9.33 g-j	7.33 k-n	10.53 B
Salicylic acid, 1 mM	15.67 a	12.00 b-e	9.67 f-j	7.67 j-n	5.67 no	10.13 CD	13.67 a	11.67 b-e	9.33 g-j	7.33 k-n	5.67 no	9.53 BC
Salicylic acid, 2 mM	15.67 a	13.00 bcd	9.34 g-k	7.33 k-o	6.00 no	10.27 BCD	13.67 a	11.33 b-f	9.33 g-j	7.67 j-m	6.00 mno	9.60 BC
Mean	15.67 A	12.76 B	10.05 C	8.05 D	6.38 E		13.67 A	11.81 B	10.00 C	8.62 D	6.90 E	

Note: Different letters indicate significantly different values by ANOVA followed by Duncan test at P≤0.05 (small letters refer to values recorder in each season, different capital letters refer to mean values)

fruits (Ding *et al*., 2007), or $CaCl_2$ on strawberry (Biten Court De Souza *et al*., 1999)

Ascorbic acid (Vitamin C) content

Ascorbic acid is an important nutrient quality parameter. However, compared to other nutrients, it is very sensitive to degradation due to its oxidation during food processing and storage (Veltman *et al.,* 2000). Pomegranates are low in vitamin C, its content ranging from 0.49 to 30 mg per 100 g of juice, depending on cultivar (Hussein and Hussein, 1972; Küpper, 1995). Table 3 shows that, in both seasons, vitamin C content significantly decreased with the increase of storage time, in accordance with O'Grady *et al.* (2014) who observed that ascorbic acid concentration reduced over time in 'Ruby' arils stored at 1 °C, 4 °C and 8 °C for 7 days. With few exceptions, SA at 1 mM gave the highest values at each storage period of both the seasons, determining also the highest mean values, with significant differences with the control. Also SA at 2 mM maintained relatively high values of vitamin C; in this context, Sayyari *et al.* (2009) found that SA at 2 mM reduced the decline rate in ascorbic acid content, compared to control fruit.

Results are consistent with the finding of Sayyari *et al.* (2011) who found that ascorbic acid losses were about 50% in control fruits at the end of storage time, and significant increases were observed for 0.5 and 1 mM acetylsalicylic acid. This could be attributed to the effects of acetylsalicylic acid on promoting the ascorbate-glutathione cycle (Wang *et al*., 2006) and improving levels of antioxidant components such as ascorbic acid (Huang *et al*., 2008). Differently from this study, Mirdehghan and Ghotbi (2014) reported that pomegranate fruits treated with 2 mM SA had the lowest ascorbic acid content, although differences between other treatments and with the untreated fruits were not significant,

Lowest mean values of ascorbic acid were always obtained with control arils, although the differences with the treated ones were only occasionally significant. Aarabi *et al.* (2008) also investigated the concentration of ascorbic acid in selected pomegranate juices during storage at 4 °C for 60 days, and reported 100% loss of initial ascorbic acid concentration after 15 days at 4 °C. Similarly, a significant loss in vitamin C concentration was observed in pomegranate fruit 'Wonderful' stored at 5 °C and 7.5 °C for 5 months (Arendse *et al*., 2014). A decrease in vitamin C may be related to the irreversible oxidation of dehydro-L-ascorbic acid (DHAA) to 2,3-diketo-L-gulonic acid (Coultate, 2007). Furthermore, ascorbic acid is affected (and its activity is reduced) by the presence of oxygen, alkalinity and high temperatures (Coultate, 2007).

Anthocyanins content

Anthocyanins are responsible for the desirable red color of pomegranate juices, as well as many other red-colored fruit juices (Li *et al*., 2010). Generally, anthocyanins are labile compounds and are easily susceptible to degradation (Mphahlele *et al*., 2014).

Table 4 shows that anthocyanin content significantly decreased throughout the storage time in both the seasons, irrespective to the treatments. No significant mean difference was observed in the first season among the different treatments and control, while in the second season arils treated with 1% Ca-lactate had the highest anthocyanin content, significantly different from control arils and the ones treated with 0.5% Ca-lactate. SA at 2 mM maintained the highest value of anthocyanin after 12 days of storage, in accordance with the results obtained by Sayyari *et al.* (2011) who showed that acetyl salicylic acid maintains high levels of bioactive compounds, such as total anthocyanins.

