

RESEARCH ARTICLE

Implication of potassium on the quality of cherry tomato fruits after postharvest during cold storage

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The influence of the potassium (K) content in tomato fruits over compounds or antioxidant characteristics during the postharvest period in cold storage is little known. The aim of this work was to determine whether the effect of a biofortification programme with K in KCl form can improve the postharvest storage of cherry tomato fruits at 4 °C. K treatments applied during the crop cycle of the plants: 5, 10 and 15 mM of KCl. Biomass parameters, levels of K, antioxidant capacity test, Vitamin C, carotenoids, phenolic compounds and free polyamines in tomato cherry fruits were measured. Our results show that the treatment with 15 mM KCl prevents weight and water loss during postharvest storage at 4 °C, increases K concentration, and bolsters the antioxidant capacity, since the concentration in lycopenes as well as flavonoids and derivatives rose, while the contents in Vitamin C together with hydroxycinnamic acids and derivatives remained stable.

Keywords

Antioxidants compounds, postharvest, potassium, tomato fruits

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The tomato (*Solanum lycopersicum* L.), an annual horticultural plant with a worldwide distribution and enormous economic values, has a global annual production of some 159.347 million of tonnes (FAOSTAT, 2011). Due to its high content in compounds that detoxify reactive oxygen species (ROS) and thus prevent oxidative changes in the human, the consumption of these fruits is considered beneficial for human health (García-Closas et al., 2004). Tomato is rich in bioactive compounds, such as lycopene (Lyc), which represents around 80% of the carotenoids and has a high capacity to eliminate ROS, being one of the phytonutrients most characteristic of tomato fruit (Rao et al., 1998); β -carotene, a precursor to Vitamin A in the human body; ascorbic acid (Vitamin C), which, apart from being the most effective antioxidant in plants (Smirnoff & Pallanca, 1996), is a major phytochemical for its antioxidant properties in eliminating ROS and regenerating Vitamin E in plants (Asada, 1994); lutein (Lut), a yellow pigment found in plants which is considered an important phytochemical for its high antioxidant capacity and which cannot be synthesized by animals; and phenolic compounds, namely flavonoids and phenolic acids (Soto-Zamora et al., 2005). Many phenolic compounds exhibit antioxidative, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory activities (Martinez-Valverde et al., 2002).

Spain has exported tomatoes since the 1940s and, in general, exportation implies the storage of fruits in cold chambers. Although cold storage is a widely used method to prolong the shelf life of climacteric fruits, it can affect their nutritional quality by provoking cold damage. This type of stress occurs during

storage below 10 °C in fleshy fruits, tomato being particularly sensitive (Stevens et al., 2008). Tomato, being climacteric and thus perishable, requires the use of conservation technologies to retard the ripening process that occurs after harvest and thereby maintain its quality and extend the shelf life of the fruit. Despite that cold storage can trigger harmful effects, this procedure has been demonstrated to be effective in maintaining the phytonutrients and other qualities that determine fruit quality. Antioxidant activity of tomatoes depends on several factors, including genetic traits, environmental conditions (temperature, light, water and nutrient availability), production techniques (plant growth regulators, date of harvest, etc.) and postharvest storage conditions (Dumas et al., 2003; Leonardi et al., 2000). As demonstrated by Wang et al. (2012) in avocado fruits harvested on different dates of the year, cold storage resulted in positive effects on the maintenance of antioxidant capacity as well as the accumulation and retention of nutrients, including phenolic compounds. In relation to the effect of cold storage on tomato fruits, Nicoletto et al. (2012) found that ripe fruits left on the plant showed increased antioxidant activity in addition to greater Vitamin C and total phenolic content in comparison with those that were stored cold, as in the latter fruits the parameters did not vary. In this sense, Kalt et al. (1999) in his study with small fruits, i.e. fresh strawberries (*Fragaria ananassa* Duch.), raspberries (*Rubus idaeus* Michx.), highbush blueberries (*Vaccinium corymbosum* L.), and lowbush blueberries (*Vaccinium angustifolium* Aiton), found losses in ascorbate after fresh storage, registering minimum values.

In terms of pigments, different authors have confirmed that cold storage retards the synthesis of Lyc and carotenoids (Gómez et al., 2009; Mejía-Torres et al., 2009). At low temperatures (below 12 °C), chlorophyll is only partially degraded while Lyc does not accumulate as it does under normal conditions (Lopez-Camelo & Gomez, 2004). On the contrary,

Farneti et al. (2012), in his work with ripe red tomato fruits, using remittance VIS spectroscopy to assess the Lyc content in the tomato pericarp tissue, concluded that tomato storage at temperatures below 12 °C (a common market practice) degrades Lyc and consequently reduces the presumed health-promoting value at the same time as lowering the external visual quality. The decrease in Lyc content induced by low-temperature storage may be caused by Lyc fragmentation. However, available published data on antioxidant active compounds Lyc, phenols, and Vitamins C and E are limited mostly to vine-ripened tomatoes or processed tomatoes. Thus, it is necessary to know more about the effects of postharvest conditions, especially at low temperatures, on the antioxidants in tomatoes, because temperature is the main factor for tomato quality in terms of antioxidants (Javanmardi & Kubota, 2006).

The main factors that can affect tomato-fruit quality at harvest as well as afterwards include the genotype cultivated, environmental conditions and the fertilizer applied (Beckles, 2012). In relation to this latter factor, in recent years, in order to improve the nutritional quality of table vegetables, biofortification programmes are being steadily more widely used, both with trace elements as well as macronutrients. Studying macronutrients, He & MacGregor (2008) have indicated that increased consumption of processed foods together with reduced consumption of fruits and vegetables results in a serious decrease in K ingestion. Evidence reveals that higher K intake has beneficial effects on human health. Epidemiological and clinical studies demonstrate that a diet rich in this nutrient lowers blood pressure, reduces mortality by cardiovascular disease, retards certain renal pathologies and appears to slow the appearance of osteoporosis (He & MacGregor, 2008).

