Journal of the Science of Food and Agriculture, Volume 99, Issue 5, 2019, pp. 2463-2472
DOI:10.1002/jsfa.9455

Effect of selenium enrichment on metabolism of tomato (*Solanum lycopersicum*) fruit during post-harvest ripening.

Running title: Ripening and post-harvest shelf-life of Se-enriched tomato fruit

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Abstract

BACKGROUND: Selenium (Se) enrichment of plants seems effective in enhancing the health-related properties of produce, and in delaying plant senescence and fruit ripening. The current study investigated the effects of Se on tomato fruit ripening. Tomato (*Solanum lycopersicum* L.) plants were grown in hydroponics with different Se-enriched nutrient solutions. Se, as sodium selenate, was added at rate of 0 mg L^{-1} (control), 1 mg L^{-1}, and 1.5 mg L^{-1}.

RESULTS: Selenium was absorbed by roots and translocated to leaves and fruit. Se enrichment did not significantly affect the qualitative parameters of fruit at commercial harvest, instead it delayed ripening by affecting specific ripening-related processes (respiration, ethylene production, color evolution) during postharvest. In the current experiment 100 g of tomato hydroponically grown with a 1.5 mg Se L^{-1} enriched solution provided a total of 23.7 µg Se. Selenium recommended daily intake is 60 µg for women and 70 µg for men, thus the daily consumption of 100 g of enriched tomato would not lead to Se toxicity, but would provide a good Se diet supplementation.

CONCLUSIONS: The cultivation of tomato plants in a Se-enriched solution appeared effective in producing tomato fruit with improved performances during storage and postharvest shelf life, and also with greater potential health-promoting properties.

Keywords: hydroponics; Se-enriched fruit; storage; shelf-life

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record.
Please cite this article as doi: 10.1002/jsfa.9455

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INTRODUCTION

Mineral nutrition is one of the main factors affecting several aspects of fruit and vegetable production: phytochemical content,[1] growth and development, susceptibility to biotic and abiotic stresses, pre- and postharvest performances.[2] The effects of essential micronutrients on fruit quality have been widely described,[3,4] whereas only few studies have reported the effects of non-essential micronutrients on the qualitative characteristics of fruit.[5]

Selenium (Se) is classified as a non-essential micronutrient for plants, but despite this, Se seems to delay plant senescence and fruit ripening, and therefore to decrease postharvest losses. These benefits are likely due to its ability in increasing antioxidant defenses as previously described.[6–8] In particular, Xue and colleagues[9] reported that selenium supplementation resulted in improved gluthatione peroxidase (GPXs; EC 1.11.1.9) and superoxide dismutase (SOD; EC 1.15.1.1) activities, together with a reduced decline in tocopherol content. In tomato fruit, Se concentration is negatively correlated with the content of some reactive oxygen species (ROS), such as hydrogen peroxide and superoxide, and positively correlated with glutathione peroxidase and glutathione reductase activity.[8,10] In tomato plants, treatments with Se induced positive effects on the oxidative status of fruit and delayed fruit ripening.[11–13]

Se has been proved to delay postharvest losses also affecting ethylene production in different produce, such as broccoli,[14] tomato[8,12] and in some leafy vegetables.[7] In peach and pear, foliar and fruit selenium spraying delayed the reduction of flesh firmness and the onset of fruit ripening, thus improving the fruit shelf-life.[15] Se plays a crucial role in human diet and animal feed. As a component of selenoaminoacids and selenoproteins, Se has been previously considered an essential trace element, a natural antioxidant, and an effective anticarcinogenic agent.[16,17] Thus, improving the dietary Se intake, without exceeding the toxic threshold, i.e. 400 µg of Se per day,[18] may have long-term health benefits.[19] The Se-enrichment of vegetables represents a safe way to increase its intake, since the organic compounds of Se contained in plants are more bioavailable than inorganic forms.[20] Vegetables grown in hydroponics can be fortified by adding Se to the nutrient solution, which allows the control of Se available for plants, avoiding hyperaccumulation.[7,12,21]

The present study aimed to better evaluate the efficacy of Se in delaying postharvest ripening and increasing fruit quality. The effects of different Se treatments (i.e, 0, 1 and 1.5 mg Se L^{-1}, added to the nutrient solution as sodium selenate) on fruit quality and postharvest ripening were investigated by measuring physiological (ethylene production, respiration rate, softening, color changes), and biochemical (non-structural carbohydrates and carotenes biosynthesis, chlorophylls degradation) parameters in fruit of Se-enriched tomato plants after harvest.