These data show that anthocyanin content is significantly influenced by storage time, but treatments have a non-significant effect on anthocyanin content on minimally processed arils. This result is in accordance with those of Caleb *et al.* (2013) on minimally processed pomegranates, as they reported a significant effect of storage duration on the total anthocyanin content, with a general trend of a decrease in total anthocyanin content as the storage time increased. Also Arendse *et al.* (2014) reported a decline in total anthocyanin concentration of 'Wonderful' pomegranate after cold storage. The decrease in phenolic concentration, including anthocyanins, in pomegranates could be attributed to the change of enzyme activities resulting to phenolic degradation (Fawole and Opara, 2013). Furthermore, loss of anthocyanins could be attributed to many other factors, such as pH and acidity, phenolic compounds, sugars and sugar degradation products, oxygen, ascorbic acid, fruit maturity and thawing time (Withy *et al.,* 1993; García-Viguera *et al*.,1998).

Firmness

As shown in Table 5, a significant reduction of arils firmness within the storage period was observed in both the investigated seasons and regardless to the treatments. As for the different treatments, calcium chloride treatments at both the tested concentrations (0.5% and 1%) maintained the highest arils firmness, with significant differences with the untreated arils. In both seasons, at every storage period, untreated arils had the lowest firmness, with values significantly different from the calcium-treated ones. In accordance with these results, Aguayo *et al.* (2012) reported that $CaCl_2$ treatments kept a better firmness of pomegranates fruits than control arils. It is well known that firmness and resistance to softening can be increased by the addition of calcium, due to the stabilization of the membrane systems and the formation of Ca-pectates which increase the rigidity of the middle lamella and cell wall, as well as the cell cohesion (White and Broadley, 2003). Manganaris *et al.* (2007) suggested the $CaCl_2$ immersion as potential postharvest treatment for whole peaches, since it increases tissue firmness and reduces susceptibility to physiological disorders. Moreover, the use of $CaCl_2$ dips is recommended as a low cost-effective method for extending the storage life of arils pomegranate (Aguayo *et al*., 2012). Mirdehghan and Ghotbi (2014) reported that calcium maintains the cell wall structure in fruit by interacting with the pectic acid in the cell walls to form calcium pectate. In addition, Garcia *et al*. (1996) and Picchioni *et al.* (1998) indicated that postharvest calcium application maintains cell turgor, membrane integrity, tissue firmness, and delays membrane lipid catabolism, extending storage life of fresh fruits and reducing physiological disorders. El-Kassas *et al.* (1995) also obtained satisfactory results on pomegranate with pre- and postharvest $CaCl_2$ treatments.

As for the treatments with SA, no significant difference in aril firmness with the control was observed at each storage period, in accordance with what observed on kiwifruits treated with acetyl salicylic acid (Sayyari *et al.,* 2011).

Table 6. Color (L* value) of minimally-processed pomegranate arils, cv Wonderful, as affected by $CaCl_2$, Ca-lactate and salicylic acid treatments during storage at 5±1 °C (2013 and 2014 seasons)

Treatments	Season 2013						Season 2014					
	Storage period (days)						Storage period (days)					
	0	3	6	9	12	Mean	0	3	6	9	12	Mean
Control	48.50 a	31.77 b-f	26.67 d-h	23.37 g-k	22.37 g-k	30.53 AB	49.40 a	29.57 cd	29.50 cd	26.87 d-g	24.77 d-j	32.02 A
$CaCl_2$, 0.5%	48.50 a	32.33 b-e	27.00 d-h	20.20 ijk	18.63 k	29.33 B	49.40 a	25.90 d-h	24.93 d-j	23.07 e-j	21.53 g-j	28.97 A
$CaCl_2$, 1%	48.50 a	34.63 bc	27.74 d-g	23.00 g-k	21.33 h-k	31.04 AB	49.40 a	27.63 c-f	27.60 c-f	20.56 hij	19.09 j	28.86 A
Ca-lactate, 0.5%	48.50 a	30.87 b-f	26.22 e-i	25.42 f-j	23.05 g-k	30.81 AB	49.40 a	29.73 cd	27.68 cde	20.84 hij	19.71 ij	29.47 A
Ca-lactate, 1%	48.50 a	31.03 b-f	30.47 c-f	23.87 g-k	21.35 h-k	31.04 AB	49.40 a	29.10 cd	25.67 d-i	21.86 e-j	20.50 hij	29.31 A
Salicylic acid, 1 mM	48.50 a	36.90 b	26.43 d-i	21.14 h-k	19.66 jk	30.53 AB	49.40 a	39.37 b	24.32 d-j	24.03 d-j	21.68 f-j	31.76 A
Salicylic acid, 2 mM	48.50 a	32.77 bcd	31.14 b-f	28.07 d-g	26.48 d-i	33.39 A	49.40 a	33.53 bc	26.06 d-h	22.93 e-j	20.50 hij	30.49 A
Mean	48.50 A	32.90 B	27.95 C	23.58 D	21.84 D		49.40 A	30.69 B	26.54 C	22.88 D	21.11 D	

