Assessment of Carbon Metabolism of Cherry Tomato Fruits: How Does Affects Potassium Biofortification During Crop Cycle at Postharvest Storage?

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Abstract

In the present work, we evaluate the implementation of a K biofortification programme that increases the dose of this macronutrient in the nutrient solution for growing tomatoes. The aim is assess whether this treatment improves the response of fruits to postharvest for 21 days at 4°C. Three K treatments were applied during the crop cycle of the plants: 5, 10, and 15 mM of KCl. The enzymes involved in carbon metabolism and sugar concentration were studied, both on the day of harvest and after 21 days of storage in cold room at 4°C. Similarly, enzymes related to the metabolism of organic acids as well as their concentration were studied. The application of this supplement, aside from increasing K concentration in the fruits, stimulated the sucrose (Suc) degradation by sucrose synthase (SuSy) activity while increasing fructose (Fruc) and glucose (Gluc) as well as raising malate accumulation induced by the activity of PEPC and MDH enzymes during storage of 21 days at 4°C. The KCl treatments could be related to changes in carbon metabolism and suggest a protective role against cold storage, improving the quality of tomato fruits.

Keywords: Carbon metabolism, postharvest, potassium biofortification, tomato fruit

Introduction

Tomato (*Solanum lycopersicum* L.) is an annual horticultural plant with a broad worldwide distribution and enormous economic value, while its high content in antioxidant compounds offers a number of health benefits to the consumer (Bilton and *al*, 2001). Tomatoes benefit human health by their high contents in phytonutrients such as lycopene, *β*-carotene, phenolic compounds, ascorbic acid, and essential nutrients, which can detoxify reactive oxygen species (ROS) and prevent oxidative changes in the human body (García-Closas and *al*, 2004). For all this, the content of these compounds is critical for assessing the quality of the fruit.

Other characteristics that determine tomato quality are related to the organoleptic properties, defined by a number of physico-chemical parameters that make the fruit satisfactory to the consumer. Numerous works have revealed that organoleptic parameters such as flavour are key components for selling the tomato (Gough and Hobson, 1990; Causse and *al*, 2002). The most important parameter for organoleptic quality of tomato fruits is flavour, produced mainly by a combination of sugars and organic acids, which implies that the level of concentration of both compounds can significantly affect consumer acceptance (Salles and *al*, 2003). Sugars and organic acids are the main metabolites in tomato fruits and constitute over 60% of the dry matter (Davies and Hobson, 1981). They not only contribute to the soluble solids but are also essential to the flavour intensity. Balanced and high levels of sugars and organic acids are important for the perception of a high-quality fruit (Bucheli and *al*, 1999; Lobit and al, 2003). Nevertheless, the choice of the tomato cultivar, the cultivation technique, and the postharvest tasks are applied primarily to reducing the loss of crop yield and do not give priority to the effect that these practices have on organoleptic characteristics.

Tomato, a very perishable climacteric fruit, requires the use of preservation technologies to slow the ripening process that occurs after harvest in order to maintain its quality and extend its shelf life. Tomato-fruit sugar content is the result of fruit physiological, metabolic, and genetic processes that are under developmental control (Baldet and *al*, 2006; Mounet and *al*, 2009; Wang and *al*, 2009). Generally, the tomato fruit accumulates sugars in the form of sucrose or reducing sugars (glucose and fructose), depending on the environmental conditions and on the growth phase of the plant (Gomez and *al*, 2009). Although sucrose is the main form of sugar translocated in tomato plants, glucose and fructose are generally present in quantities equal to or greater than sucrose in tomato fruits. Two pathways for sucrose breakdown provide the basis for a flexible system that can markedly affect the partitioning and metabolism of assimilated carbon in sink tissues (Geiger and *al*, 1996). Sucrose synthase located in the cytosol catalyses a

reversible reaction that degrades sucrose and UDP to UDP glucose and fructose, while acid (apoplast or vacuole) and neutral (cytosol or vacuole) invertase catalyse the hydrolysis of sucrose to glucose and fructose. These sucrolytic activities serve sink tissues in plants in the primary step of converting sucrose into sink-storage products, such as starch, proteins, and oil (Doehlert and Chourey, 1991; Balibrea and *al*, 1996; Rosales and *al*, 2007).