Thus, K is notable as the cation that has the greatest influence on the quality parameters determining the marketing of fruits, consumer preferences, and the concentration of vital phytonutrients for human health (Lester et al., 2010). K significantly affects the concentration of such pigments as Lyc and β -carotene, which can be used as inner-quality indicators for tomato, based on analytical and sensorial properties (Ramírez et al., 2012). Nutrition with adequate K is also associated with greater yield, larger fruit size, increased soluble solids, higher Vitamin C concentrations and improved fruit colour (Kanai et al., 2007). Studies on open-field and greenhouse tomato crops (Chapagain & Wiesman, 2004) showed that an increased K supply at specific growth stages of the tomato plant would improve fruit quality. However, the influence that the K content in fruits exerts on the compounds or antioxidant characteristics during a postharvest period of cold storage is little known. Therefore, in consideration of the functions of K described above concerning fruit quality, the aim of the present work was to evaluate a biofortification programme with K in the form of KCl in terms of nutritional quality of cherry tomato fruits (*S. lycopersicum* L. cv. Ashiari) after 21 days of cold storage at 4 °C.

Material and methods

Plant material and growth conditions

Seeds of cherry tomatoes (*S. lycopersicum* L. cv. AsHiari grafted on cv. Maxifort rootstock) were sown in flat trays (cell size 3 cm × 3 cm × 10 cm, 100 cells per tray) filled with 50% [v/v] perlite-peat mixture, and kept under greenhouse conditions for five weeks. Subsequently, the seedlings were transplanted to an experimental greenhouse at La Nacla Experimental Station (Motril, Granada, Spain). The *parral* greenhouse consisted of three modules having a symmetrical gable roof with a 27° slope and having an E-W longitudinal orientation (Soriano et al., 2004). The active environmental control was limited to a heating system

by hot-air generators, and a natural ventilation system through wall and roof windows. In the greenhouse, the cladding material was a multilayer film 0.2 mm thick, with a layer of ethylene-vinyl-acetate between two low-density polyethylene layers (inner, antiodrop; and outer, long life). The plants were grown in 40-L perlite B-12-filled sacks (1.20 m long) spaced 0.5 m apart in rows 1.4 m apart. With three tomato plants per sack and two stems per plant, the planting scheme was 3.21 plants m⁻². There were 12 rows oriented north-south in the greenhouse. The statistical design was a randomized block. Other growing conditions such as irrigation and fertilise application followed Soriano et al. (2004). The different treatments applied were as follows: 5, 10 and 15 mM KCl as liquid solution from the beginning to the end of the experiment.

Tomato fruit sampling

The cherry tomato crop cycle lasted from October 2010 to May 2011 (230 days), with a complete truss of tomatoes (10–12 tomatoes per truss) maturing every 10 days. Cherry tomato fruits were sampled in February of 2011 at 140 days after transplanting. Uniformly ripe healthy fruits, at the red-ripe stage, were harvested. Approximately 180 tomatoes fruits from each treatment were randomly collected (discarding the green fruits at the end of the truss) and were rinsed three times in distilled water after disinfection with 1% (v/v) Triton X-100 (Wolf, 1982), and then blotted on dry filter paper.

Fresh weight and percentage of lost fresh weight

For the analyses of the fruits at harvest (T0), some tomato fruits from each treatment were weighted for fresh weight (FW) (T0). The remaining tomato fruits were homogenized, and these samples of fresh tissues were stored at –80 °C, while other tomato fruits were freeze dried in a lyophilizer. The weighed fruits from each treatment were stored 21 days in a cold room at 4 °C (T21), and afterwards weighed again to record the new FW (T21). Next, the fruits were homogenized, and these samples of fresh tissues were stored at –80 °C, while another quantity of these tomato fruits were freeze dried. Samples of fresh and dry tissues from the cherry tomato fruits were used to analyse the parameters described below.

For the determination of the % of LFW, the following formula was used for each treatment:

$$\% \text{ LFW} = (\text{FW T0} - \text{FW T21}) \times 100 / \text{FW T0}$$

Analytical methods

Determination of the K concentration

For the determination of the K concentration, 0.2 g of dry cherry tomato fruits were ground and mineralized by wet digestion with H₂SO₄ 12 M and H₂O₂ at 30% and P free, at 275–300 °C. After the addition of 20 mL of deionized H₂O, the K concentration was analysed directly in this solution by flame atomic absorption spectrometry using (Perkin-Elmer AAnalyst 700, Norwalk, CT) (Wolf, 1982).

Antioxidant capacity assays

The total antioxidant capacity was measured using the Trolox equivalent antioxidant capacity (TEAC), ferric reducing ability of plasma (FRAP) assays. The TEAC was determined as described by Re et al. (1999) using 2,2-azino-bis (3-ethylbenzthiazoline-6-sulphonate) solution (ABTS) and 2,20-azo-bis (2-methylpropionamide) dihydrochloride, for the production of the ABTS radical (ABTS[•]). The TEAC value of an extract represents the

concentration of a Trolox solution that has the same antioxidant capacity as the extract. The result of TEAC was expressed as mmol Trolox g^{-1} DW. The FRAP assay was made with FRAP reagent, i.e. 1 mM 2,4,6-tripyridyl-2-triazine and 20 mM FeCl_3 in 0.25 M CH_3COONa , pH 3.6. An aliquot of 100 μL of extract (1 g per 10 mL in methanol) was added to 2 mL of FRAP reagent and mixed thoroughly. After the mixture was left at room temperature (20 °C) for 5 min, absorbance at 593 nm was measured. Calibration was against a standard curve (25–1600 mM Fe^{3+}) using freshly prepared ammonium ferrous sulphate (Benzie & Strain, 1996). The result of FRAP was expressed as mmol g^{-1} FW.