Note: Different letters indicate significantly different values by ANOVA followed by Duncan test at P≤0.05 (small letters refer to values recorded in each season, different capital letters refer to mean values)

Table 7. Hue angel (h°) values of minimally-processed pomegranate arils, cv Wonderful, as affected by $CaCl_2$, Ca-lactate and salicylic acid treatments during storage at 5±1 °C (2013 and 2014 seasons)

Treatments	Season 2013						Season 2014					
	Storage period (days)						Storage period (days)					
	0	3	6	9	12	Mean	0	3	6	9	12	Mean
Control	37.77 ab	35.80 a-f	32.57 a-i	29.63 e-i	29.27 f-i	33.01 A	37.80 a	34.17 a-f	32.80 a-g	29.20 f-j	27.57 g-j	32.31 A
$CaCl_2$, 0.5%	37.77 ab	36.97 a-d	31.53 a-i	30.73 c-i	26.60 i	32.72 A	37.80 a	35.50 a-e	30.77 e-i	27.67 g-j	26.97 hij	31.74 A
$CaCl_2$, 1%	37.77 ab	36.51 a-e	33.59 a-i	29.72 e-i	26.67 i	32.85 A	37.80 a	36.54 abc	29.76 f-i	26.96 hij	26.53 ij	31.52 A
Ca-lactate, 0.5%	37.77 ab	35.44 a-g	32.01 a-i	30.77 b-i	30.06 d-i	33.21 A	37.80 a	36.58 abc	32.20 b-h	32.61 a-g	31.10 d-i	34.06 A
Ca-lactate, 1%	37.77 ab	37.88 a	31.73 a-i	31.05 a-i	28.47 ghi	33.38 A	37.80 a	34.34 a-f	29.80 f-i	29.21 f-j	27.67 g-j	31.76 A
Salicylic acid, 1 mM	37.77 ab	33.83 a-h	36.53a-e	28.83 f-i	28.16 hi	33.02 A	37.80 a	32.03 b-h	30.63 e-i	37.17 ab	28.26 g-j	33.18 A
Salicylic acid, 2 mM	37.77 ab	37.18 abc	34.90 a-h	32.39 a-i	28.53 ghi	34.15 A	37.80 a	36.32 a-d	31.76 c-i	30.82 e-i	24.23 j	32.25 A
Mean	37.77 AB	36.23 AB	33.27 BC	30.45 CD	28.25 D		37.80 A	35.07 B	31.10 C	30.52 C	27.47 D	

Note: Different letters indicate significantly different values by ANOVA followed by Duncan test at P≤0.05 (small letters refer to values recorded in each season, different capital letters refer to mean values)

Table 8. Microbial count of fungi/yeasts (log CFU g^{-1}) of minimally-processed pomegranate arils, cv Wonderful, as affected by $CaCl_2$, Ca-lactate and salicylic acid treatments during storage at 5±1 °C (2013 and 2014 seasons)

Treatments	Season 2013				Season 2014			
	Storage period (days)				Storage period (days)			
	0	6	12	Mean	0	6	12	Mean
Control	2.50 a	2.50 a	2.57 a	2.52 A	2.50 a	2.50 a	2.50 a	2.50 A
$CaCl_2$, 0.5%	1.50 g	1.73 f	2.27 c	1.83 E	1.47 g	1.80 e	2.13 d	1.80 E
$CaCl_2$, 1%	2.03 e	2.10 de	2.17 d	2.10 D	2.10 d	2.10 d	2.30 c	2.17 D
Ca-lactate, 0.5%	2.10 de	2.27c	2.33 bc	2.23 C	2.30 c	2.30 c	2.30 c	2.30 C
Ca-lactate, 1%	2.37 b	2.50 a	2.50 a	2.46 B	2.30 c	2.37 bc	2.47 ab	2.38 B
Salicylic acid, 1 mM	1.10 i	1.30 h	1.57 g	1.32 F	1.30 h	1.30 h	1.60 f	1.40 F
Salicylic acid, 2 mM	1.00 j	1.00 j	1.00 j	1.00 G	1.10 i	1.30 h	1.50 fg	1.30 G
Mean	1.80 C	1.91 B	2.06 A		1.80 c	1.91 b	2.06 a	