Organic acid content is regarded as one of the most important quality traits of fresh tomato. The acidic taste in tomato is attributed mainly to citric acid and malic acid, which corresponds to over 90% of the organic acids in tomato (Davies and Hobson, 1981; Schauer and *al*, 2005). However, the complexity of carboxylic acid metabolism and storage means that it is difficult to predict the best way to engineer altered carboxylic acid levels. Carboxylic acids constitute a major component of the osmotic potential that drives cell expansion through water uptake in the expansion phase of fruit growth (Liu and *al*, 2007). The concentrations of citrate and other carboxylic acids fall during this expansion phase as the cell contents are diluted (Baxter and *al*, 2005; Carrari and *al*, 2006). However, during the final stages of ripening, the level of citrate (and to a lesser extent other carboxylic acids) increases again such that it is present at high abundance in the ripe fruit. The complexity of this cycle is reflected in the range of enzymes that have been proposed to control fruit citrate and malate accumulation, including phosphoenolpyruvate carboxylase (Guillet and *al*, 2002), citrate synthase (Sadka and *al*, 2000), and malate dehydrogenase. The activity of these enzymes determines the synthesis and accumulation of these organic acids. The maximal catalytic activities of enzymes of the tricarboxylic acid cycle (TCA) generally decline during fruit development, and there are no pronounced changes in activities during the later stages of ripening that correlate with the rise in organic acid levels (Steinhauser and *al*, 2010).

Postharvest storage, handling and distribution of fruit at low temperatures is the most common and manageable approach to control ripening and subsequent deterioration and to maximize product shelf life. However, tomatoes, like many other subtropical fruits, are susceptible to develop symptoms of chilling injury, a physiological disorder caused by the exposure to low temperatures above the freezing point. The effect that postharvest cold has on the organoleptic properties (organic sugar-acid relation) in fruits has been investigated in the following works. González-Aguilar and *al* (2004), working with guava fruits and methyl jasmonate (MJ) treatments, stored red and white cultivars of guava fruits at 5ºC for up to 15 days plus two days at 20ºC and found that MJ treatments reduce the chilling injury (CI) index and increased sugar content. These authors concluded that MJ reduces chilling injury and activates the fruit-defence response. Later,

Gómez and *al* (2009), studying tomato fruits, showed that cold storage retarded the accumulation of simple sugars in fruits. In ripe red fruits, the fructose level increased both during storage at 20ºC and at 6ºC. With respect to glucose, its content increased at 20ºC, while it decreased significantly during the first 18 h of chilling treatment, recovering later to levels that were somewhat higher than initial values. In addition, these authors found that cold storage slowed the metabolism of organic acids in tomato fruits. Each organic acid showed a different pattern in normally ripening fruits. While tartaric, malic, ascorbic, and citric acid tended to show modest but significant decreases during ripening, the levels of succinic acid slowly built up. These trends seem not to have been affected by the chilling treatment, which slowed but did not halt the specific kinetics of most acids. Farneti and *al* (2010) after 5 days of 4ºC storage, tomatoes generally showed depressed sugar and boosted the acid content (especially in cocktail tomatoes) compared to 15ºC stored fruit, indicating a loss of sensorial quality at 4ºC. Sánchez-Bel and *al* (2012), in peppers, found that the ones submitted to cold stress presented higher concentrations of sucrose and fructose, while glucose was not appreciably affected. With regard to the organic acid concentration, citric acid declined in concentration while malic acid augmented. The comparative proteomic analysis between control and chilled fruits revealed that the main alterations induced by CI in bell pepper fruits are linked to redox homeostasis and carbohydrate metabolism. Finally, in this sense, Cao and *al* (2013) studying loquat fruits stored in a cold chamber, examined the relationship between chilling injury and sugar metabolism. Chilling-resistant 'Ninghaibai' fruit had higher levels of glucose and fructose and higher activities of sucrose-hydrolysing enzymes, such as sucrose synthase-cleavage and invertase, than did 'Dahongpao' (sensitive). Furthermore, the chilling-resistant 'Ninghaibai' fruit also showed higher activities of hexokinase and fructokinase, involved in hexose phosphorylation and sugar signal generation. These results suggest that the higher content of hexoses and activities of hexose sensors were likely part of the mechanism for chilling tolerance of loquat fruit.

One of the factors that can *a priori* improve cold tolerance in fruit is the presence of high potassium (K) concentrations. In this sense, Beringer and Trolldenier (1980) indicated that a high K concentration in cells can improve cold tolerance by lowering the osmotic potential Later works have revealed a positive correlation between K availability and cold-stress tolerance, revealing, furthermore, that suboptimal K concentrations intensify the negative effects of cold stress (Kafkafi, 1990; Yermiyahu and Kafkafi, 1990). In addition, significant yield losses and extensive leaf damage due to cold temperatures reportedly occurred under low K fertilization, while the effects were alleviated once the K supply was increased in a number of

vegetable crops such as potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* Mill.) (Hakerlerler and *al*, 1997). This could be due to the effect of K on sugar accumulation and the essential role that this physiological process plays in the resistance to chilling injury in plants. Javaria and *al* (2012) in their investigations with different rates of K fertilizer in relation to chemical and sensory attributes of tomato, observed that total solids, sugars, and titratable acidity increased significantly with higher rates of K. It was concluded that increasing the K concentrations improved the quality of tomato fruit parameters. Similar results were found by Han and *al* (2012) on applying K at different growth phases in tomato plants. These authors found an increase in soluble sugar, while soluble sugar, organic acid, and soluble solids were the highest among all the treatments (increases of 44.7%, 28.8% and 7.1% as compared with control treatment, respectively). Potash applied during the fruit-growth phase reached the highest ratio of sugar to acid.