For reducing power assays, tomato fruits were homogenized in methanol 80%, and centrifuged at $3000 \times g$ for 10 min. The reducing power of tomato fruits was measured following Hsu et al. (2009). Tomato extract, phosphate buffer (0.2 mol L^{-1} , pH 6.6) and $\text{K}_3\text{Fe}(\text{CN})_6$ (1% v/w) was mixed and allowed to react for 20 min at 50 °C. The sample was immediately cooled and then Cl_3CCOOH 10% was added. After centrifugation at $3000 \times g$ for 10 min, the supernatant was mixed with distilled water and FeCl_3 (0.1%), and allowed to react for 10 min. Increased absorbance of the reaction mixture at 700 nm indicated greater reducing power.

Pigment concentrations

Carotenoids were extracted directly in a 1.5-mL Eppendorf tube containing an assay sample of approximately 400 mg of tomato powder. This was achieved by means of alternating periods of stirring and centrifugation (19 500 g), in the following order: the addition of 100 μL of saturated aqueous NaCl solution and 50 μL of Hex, agitation for 30 s and centrifugation for 2 min; the addition of 200 μL of dichloromethane, stirring for 30 s and centrifugation for 2 min; the addition of 1000 μL of ethyl acetate (EA), stirring for 30 s and centrifugation for 5 min. An aliquot of the organic fraction (upper phase) was filtered and assayed by HPLC (Sérino et al., 2009). The assay was performed using HPLC with a DAD UV-Visible detector (Agilent Technologies, Santa Clara, CA) under the following conditions: Phenomenex reverse-phase column, 250×4.6 mm i.d., 5 μm , Li-Chrospher 100 RP-18, with a 4×4 mm i.d. guard column of the same material (Luna, Phenomenex, Utrecht, Belgium). The column oven temperature, 28 °C; mobile phase, acetonitrile (ACN):UP water:EA (53:7:40, v/v/v); flow rate of mobile phase, 1 mL min^{-1} ; injection volume, 10 μL ; wavelength range, 200–750 nm; two working wavelengths, 474 nm for Lyc, 454 nm for β -carotene, and 448 nm for Lut. These chromatographic conditions allow good separation of the different carotenoids present in tomato. Lut, Lyc and β -carotene were used as a standard (Sigma-Aldrich, Steinheim, Germany), eluting at 4, 13 and 23 min, respectively.

Anthocyanins were determined according to Lange et al. (1971) with some modifications. Tomato fruits were homogenized in propanol:HCl:H₂O (18:1:81) and further extracted in boiling water for 3 min. After centrifugation at $5000 \times g$ for 40 min at 4 °C, the absorbance of the supernatant was measured at 535 and 650 nm. The absorbance due to anthocyanins was calculated as $A = A_{535} - A_{650}$.

Vitamin C concentration

The determination of ascorbic acid was based on the method of Hejtmánková et al. (2009) with slight modifications. About 0.2 g of freeze-dried tomato samples were homogenized with 10 mL of 3% meta-phosphoric acid. The resulting mixture was centrifuged for 10 min and then filtered through a 0.45 μm membrane filter, and triplicates of 10 mL for each sample were analysed by HPLC-DAD. HPLC analysis of ascorbic acid was carried out using the same

equipment as described above. Samples were injected into an ACE 5C18 column, 250×4.6 mm (Hichrom, Berkshire, UK) operating at 30 °C. A single mobile phase consisting of 2.5 mM sulphuric acid at 1.0 mL/min was used. The elution was monitored at 250 nm. L-Ascorbic acid was used as a standard (Sigma-Aldrich), eluting at 4.1 min.

Concentration of phenol compounds

For the identification and characterization of phenolics, 0.1 g of lyophilized tomatoes was extracted with 1 mL of water/methanol (1:1) by sonication for 1 h, followed by overnight maceration and another sonication period (1 h). The resulting extract was centrifuged and filtered through a 0.45 μm PVDF membrane (Sánchez-Rodríguez et al., 2011). Chromatographic analyses were made in an ACE 5C18 column, 250×4.6 mm (Hichrom). The mobile phase consisted of two solvents: water/acetic acid (1%) (A) and ACN (B), starting with 5% B and using a gradient to obtain 50% at 30 min and 80% at 37 min. The flow rate was 1 mL/min and the injection volume, 20 μL . Spectroscopic data from all peaks were accumulated in the range of 200–400 nm, and chromatograms were recorded at 280, 320 and 360 nm. The identified analytes were quantified by HPLC-PDA detection using the external standard method with calibration graphs, as a function of concentration based on peak area, detected at the wavelength corresponding to their maximum absorbance.

Concentration of free polyamines

In 1 mL of 6% (v/v) cold perchloric acid (PCA), 1.5 g of tomato were homogenized, kept on ice for 1 h, and then centrifuged at $21\,000 \times g$ for 30 min. The pellet was extracted once with 1 mL of 5% PCA and recentrifuged. The supernatant was benzoylated following the method of Aziz & Larher (1995) to determine the levels of free PAs. The benzoyl derivatives were separated and analysed by a HPLC (Agilent 1100 system, Santa Clara, CA). Next, 10 μL of ACN solution of benzoyl polyamines (PAs) was injected into an ACE 5C18 column, 250×4.6 mm (Hichrom). The column temperature was maintained at 30 °C. Samples were eluted from the column with 40% ACN at a flow rate of 1 mL/min. PA peaks were detected with a UV detector at 254 nm, and 1,6-hexanediamine was used as an internal standard.

Statistical analysis

Data were analysed using one-way analysis of variance to determine significance and Fisher's protected least-significant difference (LSD) test to separate means. Standard errors of the means were also calculated. The significance levels were expressed as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ and ns (not significant) $p > 0.05$.