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MATERIALS AND METHODS

Plant material and experimental design

Tomato plants (*Solanum lycopersicum* L. cv. Red Bunch) were grown from February to June 2016, at the Department of Agriculture, Food and Environment of the University of Pisa, Italy (lat. 43° 40' N). Tomato seeds were sown, on 12th February, in 254-cell plug-trays filled with rock wool and vermiculite, and germinated in a growth chamber at 25°C. 14 days after sowing, seedlings were placed into rock wool blocks (75x75x65 mm) and transferred to a heated greenhouse. After 22 days, three rock wool blocks were placed on each rock wool slabs (1.000x150x75 mm) distributed on six benches. Thirty plants were placed on each bench.

The plants were grown vertically with a single stem at a density of three plants m^{-2}, and pollination was done by mechanical vibration of the flower clusters. Drip irrigation was performed two times per day for the first two weeks, then four times per day till the end of the experiment, according to the growth stage of tomato plant.

Sodium selenate was added once to the nutrient solution at a rate of 0 (control), 1, and 1.5 mg Se L^{-1} two weeks after transplanting (April 1). Two benches, for a total of 60 plants, were allocated to each treatment.

The nutrient solution contained 14 mM N-NO_3, 1 mM P-H_2PO_4, 2.77 mM S-SO_4, 4 mM Ca, 8 mM K, 1.5 mM Mg, 1 µM Cu, 15 µM Fe, 10 µM Mn, 1 µM Mo, 5 µM Zn. The pH and electrical conductivity (EC) values were 5.6 and 2.29 dS m^{-1} respectively, and were checked every 2 days. The nutrient solution was renewed every three weeks or whenever the EC was higher than 6 dS m^{-1}.

Climatic parameters were continuously monitored by a weather station located inside the glasshouse. The minimum, maximum and mean air temperatures were 11.2, 39.4 and 22.6 °C, respectively. The relative humidity was 58.7 %. The daily mean and the cumulative solar radiation were 8.7 and 743 MJ m^{-2} respectively.

Two different fruit collections were performed in order to evaluate the effects of Se enrichment on tomato fruit quality (first collection) and on postharvest ripening (second collection). Ten plants per treatment were randomly selected for each harvest.

Lateral shoots and the leaves below the bottom of most-truss with ripening fruit were regularly removed.

Tomatoes were collected at commercial maturity when 50 % of fruit was at the red ripe stage (i.e. 71, 82 and 85 days after transplanting for the first, second and third truss, respectively) to evaluate the effects of Se treatments on tomato quality. The red fruit were analyzed for Se content.

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Qualitative analyses, oxidative stress markers analyses and antioxidant enzyme activity were carried out on fruit of the first truss of 6 plants for each treatment.

16 fruit from each of the first three trusses were harvested at colour break stage (i.e. 63, 74 and 85 days after transplanting for the first, second and third truss, respectively) to evaluate the effects of Se on post-harvest ripening. Fruit of each truss from each treatment were kept separately and stored at 21°C and 70-75% RH. The following parameters were periodically measured during storage: visual evaluation of ripening, weight loss, fruit elasticity, ethylene production, respiration rate. In addition, non-structural carbohydrates and pigments contents were measured only in fruit of the first truss. Fresh (FW) and dry (DW) weights of red fruit were recorded at each harvest.

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Fruit analyses

Se concentration

Total selenium concentration was determined in fruit samples oven-dried at 50 °C for 1 week. For each replicate, around 100 fruit were oven-dried and then ground in a mortar. 0.5 g of powder were taken for each replicate and mineralized with nitric and perchloric acids and reduced by hydrochloric acid, following Zasoski and Burau.[22] The digests were analyzed by hydride generation atomic absorption spectrophotometry (Varian VGA 77). [23]

Qualitative analysis

For each replicate, 20 fruit were grounded and 50 mL of tomato purée were used to conduct the qualitative analysis. Three replicates were made for each treatment. Soluble solid content (SSC) was directly determined in the fruit juice using a digital refractometer (model 53011, Turoni, Italy). Titratable acidity was determined using NaOH 0.1 N to the end point of pH 8.2 and expressed as citric acidity percentage. The taste index and maturity were calculated using the equation proposed by Navez et al.[24] starting from the Brix and the titratable acidity values as indicated in Hernández Suárez et al.[25]: maturity =°Brix/acidity; taste index = (°Brix/20 × acidity) + acidity.