In view of the above-mentioned functions of K in fruit quality, the aim of the present study was to examine how the application programme of biofortification with different application rates of K in the form of KCl influences the C metabolism of cherry tomato (*Solanum lycopersicum* L. cv. AsHiari) fruits and therefore their organoleptic properties after storage for 21 days in a cold chamber at 4ºC. In addition, whether the metabolism of sugars and organic acids is determinant in the resistance to chilling injury was evaluated.

Materials and Methodology
Plant material, growth conditions and Sampling ot tomato fruits

Seeds of cherry tomatoes (*Solanum lycopersicum* L. cv. AsHiari grafted on cv. Maxifort rootstock) were sown in flat trays (cell size 3 cm x 3 cm x 10 cm, 100 cells per tray) filled with 50% [v/v] perlite-peat mixture, and kept under greenhouse conditions for 5 weeks. Subsequently, the seedlings were transplanted to an experimental greenhouse at La Nacla Experimental Station (Motril), near the Granada coast in southern Spain (36º 45'N; 3º 30'W; altitude 130 m). The "*parral*" greenhouse consisted of three modules having a symmetrical gable roof with a slope of 27º and having an E-W longitudinal orientation (Soriano and *al*, 2004; Constán-Aguilar and *al*, 2015). 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 layers (inner, antidrop; and outer, long life) of low-density polyethylene. 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 3 tomato plants per sack and 2 stems per plant, the

planting scheme was 3.21 plants m^{-2}. Their arrangement in the greenhouse was in 12 rows with North-South orientation. The statistical design was on randomized block. Other growing conditions such as irrigation and fertilization followed (Soriano and *al*, 2004; Constán-Aguilar and *al*, 2015). The different treatments applied were: 5 mM KCl, 10 mM KCl y 15 mM KCl as liquid solution from the beginning to the end of the experiment.

The cherry tomato crop cycle lasted from October to May (230 days), with a complete truss of tomatoes (10-12 tomatoes per truss) maturing every 10 days. Cherry tomato fruits were sampled in February at 140 days after transplanting (DAT). Uniformly ripe healthy fruits, at the red-ripe stage, were harvested. Approximately 200 tomato 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.

Biomass parameters

From 180 tomatoes harvested from each treatment, 90 tomatoes were intended for analysis at harvest day (T0) being clustered in 9 replicates of 10 fruits. 5 tomato fruits from each replicate were weighed obtaining fresh weight (FW) and then were dried in a lyophilizer to determine the dry weight (DW) and percentage of dry matter (% DM). Another 5 tomato fruits from each replicate were homogenized, and these samples of fresh tissues were stored at -80ºC. For analyzing the fruits after 21 days of storage in a cold room at 4°C (T21), in the same way, 90 tomatoes were intended for analysis being clustered in 9 replicates of 10 fruits. 5 tomato fruits from each replicate were weighed obtaining FW at (T0) and stored 21 days in a cold room at 4°C, after this period were reweighed (T21), and then were dried in a lyophilizer to determine the DW and % of DM. Another 5 tomato fruits from each replicate were homogenized, and these samples of fresh tissues were stored at -80ºC. Samples of fresh and dry tissues from the cherry tomato fruits were used to analyse the parameters described below.

For the determination of the percentage of lost of fresh weight (% LFW), the following formula was used for each treatment:

% LFW= (FW T0- FW T21)*100/FW T0

To calculate percentage of dry matter (% DM) was followed the method proposed by Garg and Cheema (2011). Some tomatoes were weighed and then lyophilized for 72 h. After freeze drying, the samples are reweighed. The% DM was calculated as:

DM (%) = (B/A) x100

Where A is the total fresh weight of sample (g), and B, the total weight of dry sample (g).

Determination of K concentration

For the determination of K concentration, 0.2 g of dry cherry tomato fruits were ground and mineralised by wet digestion with H_2SO_4 12 M and H_2O_2 at 30% and P free, at a temperature of 275–300 ºC. After this step were added 20 mL of deionised H_2O, and finally K concentration was analysed by flame photometry (Wolf, 1982).