Results

In relation to FW of the cherry tomato fruits, after 21 days of cold storage (T21), the FW of the fruits treated with 5 and 10 mM of KCl lost weight, while in the treatment with 15 mM of KCl no significant differences were found between T0 and T21 ($p < 0.001$, Figure 1). In terms of percentage of LFW, after postharvest storage of the fruits, the 15 mM KCl treatment registered the lowest value for this parameter ($p < 0.001$, Figure 2). With respect to the K concentrations, a proportional response was found according to the KCl applied, the highest concentration being recorded in the tomato fruits grown with 15 mM KCl ($p < 0.001$, Figure 3).

The cold storage for 21 days in the treatment of 5 mM KCl resulted in a decline in values of the antioxidant tests TEAC and FRAP (Table 1). On the contrary, at the rate of 15 mM KCl,

Figure 1. Effect of KCl treatments at the day of harvest over fresh weight (FW) in cherry tomato fruits. Values are means ($n=9$) and differences between means were compared by Fisher's LSD test ($p=0.05$). Means followed by the same letter do not differ significantly.

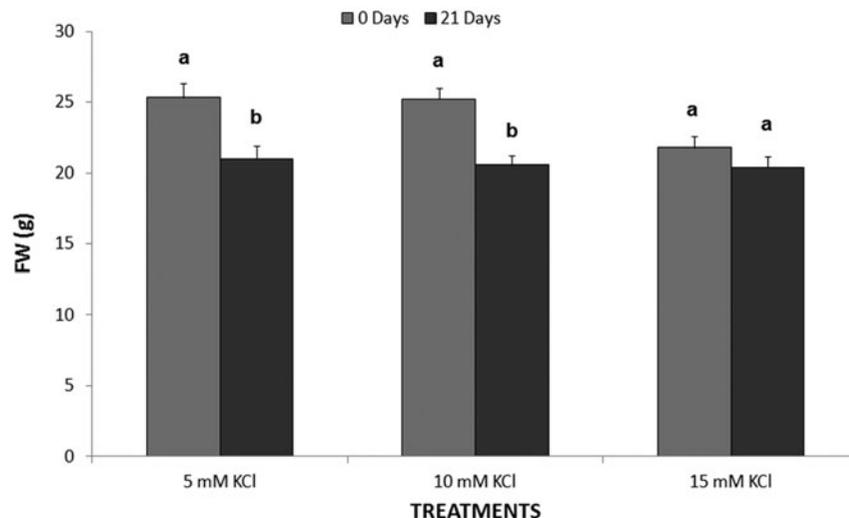


Figure 2. Effect of KCl treatments after 21 days of postharvest in cold storage at 4°C over % LFW in cherry tomato fruits. Values are means ($n=9$) and differences between means were compared by Fisher's LSD test ($p=0.05$). Means followed by the same letter do not significantly differ.

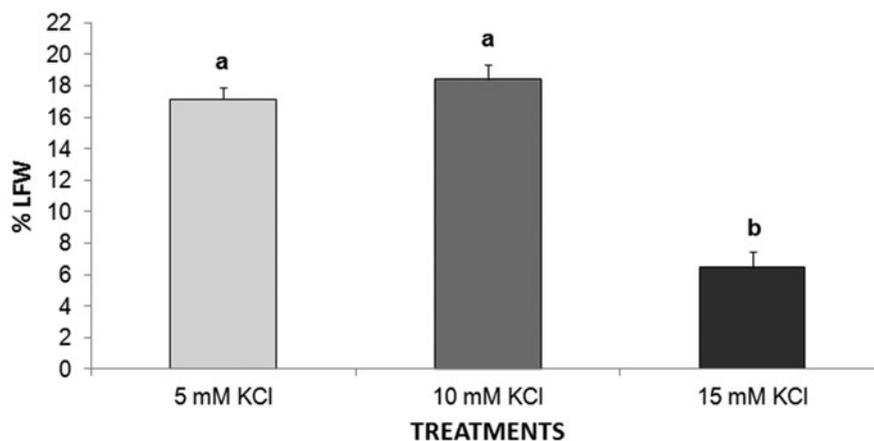
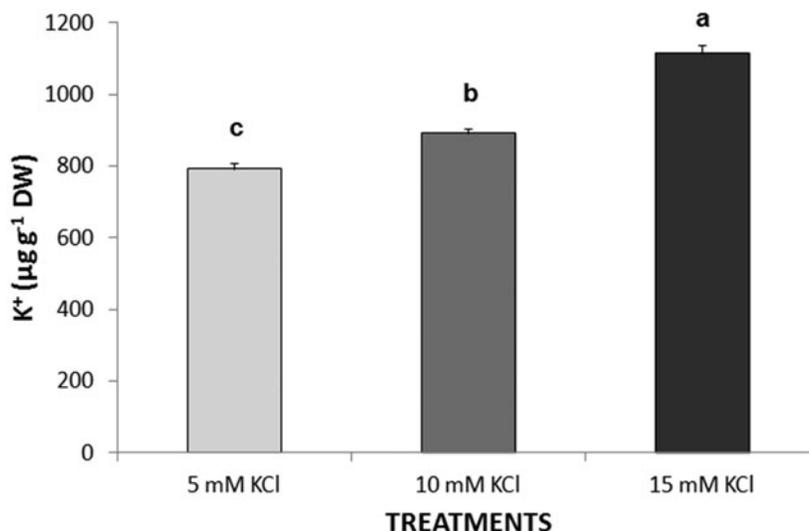


Figure 3. Effect of KCl treatments at the day of harvest over concentration of K in cherry tomato fruits. Values are means ($n=9$) and differences between means were compared by Fisher's LSD test ($p=0.05$). Means followed by the same letter do not significantly differ; DW, dry weight.



the postharvest values increased in these antioxidant tests, reaching maximum values at T21 (Table 1). For the fruits harvested from plants grown with 10 mM of KCl, no significant differences were found between T0 and T21 for these tests (Table 1). Finally, with respect to the reducing power, no differences appeared between T0 and T21 for any K treatment applied (Table 1).