Malondialdehyde (MDA) assay

For the measurement of malondialdehyde (MDA) content, fresh fruit material was extracted with ethanol. The absorbance of reaction mixture was measured at 600, 532 and 440 nm, and the results expressed as mmol of MDA equivalents g^{-1} FW.[26]

Antioxidant Enzymes activity

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For the measurement of Ascorbate Peroxidase (APX; EC 1.11.1.11), catalase (CAT; EC 1.11.1.7) and superoxide dismutase (SOD; EC 1.15.1.1) activity, 0.5 g of fresh material were extracted with Na-P buffer 66 mM pH 7 + EDTA 1 mM. APX, CAT and SOD activities were evaluated as described by Lyons et al. [27] measuring the extract absorbance at 290, 240 and 560 nm, respectively. Results were corrected for the relative extinction coefficient for APX and CAT, and expressed as µmol AsA (oxidized ascorbate) g^{-1} FW min^{-1} and as µmol H_2O_2 g^{-1} FW min^{-1}, respectively. SOD activity was expressed in unit (U). One unit is defined as the amount of SOD required to inhibit the rate of reduction of cytochrome C by 50 % at 30 °C.

For the analysis of glutathione peroxidase (GPXs; EC 1.11.1.9) activity, 1 g of fresh material was extracted with Na-P buffer 66 mM pH 7 and centrifuged at 1100 g for 10 minutes. The reaction mixture containing 0.2 mL of extract, 0.4 mL of GSH 0.3 mM, and 0.2 mL Na-P buffer 66 mM pH 7 was preincubated for 5 minutes at 25°C. After the preincubation, the reaction was started by the addition of 0.2 mL of H_2O_2 1.3 mM. After 10 minutes the reaction was stopped by the addition of 1 mL of trichloroacetic acid. Then, the samples were put in the ice for 30 minutes and, at the end, centrifuged at 100 g for 10 minutes. The assay mixture contained 0.24 mL of supernatant, 1.10 mL of buffer Na-P 0.32 M pH 7, 0.16 mL of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) 1 mM. The absorbance of the assay mixture was measured at 412 nm and the results expressed as µmoli GSH g^{-1} FW min^{-1}. For the calibration curve growing concentrations of glutathione (GSH) were used.[28]

Visual evaluation

The visual evaluation of fruit post-harvest ripening was performed 1, 4, 6, 8 and 10 days after harvest for the first truss, 1, 3, 8, 10, 12 and 15 days after harvest for the second truss, and 3, 6, 10 and 12 days after harvest for the third truss. Fruit were classified by comparing the individual color to the ripening stages reported in the "United States standards for grades of fresh tomatoes".[29] The number of fruit at each ripening stage was recorded and a ranking was used to evaluate the development of fruit colour.

At each ripening stage was assigned a growing score from breakers to red fruit.[29] The average ripening stage of each set of fruit was calculated according to the formula:

$$ripening\ stage = \frac{n.f.\ breakers \times 2 + n.f.\ turning \times 3 + n.f.\ pink \times 4 + n.f.\ light\ red \times 5 + n.f.\ red \times 6}{n.f.\ total}$$

Weight loss

6 fruit for each truss of each treatment were weighted at harvest, and then every 3 days during storage at 21°C and 70-75% RH for 15 days.

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Fruit elasticity

A Shore durometer type A was used to measure fruit elasticity 0, 4, 6, 8, 11 and 13 days after harvest for the first truss, 0, 2, 7, 9, 11 and 14 days after harvest for the second truss, and 0, 3, 6, 10 and 12 days after harvest for the third truss. The durometer measures the depth of an indentation in the material created by a given force on a standardized presser foot. There are several scales of durometers, used for materials with different properties. The scale used was the ASTM D2240 type A. However, the ASTM D2240-00 testing standard calls for a total of 12 scales, depending on the intended use. Each scale results in a value between 0 and 100, with higher values indicating a harder material.