Sugars metabolism

For extraction of sucrose synthase (EC 2.4.1.13), a quantity of 0.2 g of tomato fruits were homogenized in 1 ml of buffer Heppes-HCl 50 mM, pH 7.5, which contained: magnesium chloride 0.5 mM, sodium EDTA 1 mM, DTT 2.5 mM, Triton X-100 at 0.05%, based on the method of Cheikh and Brenner (1992). The homogenate was filtered and centrifuged at 12000 g for 10 min. The determination of the SuSy activity followed the method of Kerr and *al* (1984). The reactions were started by the addition of an aliquot of 100 µL of the leaf extract previously centrifuged in a reaction buffer adapted from Cheikh and Brenner (1992), composed of sodium Heppes 50 mM (pH 7.5) that contained: MgCl 15 mM, fructose-6-phosphate (F-6-P) 25 mM, UDP-G 25 mM, Potassium fluoride (FK) 10 mM. The absorbance was measured at 520 nm against a standard sucrose curve.

Acid (EC 3.2.1.25) and neutral (EC 3.2.1.26) invertases were extracted in KH_2PO_4–K_2HPO_4 buffer (0.2 M, pH 7.0) and (20 mM) 2-mercaptoethanol, as described by Hubbard and *al* (1989). The extracts were assayed for acid invertase by addition of 30 µL enzyme preparation to test tubes containing 600 µL sodium acetate buffer (0.1 M, pH 4.5) and 200 µL sucrose (0.75 M) equilibrated in a 37ºC water bath. The reaction was allowed to proceed for 30 min and was stopped by addition of 1 mL dinitrosalicylic acid reagent. After that, being incubated for 5 min at 100 ° C and allowed to cool to room temperature. Finally was added 1 mL of Rochelle salt at 40%. The absorbance was measured at 575 nm against a standard glucose curve. Neutral invertase activity was determined as described above, except that Na-acetate buffer (0.1 M, pH 4.5) was substituted for K_2HPO_4-citrate buffer (0.1 M, pH 7.0) (Hubbard and *al*, 1989).

Organic acids metabolism

Extracts for measuring enzyme activities tomato fruit (0.2 g) was homogenized with 1 ml of extraction buffer containing 30 mM sorbitol, 1% bovine serum albumin (BSA) and 1% polyvinylpyrrolidone (PVP) in 100 mM N-2-hydroxyethylpiperazine- N%-2-ethanesulphonic acid (HEPES)-KOH, pH 8.0. The slurry was centrifuged for 15 min at 10.000 g and 4°C, and the supernatant was collected and analysed immediately. The activities of all enzymes were analysed in 1 ml (final volume) of the media indicated

below. Malate dehydrogenase (MDH; EC 1.1.1.37) activity was determined with oxalacetate as substrate (Dannel and *al*, 1995) by measuring the decrease in absorbance at 340 nm due to the enzymatic oxidation of NADH. The reaction was carried out with 70 µL of extract in 0.1 mM NADH, 0.4 mM oxalacetate and 46.5 mM Tris-HCl, pH 9.5. Citrate synthase (CS; EC 4.1.3.7) was assayed spectrophotometrically according to Srere (1967) by monitoring the reduction of acetyl coenzyme A (CoA) to CoA with 5-5%-dithio-bis-2-nitrobenzoic acid (DTNB) at 412 nm. The reaction was carried out with 50 ml of extract in 0.1 mM DTNB, 0.36 mM acetyl CoA, 0.5 mM oxalacetate and 100 mM Tris- HCl, pH 8.1. Phosphoenol pyruvate carboxilase (PEPC; EC 4.1.1.31) activity was measured in a coupled enzymatic assay with MDH according to López-Millan and al (2001) with 70 µL of extract in 2 mM phosphoenol pyruvate (PEP), 10 mM $NaHCO_3$, 5 mM $MgCl_2$, 0.16 mM NADH and 100 mM N,N-bis[2-hydroxyethyl]glycine (Bicine)-HCl, pH 8.5.

Sugars and organic acids content

Hexose (glucose and fructose) and sucrose contents were extracted and quantified using a kit (Roche Biopharm, St Didier au Mont d'Or, France) based on enzyme-linked formation of nicotinamide adenine dinucleotide phosphate (NADPH).

The determination of organic acids was based on the method of Scherer and *al* (2012) with slight modifications and was performed using HPLC with a DAD UV-visible detector (Agilent Technologies, USA) under the following conditions: Phenomenex reverse-phase column, 250×4.6mm i.d., 5 µm, Li-Chrospher 100 RP-18, with a 4×4mm i.d. guard column of the same material (Luna, Phenomenex, Utrecht, Belgium). About 0.2 g of freeze-dried tomato samples were homogenized with H_2O milliQ. The resulting mixture was centrifuged for 400 g 2 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 organic acids was carried out using the same equipment as described above. Samples were injected into an ACE 5C18 column, 250 x 4.6mm (HICHROM) operating at 25ºC. A single mobile phase consisting of 0.01M of KH_2PO_4 (pH 2.6) at 0.5 ml/min was used. The elution was monitored at 210 nm. Malic and citric acid was used as a standard (SIGMA-ALDRICH), eluting at 7.27 min and 10.57 min respectively.