With respect to Lyc, the cold storage increased values, and significant differences appeared for all the treatments applied.

In all cases, the maximum values of this compound were reached at T21, the highest corresponding to 15 mM KCl (Table 2). In the concentration of β -carotene, no significant differences were found for any K treatment between T0 and T21 (Table 2). Finally, with respect to Lut, the treatments with 5 and 15 mM of KCl presented no significant differences between T0 and T21. On the contrary, for the fruits treated at the rate of 10 mM KCl, postharvest values increased for Lut (Table 2). With respect to the

Table 1. Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4 °C over: TEAC, FRAP and reducing power in cherry tomato fruits.

KCl (mM)	Days	TEAC (mmol g ⁻¹ DW)	FRAP (mmol g ⁻¹ DW)	Reducing power (Abs g ⁻¹ DW)
5	0	16.72 ± 0.26 ^a	61.14 ± 0.81 ^a	14.12 ± 1.44
	21	10.67 ± 0.38 ^b	33.44 ± 0.67 ^b	14.27 ± 0.86
	<i>p</i> Value	***	***	NS
10	0	10.66 ± 0.37	34.78 ± 0.37	14.02 ± 0.71
	21	9.93 ± 0.41	33.78 ± 0.46	13.45 ± 0.46
	<i>p</i> Value	NS	NS	NS
15	0	8.33 ± 0.15 ^b	30.68 ± 0.41 ^b	13.19 ± 0.68
	21	13.31 ± 1.07 ^a	40.47 ± 0.47 ^a	13.82 ± 0.53
	<i>p</i> Value	***	***	NS

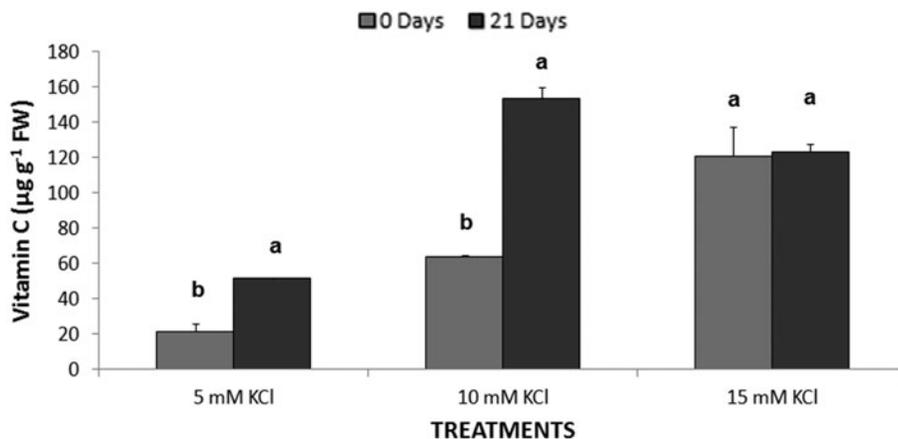
Values are mean ($n=9$) and differences between means were compared by Fisher's LSD test ($p=0.05$). Significance levels are represented by $p>0.05$; NS, not significant. Means followed by the same letter do not significantly differ; DW, dry weight. *** $p<0.001$.

Table 2. Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4 °C over: Lyc, β -carotene, Lut and anthocyanins in cherry tomato fruits.

KCl (mM)	Days	Lyc ($\mu\text{g g}^{-1}$ DW)	β -Carotene ($\mu\text{g g}^{-1}$ DW)	Lut ($\mu\text{g g}^{-1}$ DW)	Anthocyanins ($\Delta\text{Abs g}^{-1}$ DW)
5	0	331.71 ± 22.41 ^a	73.49 ± 7.40	5.98 ± 0.51	0.93 ± 0.12
	21	434.85 ± 29.36 ^b	53.67 ± 15.57	6.92 ± 0.94	0.88 ± 0.03
	<i>p</i> Value	**	NS	NS	NS
10	0	300.24 ± 16.60 ^b	62.01 ± 2.99	5.02 ± 0.46 ^b	0.82 ± 0.02
	21	507.65 ± 20.49 ^a	60.55 ± 6.04	8.89 ± 0.65 ^a	0.77 ± 0.02
	<i>p</i> Value	**	NS	**	NS
15	0	166.53 ± 55.20 ^b	60.69 ± 0.00	4.42 ± 0.39	0.81 ± 0.02
	21	531.31 ± 65.41 ^a	51.80 ± 3.86	6.19 ± 0.64	0.76 ± 0.01
	<i>p</i> Value	**	NS	NS	NS

Values are mean ($n=9$) and differences between means were compared by Fisher's LSD test ($p=0.05$). Significance levels are represented by $p>0.05$; NS, not significant. Means followed by the same letter do not significantly differ; DW, dry weight. ** $p<0.01$.

Figure 4. Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4 °C over Vitamin C concentration in cherry tomato fruits. Values are means ($n=9$) and differences between means were compared by Fisher's LSD test ($p=0.05$). Means followed by the same letter do not significantly differ; FW, fresh weight.



concentration of anthocyanins, no significant differences were found for any K treatment between T0 and T21 (Table 2).

The quantity of Vitamin C in the fruits treated with the rate of 5 and 10 mM KCl showed significant differences, both rates raising the levels of this compound at T21 (Figure 4), with the highest levels of this compound being reached in the treatment of 10 mM KCl ($p<0.001$, Figure 4). By contrast, the treatment of 15 mM KCl presented no differences between T0 and T21 (Figure 4).