Note: Different letters indicate significantly different values by ANOVA followed by Duncan test at P≤0.05 (small letters refer to values recorded in each season, different capital letters refer to mean values)

Table 9. Microbial count of bacteria (log CFU+1 g^{-1}) of minimally-processed pomegranate arils, cv Wonderful, as affected by $CaCl_2$, Ca-lactate and salicylic acid treatments during storage at 5±1 °C (2013 and 2014 seasons)

Treatment	Season 2013				Season 2014			
	Storage period (days)				Storage period (days)			
	0	6	12	Mean	0	6	12	Mean
Control	2.77 bc	2.90 b	3.50 a	3.06 A	2.70 bcd	2.70 bcd	3.50 a	2.97 A
$CaCl_2$, 0.5%	2.67 cd	2.60 de	2.47 e	2.58 C	2.67 cd	2.00 f	2.70 bcd	2.46 C
$CaCl_2$, 1%	2.30 f	2.70 cd	2.77 bc	2.59 C	2.60 d	2.63 d	2.67 cd	2.63 B
Ca-lactate, 0.5%	2.30 f	2.30 f	2.73 cd	2.44 D	2.00 f	2.30 e	2.83 b	2.38 C
Ca-lactate, 1%	2.70 cd	2.73 cd	2.70 cd	2.71 B	2.30 e	2.80 bc	2.70 bcd	2.60 B
Salicylic acid, 1 mM	2.00 g	2.00 g	2.00 g	2.00 F	2.00 f	2.00 f	2.30 e	2.13 D
Salicylic acid, 2 mM	2.00 g	2.00 g	2.77 bc	2.26 E	2.00 f	2.00 f	2.63 d	2.21 D
Mean	2.39 B	2.46 B	2.71 A		2.32 B	2.36 B	2.76 A	

Note: Different letters indicate significantly different values by ANOVA followed by Duncan test at P≤0.05 (small letters refer to values recorded in each season, different capital letters refer to mean values)

Table 10. Sensory evaluation (score) of minimally-processed pomegranate arils, cv Wonderful, as affected by CaCl2, Ca-lactate and salicylic acid treatments after 12 days of storage at 5 ±1 °C (2013 and 2014 seasons). GA, general appearance

Treatment	Season 2013				Season 2014			
	GA	Decay	Flavor	Off odors	GA	Decay	Flavor	Off odors
Control	7.17 b	2.00 a	3.75 b	1.58 a	6.83 c	2.00 a	3.58 b	2.00 a
CaCl2, 0.5%	7.83 ab	1.00 b	4.33 a	1.00 b	7.67 abc	1.00 b	4.33 a	1.00 b
CaCl2, 1%	7.67 ab	1.00 b	4.25 a	1.00 b	7.67 abc	1.00 b	4.25 a	1.00 b
Ca-lactate, 0.5%	7.67 ab	1.08 b	3.92 ab	1.00 b	7.67 abc	1.17 b	3.97 ab	1.67 b
Ca-lactate, 1%	7.67 ab	1.00 b	4.00 ab	1.00 b	7.67 abc	1.08 b	4.00 ab	1.08 b
Salicylic acid, 1 mM	8.17 a	1.00 b	4.25 a	1.00 b	8.30 a	1.08 b	4.25 a	1.00 b
Salicylic acid, 2 mM	8.00 a	1.00 b	4.25 a	1.00 b	8.00 a	1.00 b	4.25 a	1.00 b

Note: different letters indicate significantly different values by ANOVA followed by Duncan test at P≤0.05