Sweetness index

The sweetness index of fruits, an estimate of total sweetness perception, was calculated, based on the amount and sweetness properties of individual carbohydrates (Keutgen and Pawelzik, 2008). The contribution of

each carbohydrate was calculated, based on the fact that fructose is 2.30 and sucrose 1.35 times sweeter than glucose and, hence, the sweetness index was calculated as (1.00 [glucose])+(2.30 [fructose])+(1.35 [sucrose]).

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) 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 and Discussion
Biomass parameters and K concentration

The postharvest water loss from fresh products is a major problem because it provokes the weight loss, most products becoming unsellable as fresh products after losing 3-10% of their weight (Ben-Yehoshua and Rodov, 2003). In all the treatments, after 21 days of storage in a cold chamber, the FW decreased (Figure 1), although with the treatment 15 mM KCl the loss was less than in the rest of the treatments, with only 6% with respect to T0 ($P<0.001$, Figure 1). For the treatments 5 and 10 mM KCl the loss of FW at T21 was 17 and 18%, respectively, in relation to T0. In the present work, the fruits harvested from the plants treated with 15 mM KCl at T21 presented a lower percentage of LFW (6%) (Figure 2) while the treatments 5 and 10 mM KCl showed a LFW percentage of 17% and 18%, respectively (Figure 2), the treatment 15 mM KCl improving the postharvest response ($P<0.001$, Figure 2). 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 and *al* (2010) observed that an extra provision of K in the fertilization of tomato plants can help to preserve fruits during storage.

Figure 1: Effect of KCl treatments at the day of harvest over FW in cherry tomato fruits. Values are means (*n*=9) and differences between means were compared by Fisher´s least-significant difference test of (LSD, *p*=0.05). Means followed by the same letter do not differ significantly.
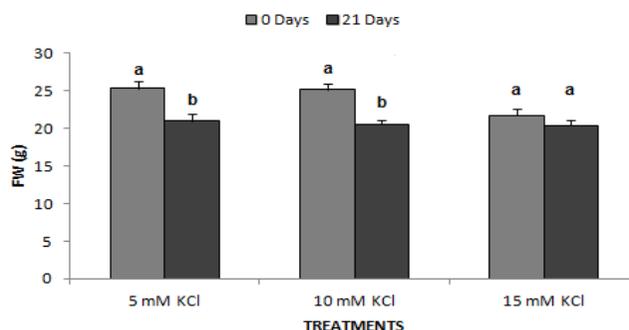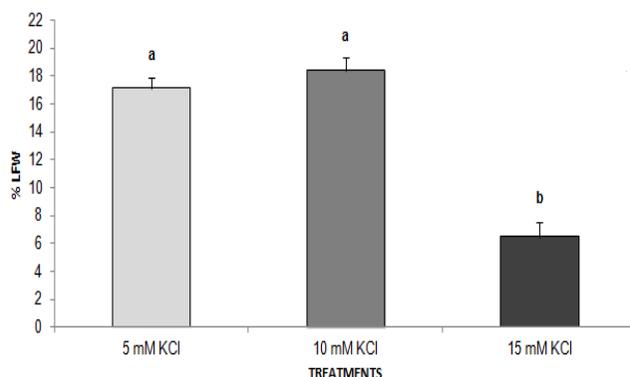
Figure 2: Effect of KCl treatments at the day of harvest over % LFW in cherry tomato fruits. Values are means (*n*=9) and differences between means were compared by Fisher´s least-significant difference test of (LSD, *p*=0.05). Means followed by the same letter do not differ significantly.

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 (*P*<0.001, 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 (He and MacGregor, 2008).

Figure 3: Effect of KCl treatments at the day of harvest over concentration of K cherry tomato fruits. Values are means (*n*=9) and differences between means were compared by Fisher´s least-significant difference test of (LSD, *p*=0.05). Means followed by the same letter do not differ significantly.
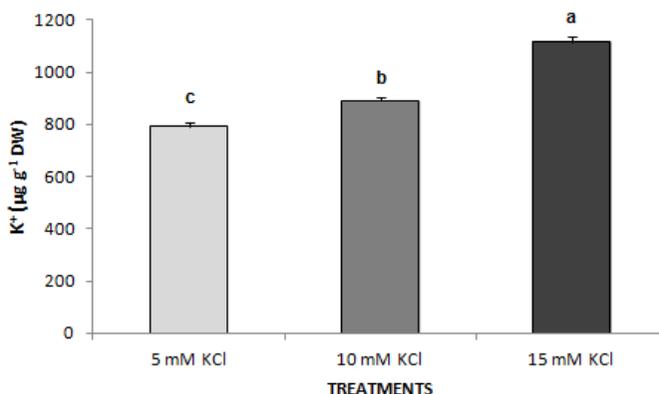
Sugar metabolism