For the hydroxycinnamic acids and derivatives, no significant differences were found for any K treatment between T0 and T21 (Table 3). With respect to the content in flavonoids and

derivatives, the treatment of 5 mM KCl showed significant differences, with a decline in these types of phenols at T21 (Table 3). Contrarily, the application of 15 mM KCl augmented these phenols at T21 with respect to T0 (Table 3). Finally, with respect to the other phenols and the total phenolic content, no differences were found between T0 and T21 for any of the K treatments (Table 3).

With respect to Put in all the K treatments, the concentration rose at T21 (5 mM KCl: $p<0.01$; 10 mM KCl: $p<0.001$; 15 mM KCl: $p<0.001$, Figure 5A). Also, Spd presented significant differences in the treatments of 5 and 10 mM KCl, in which cold storage raised the concentration of this polyamine

Table 3. Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4 °C over: content of phenolic compounds in cherry tomato fruits.

KCl (mM)	Days	Hydroxycinnamic acids and derivatives (mg g ⁻¹ DW)	Flavonoids and derivatives (mg g ⁻¹ DW)	Other phenols (mg g ⁻¹ DW)	Total phenols (mg g ⁻¹ DW)
5	0	2.30 ± 0.12	3.80 ± 0.19 ^a	2.73 ± 0.49	8.83 ± 0.79
	21	1.85 ± 0.21	2.76 ± 0.11 ^b	2.31 ± 0.62	6.92 ± 0.84
	<i>p</i> Value	NS	**	NS	NS
10	0	2.81 ± 0.24	3.68 ± 0.41	4.50 ± 0.79	10.99 ± 1.43
	21	2.50 ± 0.19	3.32 ± 0.12	4.18 ± 0.81	10.00 ± 0.88
	<i>p</i> Value	NS	NS	NS	NS
15	0	2.40 ± 0.10	3.15 ± 0.14 ^b	5.66 ± 1.19	11.21 ± 1.15
	21	2.30 ± 0.17	3.55 ± 0.52 ^a	4.28 ± 1.25	10.13 ± 1.92
	<i>p</i> Value	NS	*	NS	NS

Notes: Values are mean ($n=9$) and differences between means were compared by Fisher's LSD test ($p=0.05$). Significance levels are represented by $p>0.05$; NS, not significant. Means followed by the same letter do not significantly differ; DW, dry weight.

* $p<0.05$, ** $p<0.01$.

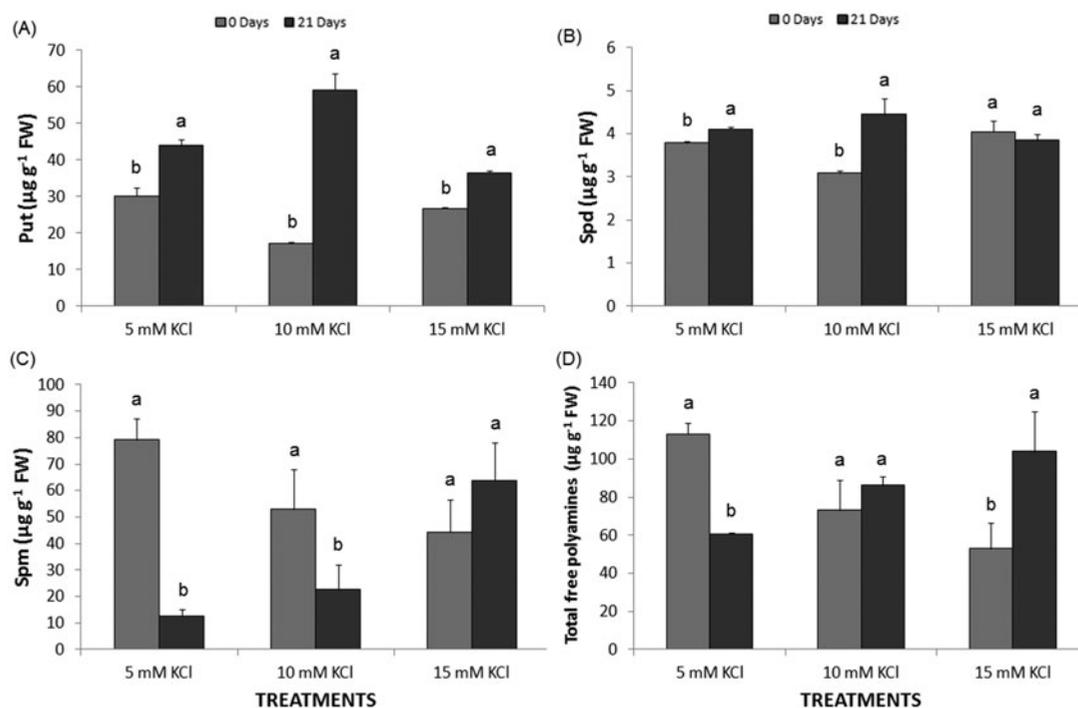


Figure 5. Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4 °C over PAs concentration in cherry tomato fruits. Values are means ($n=9$) and differences between means were compared by Fisher's LSD test ($p=0.05$). Means followed by the same letter do not significantly differ.

(5 mM KCl: $p<0.01$; 10 mM KCl: $p<0.05$, Figure 5B). On the other hand, the treatment of 15 mM KCl did not give rise to significant differences between T0 and T21. In relation to Spm, the rate of 5 and 10 mM KCl led to a decline at T21 ($p<0.001$ and $p<0.01$, respectively, Figure 5C), while the application of 15 mM KCl caused no significant differences between T0 and T21 (Figure 5C). Finally, regarding total free PAs, the application of 5 mM KCl lowered values at T21 compared with T0 ($p<0.001$, Figure 5D), whereas the application of 15 mM KCl raised the concentration of total free PAs at T21 with respect to T0 ($p<0.05$, Figure 5D). The treatment of 10 mM KCl showed no significant differences between T0 and T21 (Figure 5D).