Color measurements

L* value

With regard to color characteristics of pomegranate arils, the L* value showed a significant decrease along storage period up to 9 days in both studied seasons (Table 6), indicating a darker coloration, in accordance with previous reports (Toor and Savage, 2006; Ashebir *et al*., 2009; Sanchís *et al*., 2015). As for the different treatments, no significant difference was observed. In accordance, Khademi *et al.* (2012) reported that SA at 1 and 2 mM determined no significant difference of color index among treatments, and also Sepúlveda *et al*. (2000) and Artés *et al*. (2000) observed no color change in minimally processed 'Wonderful' arils stored at 4±0.5 °C in semi- permeable films for 14 days. Gil *et al.* (1996) reported a relatively small change in L* values for 'Mollar' arils, packed in oriented polypropylene (OPP) bags stored at 8, 4 and 1 °C for 7 days. These results are also in agreement with the pattern of decline in anthocyanins, observed by Meighani *et al.* (2014).

Hue angle (h°)

As for of hue angle (h°), Table 7 shows that values regularly decreased along the storage period. In both seasons, at the end of the storage period the h° values were significantly lower than at time '0'. No significant difference in h° mean values was observed among the different treatments and untreated arils, similarly with what previously reported by Mirdehghan *et al.* (2012) and Artés *et al.* (2000). Also Belay *et al.* (2016) didn't obtain significant differences between treatments with atmospheric oxygen at the end of 12 days of cold storage (5 °C) of minimally processed pomegranate arils.

Microbial quality

Total fungal and bacterial counts were assessed at the beginning of the trial (day 0), then at 6 and 12 days of storage. In both seasons, Tables 8 and 9 show a slight increment, but often significant, in both fungal and bacterial counts during the storage period. However, counts remained fewer than 5 log CFU g^{-1}, which is the maximum limit for yeasts and moulds in raw and fresh-cut fruits by the South African legislation (FCD, Act 54 1979; Caleb *et al.,* 2013). Data also show that control arils had always the highest count of fungi/yeasts and bacteria, with significant mean values. Lowest counts were obtained with SA treatment at both the tested concentrations, with significant differences with the other treatments and untreated arils. Among the different treatments, 1% Ca-lactate was the one that produced the highest counts of fungi/yeasts and bacteria both along the storage period, and as mean values.

Overall, data obtained in these trials were in agreement with those reported by Arendse *et al.* (2014). Several types of moulds and bacteria are associated with pomegranate fruit, affecting its overall quality. In addition, Soliva-Fortuny and Martín-Belloso (2003) reported that physicochemical properties of fruit, such as pH and TA, have an important effect on microbial shelf-life of fresh-cut fruit.

Sensory evaluation

Sensory descriptive analysis, coupled with a consumer preference test, can establish the relative importance of the characteristics that drive acceptability. Difference tests can be used to select the best treatments for use in a consumer preference test (Barrett *et al*., 2010). As shown in Table 10, data indicate that arils treated with SA (1 and 2 mM) maintained the best general appearance up to the end of the storage period, as the scores were significantly higher in comparison with untreated ones. However, differences were not significant among the various treatments. In both seasons, all treatments showed a minimal or nil visible decay after 12 days of storage, while control arils recorded a slight visible decay.

Flavor may be evaluated with either instrumental or sensory methods, but most scientists agree that sensory methods are the most critical for this particular quality attribute (Barrett *et al*., 2010). Both calcium (0.5% and 1%) and SA (1 and 2 mM) treatments obtained the highest scores of flavors, while untreated (control) arils showed the lowest score, with no significant differences with arils treated with Ca-lactate at both tested concentrations.

Untreated arils (control) recorded the highest scores of off odor detection. On the other hand, differences between treatments failed to show any significance, although 0.5% Ca-lactate showed a slight increase in the off odour in the second season of investigation.

Conclusions

Treatments with different compounds can help the maintenance of post-harvest quality of fruits. However, in order to achieve maximum time of conservation, the best combination compound/concentration should be always tested. In this study, the post-harvest assessment was performed at 3-day intervals with several parameters (appearance, decay, off odor, flavor, total soluble solids, acidity, anthocyanin and vitamin C content, firmness, color development by L* value and hue angle), as well as with microbial load assessed at 0,6 and 12 days of storage, achieving a clear view of the effectiveness of calcium chloride, Ca-lactate and SA in the maintenance of pomegranate aril quality. Based on the data it can be concluded that SA had a significant effect on quality parameters changes and sensorial quality of pomegranate arils, determining the lowest microbial counts and the highest values in all the tested quality attributes.

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