Tomato fruit sugar content is the outcome of fruit physiological, metabolic, and genetic processes that are under developmental control (Ho and Hewitt, 1986; Baldet and *al*, 2006; Mounet and *al,* 2009; Wang and *al,*

2009). Sugar production begins with leaf photosynthesis, the product of which is translocated to developing fruits. Although Suc is the main form of sugar translocated in tomato plants, Glu and Fruc are present generally in higher quantities than sucrose in tomato fruits. Among the factors that can alter the concentration of these sugars in fruits, as mentioned in the introduction, K availability and cold storage are notable. In our work, we found that the Suc concentration in cherry tomato fruits was not altered either by K treatments or by storage for 21 days at 4ºC (Table I). On the contrary, the Gluc and Fruc concentrations increased more significantly after 21 days of storage at 4ºC with the treatments 10 and 15 mM de KCl, with no appreciable variations with respect to the treatment 5 mM KCl (Table I).

Table I: Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4°C over: sugars in cherry tomato fruits

KCl (mM)	DAYS	SACAROSE (mg g^{-1} FW)	GLUCOSE (mg g^{-1} FW)	FRUCTOSE (mg g^{-1} FW)
5	0	2.20±0.18	0.96±0.13	1.83±0.13
	21	1.97±0.13	0.86±0.11	1.53±0.18
	P-value	NS	NS	NS
	LSD	0.57	0.41	0.47
10	0	1.65±0.16	0.78±0.16b	1.91±0.19b
	21	2.03±0.29	1.38±0.17a	2.33±0.15a
	P-value	NS	***	*
	LSD	0.71	0.49	0.20
15	0	1.75±0.19	0.69±0.11b	2.06±0.09b
	21	1.85±0.35	1.34±0.05a	2.33±0.05a
	P-value	NS	*	*
	LSD	0.89	0.26	0.12

Values are mean (*n*=9) and differences between means were compared by Fisher's least-significant difference test (LSD; *p*=0.05). Significance levels are represented by *p*>0.05, NS, not significant, *p <0.05, **p<0.01, ***p<0.001. Means followed by the same letter do not differ significantly. Fresh weight (FW).

Sucrose can be converted to hexose phosphate by the ATP-dependent invertase pathway or the sucrose synthase pathway (SuSy), depending on the pyrophosphate (PPi) (Plaxton, 1996). As reflected in Table II, the neutral and acidic invertase activities diminished at T21 with the 5 mM KCl treatment (Table II), while for treatments 10 and 15 mM KCl no variations in T21 were found with respect to T0 (Table II). With respect to the SuSy activity, its response was totally contrary to that of invertase, since its activity intensified only at T21 for the treatments 10 and 15 mM KCl (Table II). In short, according to our results, we can conclude that in our work the degradation of Suc to Gluc and Fruc was due to the activity of SuSy, which was induced during postharvest at 4ºC by the high treatments of KCl (10 and 15 mM).

One possible explanation for these results could be that the cold stress brings about ATP-depletion conditions (Atkin and *al*, 2000), which could explain the absence of participation of invertase activity in our work. The increase in Gluc and Fruc for 21 days of postharvest storage at 4ºC in the treatments 10 and 15 mM KCl (Table I) appears to confirm the possible role of these sugars in cold resistance in tomato fruits, since, for these treatments, and especially for treatment 15 mM KCl, the sugar concentration was directly correlated with the minimum reduction of fruit FW after 21 days of postharvest at 4ºC.

Finally, it bears highlighting two aspects that could also contribute to improving the resistance to chilling injury for the treatment 15 mM KCl: (i) the greater K concentration in fruits submitted to this treatment (Figure 3) could contribute, together with the accumulation of sugars (Table I), to a fall in the cellular osmotic potential and thereby to greater cold-stress resistance; and (ii) the greater Gluc accumulation in these fruits could act as a substrate for the synthesis of ascorbate, one of the early precursors in ascorbate biosynthesis (Cervilla and *al*, 2007), and thereby improve the antioxidant response to this type of stress, as shown Constán-Aguilar and *al* (2014a,b) in previous works.

Table II: Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4°C over: sucrose synthase, neutral and acid invertases in cherry tomato fruits

KCl (mM)	DAYS	NEUTRAL INVERTASE (mg Gluc min^{-1} mg^{-1} prot)	ACID INVERTASE (mg Gluc min^{-1} mg^{-1} prot)	SuSy (mg Fruc min^{-1} mg^{-1} prot)
5	0	35.37±2.33a	79.20±5.89a	49.43±5.88
	21	25.73±1.10b	55.33±4.89b	42.43±4.75
	P-value	**	*	NS
	LSD	7.03	16.68	9.56
10	0	35.56±1.13	89.68±1.79	34.69±6.38b
	21	36.69±1.49	91.30±2.24	43.83±9.13ª
	P-value	NS	NS	*
	LSD	3.96	6.07	7.97
15	0	39.99±0.58	100.22±2.27	52.11±7.68b
	21	36.58±0.40	85.75±1.52	55.48±3.83ª
	P-value	NS	NS	*
	LSD	3.49	15.78	2.91

Values are mean (*n*=9) and differences between means were compared by Fisher's least-significant difference test (LSD; *p*=0.05). Significance levels are represented by *p*>0.05, NS, not significant, **p*<0.05, ***p*< 0.01, ****p*<0.001. Means followed by the same letter do not differ significantly.