Discussion

Fresh weight, percentage of lost fresh weight and K concentration

The postharvest water loss from fresh products is a major problem because it implies the weight loss, most products becoming

unsellable as fresh products after losing 3–10% of their weight (Ben-Yehoshua & Rodov, 2003). In this experiment, although the harvested cherry tomato fruits treated with the rate 15 mM KCl presented a lower FW (Figure 1), yield was not compromised, as these plants had a higher number of fruits (data not shown). Furthermore, in the present work, the fruits from the plants treated with 15 mM KCl at T21 presented a lower percentage of lost fresh weight (% LFW) (6%) while the treatments of 5 and 10 mM KCl showed an LFW of 17% and 18%, respectively (Figure 2), with the treatment of 15 mM KCl most improving the postharvest response. These results suggest that the application of the highest K rate in KCl form prevents weight and water loss during postharvest storage. In this context, Almeselmani et al. (2010) observed that an extra provision of K in the fertilizer applied to tomato plants can help to preserve fruits during postharvest storage.

Finally, with respect to the K concentrations at harvest, in the present experiment a proportional response was observed in relation to the KCl rate applied, and the highest concentration

was registered in the fruits from plants grown with the rate of 15 mM KCl (Figure 3). These results demonstrate the validity of the biofortification programme with K in tomato plants, since the consumption of fruits treated with 15 mM KCl provide added intake of this macronutrient, this being a potential benefit to human health, as demonstrated elsewhere (He & MacGregor, 2008).

Antioxidant capacity assays

For the determination of the antioxidant capacity in the most precise way possible, the use of several quantification methods is recommended. In our work, we used the methods TEAC, FRAP and reducing power to quantify the antioxidant activity. It was found that after 21 days of postharvest cold storage at 4 °C, for both the TEAC and FRAP tests, the treatment of 15 mM KCl caused increases of 60% and 32%, respectively (Table 1). However, when the treatment of 5 mM KCl was applied, the trend for the TEAC and FRAP tests was the opposite to the previous rate, with values falling in both tests at T21 with respect to T0 (Table 1). Also, it bears emphasizing that the reducing-power test presented the highest value in the treatment of 15 mM KCl at T21 (Table 1). All these results could indicate a benefit of applying the rate of 15 mM KCl regarding the antioxidant capacity of cherry tomato fruits during the period of cold-storage stress. Similar results were reported by Javanmardi & Kubota (2006), who found that tomato fruits in cold storage showed significantly increased antioxidant activity, which they related to phenolic compounds.

Concentration of pigments in cherry tomato fruits

A large group of phytonutrients are found in fruits and vegetables of the Mediterranean diet, among these the tomato, are carotenoids, including Lyc and β -carotene. Lyc represents roughly 80% of all carotenoids and has a high capacity to eliminate ROS, being one of the most characteristic phytonutrients in tomato fruit (Rao et al., 1998). Many studies have demonstrated a strong relation between the nutritional quality of tomato and its Lyc content (Rosales et al., 2006). Tomatoes contain moderate amounts of β -carotene, a potent dietary precursor of Vitamin A (Nguyen & Schwartz, 1999). It has been demonstrated that the highest amount of Lyc and β -carotene in the tomato are strong contributors to the major antioxidants of tomatoes (Toor et al., 2006). Finally, Lut is a compound belonging to the group of carotenoids with a high antioxidant capacity (Jahns & Holzwarth, 2012).

Anthocyanins are the most important group of water-soluble pigments in plants. Their biological interest stems from their antioxidant function and their effects reinforce certain compounds such as ascorbic acid (García-Alonso, 2004).

In our work, the majority of the pigments studied (β -carotene, Lut and anthocyanins) registered no significant differences between treatments (Table 2). Similar results have been reported by Rivera-Pastrana et al. (2010) in papaya fruits in which postharvest cold storage did not negatively influence the β -carotene concentration. Meanwhile, Rugkong et al. (2011), in a work on gene expression related to tomato fruit ripening in cold storage, did not find a decrease in Lut, either. In terms of Lyc, the ripening processes that are associated with the increase in their content were found to be retarded by low temperatures (Gómez et al., 2009) and, in this sense, Javanmardi & Kubota (2006), studying tomato fruits, found that the Lyc content in tomatoes stored at 12 °C and 5 °C decreased in comparison with those stored at room temperature. Rivera-Pastrana et al. (2010) also found a decline in Lyc after postharvest storage. On the contrary, our work indicates an increase in the Lyc content for

all the treatments after 21 days of cold storage, with a notable increase of 219% in the treatment of 15 mM KCl (Table 2). Given the fundamental importance of Lyc in the nutritional quality of the tomato, the application of 15 mM KCl could be beneficial to increase this pigment in cold storage, as K boosts the synthesis of Lyc, as demonstrated by Ramírez et al. (2012) in recently harvested tomato fruits.

Concentration of Vitamin C in cherry tomato fruits

Antioxidation mechanisms and the protection of metabolites in plants include a number of non-enzymatic antioxidants such as Vitamin C, and one of the main functions is to interrupt the uncontrolled oxidation cascades in some organelles and eliminate ROS. Antioxidant compounds are the essential determinants of nutritional quality in tomato fruits. Among the phytochemicals present in tomato fruits, the Vitamin C content is considered a key factor to determine the commercial value of the tomato yield, thanks to the nutritional benefit associated with its consumption (Frusciante et al., 2007). The antioxidant levels of a plant also constitute a good indicator of the redox state, which is indispensable for stress tolerance. In the present study, after the application of the biofortification programme with K in the form of KCl, the Vitamin C (Figure 4) presented the highest concentration for the treatments of 10 and 15 mM KCl at T21, this latter treatment being the one that best maintained the Vitamin C concentration. Lester et al. (2010), studying melon fruits (*Cucumis melo L.*), found that the fruits treated with the different K forms presented generally higher Vitamin C contents than did control fruits. The beneficial effects of the K supplement to the plant were presumably the result of a combination of improved photosynthetic assimilation of CO₂ by the leaves, greater translocation of assimilates from the leaves to the fruits, better leaf-fruit water relations, as well as more vigorous enzymatic activity and better availability of substrate for Vitamin C biosynthesis (Gross, 1991). Thus, an adequate biofortification programme with K has been associated with increased Vitamin C (Panda & Upadhyay, 2003), as confirmed by our results both at harvest and afterwards.