Organic acid metabolism

The TCA cycle connects glycolysis to amino acid biosynthesis and is important in the regulation of respiration and energy generation by producing ATP and NADH. In fruit, the TCA cycle is involved in organic acid biosynthesis, but little information is available on the dynamics of tricarboxylic acid cycle (TCA)-related metabolism.

With respect to the organic acids malate and citrate, Figure 4A ($P<0.05$) shows how with the treatment 5 mM KCl, 21 days postharvest at 4ºC, malate significantly diminished. On the contrary, the application of 10 and 15 mM of KCl increased this organic acid at T21 with respect to T0 (Figure 4A). Citric acid, independently of the K treatment applied during storage for 21 days at 4ºC, prompted a significant decline in this organic acid (Figure 4B, $P<0.05$).

The response in our work of the different concentrations in cherry tomato fruit of malic and citric acid can be explained by the activities of PEPC, MDH, and CS. Table III shows that, due to the PEPC activity, cold storage for 21 days resulted in a decline in the activity with the treatment 5 mM KCl (Table III), which, together with the increased MDH activity (Table III) at T21 indicated that the tricarboxylic acid cycle was functioning in the sense that MDH degrades malate to form oxaloacetate. This could have been accumulating, since the CS activity fell in this treatment (Table III). In short, the behaviour of these enzymes would explain the decline in the treatment 5 mM de KCl of the malic and citric acid concentrations at T21 (Figures 4A and 4B). However, with the treatments 10 and 15 mM of KCl, high PEPC and MDH activity at T21 (Table III) would explain why malate increased, since MDH would be converted oxaloacetate generated from PEPC into malate. Therefore, in these treatments, the synthesis of this organic acid could be occurring by the anaplerotic pathway. The accumulation of malate in the treatments 10 and 15 mM at T21 could also be explained by the behaviour of the CS activity in these treatments, since in none of them significantly stimulated this enzymatic activity at T21 (Table III).

Figure 4: Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4°C over: malic acid and citric acid in cherry tomato fruits. Values are means (*n*=9) and differences between means were compared by Fisher´s least-significant difference test of (LSD, *p*=0.05). Means followed by the same letter do not differ significantly.
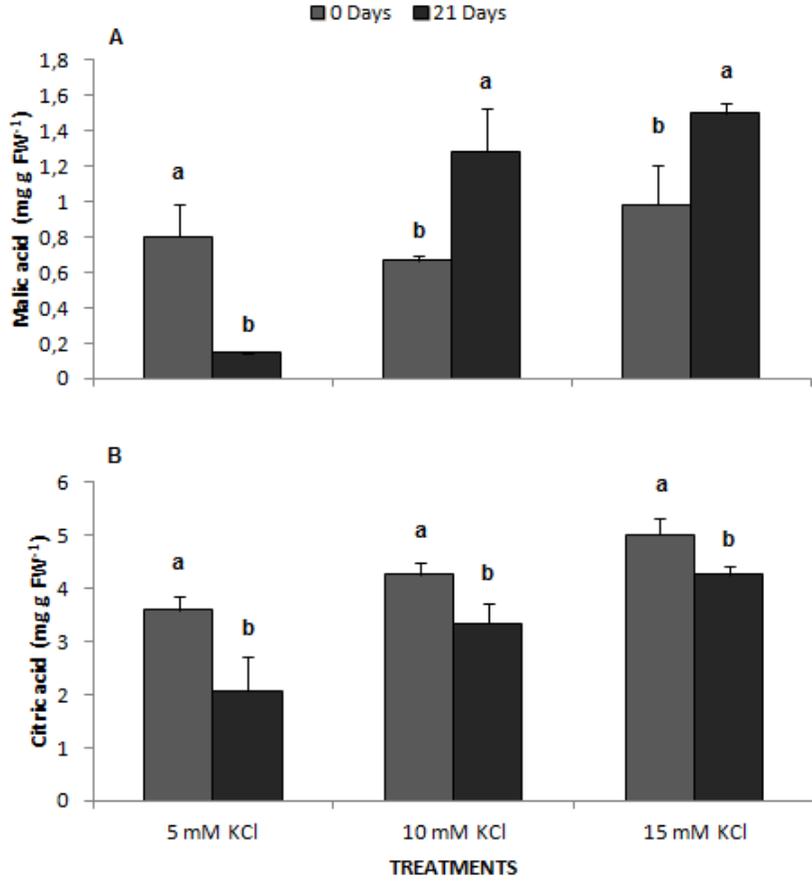
Table III: Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4°C over: PEPC, MDH, CS in cherry tomato fruits

KCl (mM)	DAYS	PEPC (ΔAbs hour^{-1} mg^{-1} prot)	MDH (ΔAbs hour^{-1} mg^{-1} prot)	CS (ΔAbs hour^{-1} mg^{-1} prot)
5	0	1.61±0.20a	1.07±0.20b	0.63±0.13a
	21	0.12±0.03b	3.07±0.18a	0.30±0.04b
	P-value	**	***	**
	LSD	0.71	0.74	0.15
10	0	1.21±0.08b	1.81±0.50b	0.95±0.04a
	21	2.97±0.67a	3.41±0.03a	0.73±0.00b
	P-value	**	*	**
	LSD	0.57	1.39	0.12
15	0	0.34±0.22b	4.26±0.62b	0.81±0.21
	21	1.06±0.04a	8.64±1.13a	1.12±0.33
	P-value	*	*	NS
	LSD	0.61	3.57	1.09

Values are mean (*n*=9) and differences between means were compared by Fisher's least-significant difference test (LSD; *p*=0.05). Significance levels are represented by *p*>0.05, NS, not significant, **p*<0.05, ***p*<0.01, ****p*<0.001. Means followed by the same letter do not differ significantly.

Sweetness index

The sweetness index is a parameter frequently used in many fruits to describe their flavour and commercial acceptability (Keutgen and Pawelzik, 2008). In our work, with regard to the sugar:acid ratio, the postharvest cold storage for 21 days for the 5 mM KCl treatment diminished this index with respect to that of T0 (*P*<0.05, Figure 5). On the contrary, the treatments 10 and 15 mM KCl, after the cold-storage period showed a rise in this index at T21 (*P*<0.05, Figure 5), indicating that the treatments K increased the sugar involved in the determination of this index after the postharvest, and therefore improved the likelihood of customer acceptance.

Figure 5: Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4°C over: Sweetness index in cherry tomato fruits. Values are means (*n*=9) and differences between means were compared by Fisher´s least-significant difference test of (LSD, *p*=0.05). Means followed by the same letter do not differ significantly.
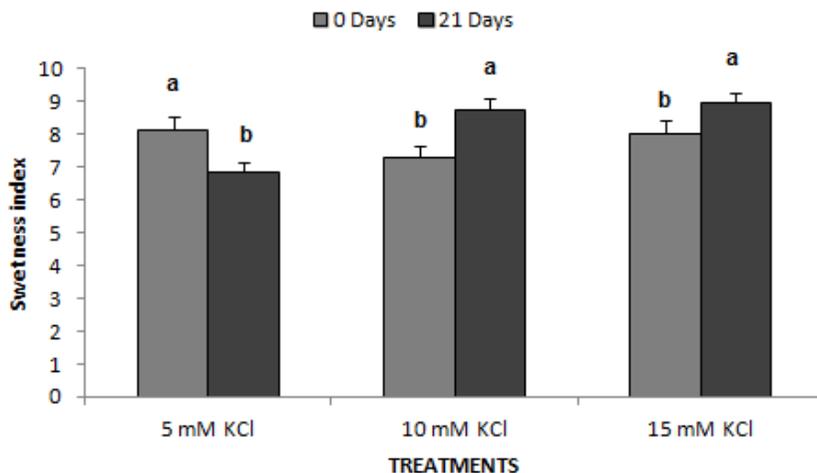
Conclusion

Application of a biofortification programme with K, increasing the application rates of this nutrient in the nutrient solution during tomato cultivation, improved the fruit response to postharvest storage at 4ºC for 21 days. This improved response was found in our work at 10 mM and specifically with the rate of 15 mM of KCl, and this could be related to changes in the carbon metabolism. Thus, the application of high amounts of K, in addition to increasing the concentration of this nutrient in fruits, stimulated the Suc degradation by SuSy activity, raising the levels of Glu and Fruc and inducing the accumulation of malate by the activity of the enzymes PEPC and MDH during storage for 21 days at 4ºC. Therefore, in our work the accumulation of Glu, Fruc, and malate could explain the protective role, during cold storage, of the treatment 15 mM of KCl, showing the minimum reduction of the % LFW. Finally, it bears indicating that the implementation of biofortification programmes with high rates of K application (in our case 10 and 15 mM of KCl) clearly improves the organoleptic quality of cherry tomato fruits during postharvest at 4ºC, with an increase in the sweetness index. In addition, the consumption of these fruits provides added intake of this element as a potential benefit to human health.

Acknowledgments

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