Vitamin C might also be involved in antioxidant capacity. Numerous studies in fruits and vegetables have demonstrated by different means a directly proportional relation between antioxidant capacity and the total phenol content and Vitamin C (Wang et al., 2012).

Concentration of phenols in cherry tomato fruits

Phenolics, ubiquitous secondary metabolites in plants, include a large group of biologically active components, from simple phenol molecules to polymeric structures with a molecular mass above 30 kDa (Dreosti, 2000). As demonstrated by Wang et al. (2012) in avocado fruits harvested on different dates of the year and kept in cold storage, the storage had positive effects on the accumulation and retention of compounds of nutritional interest, such as phenolics. In our study, although no significant differences were detected either at T0 or at T21 in the content of hydroxycinnamic acids and derivatives or of flavonoids and derivatives for any of the treatments applied (Table 3), the treatment of 5 mM KCl did register a 27% decrease in the content of flavonoids and derivatives during the postharvest period (Table 3). It bears highlighting that the treatment of 15 mM KCl best maintained the concentration of hydroxycinnamic acids and derivatives between T0 and T21, and for the flavonoids and derivatives it was the only treatment that led to an increase of 13% between T0 and T21 (Table 3). These results may be related to the high antioxidant capacity presented by the fruits of the plants grown at this treatment rate, as reflected by Wang et al. (2012)

in avocado fruits, and as has been found in numerous fruits and vegetables.

Concentration of free PAs in cherry tomato fruits

Other antioxidant compounds that in addition to participating in the responses or adaptation of different adverse environmental conditions, including cold stress (Alcázar et al., 2010), may also influence the nutritional quality of tomato fruits are PAs. However, the physiological significance of these compounds remains unclear, and it needs to be evaluated whether elevated polyamine levels were a result of stress-induced injury or a protective response to abiotic stress. Low-temperature conditioning has been shown to raise polyamine levels and stimulate *S*-adenosylmethionine decarboxylase activity (Wang, 1994). PAs may be associated with anionic components of the membrane such as phospholipids (Ballas et al., 1993) and this interaction serves to stabilize the bilayer surface and may thus retard membrane deterioration. PAs also have free-radical-scavenging properties (Drolet et al., 1986). Membrane protection from peroxidation by PAs could involve both their ability to interact with phospholipids and their antioxidant activity. Given the relationship between PAs and membrane protection, and between chilling injury (CI) and membrane damage, the possible connection between PAs and CI is of great interest. Zhang et al. (2013) have found an increase in the Put in tomato fruits treated with arginine and submitted to cold stress. These authors contend that in view of the protective function of PAs, especially Put against CI in many horticultural crops, it cannot be ruled out that the tolerance of fruits to refrigeration induced by the arginine treatment could be related to the increase in the Put concentrations found in those fruits. Similarly, accumulation of Put was also detected in chilling-injured peach and tomato fruit (Xu et al., 2005; Zhang et al., 2011). In our work, we noted an increase in Put between T0 and T21 for all the KCl treatments applied. However, the 15 mM KCl treatment presented the least increase at T21, of only 39% (Figure 5A), suggesting that the K applied in the form of KCl at this rate could boost the protection against cold stress of tomato fruits, reflecting a lower increase in Put. This hypothesis is confirmed by the data of Spd and Smp, PAs that are also important in the response to abiotic stress, such as that caused by CI (Alcázar et al., 2010). Specifically, in Spd, we found an increase in concentration at T21 only at the rate of 5 and 10 mM of KCl (Figure 5B), which were the treatments that most affected the cold stress (Figure 2). With respect to Spm, on the contrary, the treatments of 5 and 10 mM of KCl (Figure 5C) diminished its concentration at T21, while 15 mM of KCl showed no changes between T0 and T21 (Figure 5C), suggesting a strong protective role of this PA in our work. Similar results were reported by Zhang et al. (2013) for Spd and Spm in tomato fruits after cold storage ($2 \pm 1^\circ\text{C}$) for 28 days. These authors concluded that of the PAs studied, Put was predominant, followed by Spd and Spm. In this sense, they found that the Put concentration in fruits increased in response to cold stress, while by contrast the Spd and Spm concentrations fluctuated during the storage period. Finally, with respect to total free PAs (Figure 5D), significant differences were found for the treatment of 5 mM KCl, which presented a decline in these compounds of 46%, while in the treatment of 15 mM KCl, values rose 96.51%. Meanwhile, the treatment of 10 mM KCl showed no significant differences. In short, the greatest accumulation of free PAs in cherry tomato fruits treated with 15 mM of KCl after 21 days of postharvest cold storage could have a protective role against these stress conditions together with K (Figure 2).

Conclusions

In our work, we demonstrate how the application of a biofortification programme of K in the form of KCl at high application rates (15 mM) could constitute a beneficial strategy for improving the quality and antioxidant capacity of cherry tomato fruits to be stored cold before consumption. The treatment of 15 mM of KCl furthermore prevents weight and water loss in tomato cherry fruits during postharvest storage at 4°C , raises the K concentration and the antioxidant capacity by increasing the Lyc concentration, maintains the contents in Vitamin C, hydroxycinnamic acid and derivatives, and increases the flavonoids and derivatives, signifying that the consumption of these fruits could offer benefits for human health.

Declaration of interest

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