Six fruit for each truss of all treatments were pressed at the opposite sides of their equatorial axes, and results were expressed as shore hardness. Shore values are internationally defined as resistance to a 2.4 mm diameter plunger to penetrate an intact fruit using a Turioni Durometer.[30]

Ethylene production and respiration rate

Ethylene production and respiration rate were measured 0, 3, 5, 7 and 9 days after harvest in fruit of the first truss, 0, 3, 5, 7, 9 and 11 days after harvest in the fruit of the second and third truss. During this period fruit were stored at 21°C. Four fruit for each truss of each treatment were placed in four 85 mL glass tubes (Pyrex, France) and closed with holed plastic screw caps supplied with caoutchouc rubber septa. Analysis was performed according to Malorgio et al.[7]

Non-structural carbohydrate content

Content of non-structural carbohydrates was measured in fruit of the first truss at the red stage. Four replicates were made for each treatment. Each replicate consisted of 6 fruits. Carbohydrates were extracted from ground samples as previously reported. [31] 150 mg of freeze-dried tomato samples were grinded, combined and mixed with 3 mL of 62.5:37.5 HPLC grade methanol:water (v/v). The samples were placed in a water bath at 55°C and shaked for 15 min. Thereafter, the samples were cooled, filtered through 0.2µm filter and stored at -40°C. 50 µL of the extract was diluted in 1 mL using HPLC grade water immediately prior the analysis. Non-structural carbohydrates were quantified using a Agilent 1200 series HPLC binary pump system (Agilent, Berks., UK) coupled with either an Agilent refractive index detector (RID) G1312A. Chromatography was carried out with a Phenomenex Rezex RCM monosaccharide Ca+2 (8%) 300x7.8 mm column fitted with a Phenomenex Carbo Ca+2 4x3 mm guard column. The mobile phase was HPLC grade water (filtered through a 0.4µm filter and degassed using He) at a flow rate of 0.6 mL min^{-1}. Temperature of the optical unit in the detector was set up at 35°C and the column at 80°C. The autosampler was

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cooled at 5°C. The concentrations of glucose, fructose and sucrose were calculated against a calibration curve prepared with authentic standards (Sigma-Aldrich, Dorset, UK) and the results were expressed on a fresh weight basis.

Pigments content

Pigments content was measured in fruit of the first truss at the red stage. Four replicates were made for each treatment. Each replicate consisted of 6 fruit. Pigments were extracted from 100 mg ground freeze-dried tomato fruit samples as previously reported [32] and quantified using an Agilent 1200 series HPLC binary pump system (Agilent, Berks., UK) coupled with either an Diode-Array Detection (DAD). Chromatography was carried out with a reverse phase column 250x4 mm i.d., 5 µm particle size. The concentrations of lycopene, β-carotene and chlorophylls were calculated by comparison against an external calibration curve prepared with authentic standards (Sigma-Aldrich, Dorset, UK) and the results expressed on a fresh weight basis.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) with Se treatments as variables. The means were separated using the least significance difference (LSD) ($P<0.05$). Statistical analysis was performed using Statgraphics Plus 5.1.

RESULTS AND DISCUSSION

Effects of selenium on tomato fruit quality at harvest

The addition of Se to the nutrient solution determined a significantly dose-dependent increase of Se concentration in tomato fruit of all trusses, and the average Se concentration decreased from the first to the second and third trusses (Table 1). The fruit Se concentration was in the range of 0.94-2.76 mg kg^{-1} DW when plants were treated with 1 mg Se L^{-1}, and 2.08-3.54 mg kg^{-1} DW when plants were added with 1.5 mg Se L^{-1}. These values are approximately three times lower than those reported by Pezzarossa et al.[12] which found a fruit Se concentration of 10.33 mg kg^{-1} DW in plants added with 1 mg Se L^{-1} as sodium selenate. These differences could be ascribed to the different growing period that in the experiment conducted by Pezzarossa et al.[12] lasted from the beginning of May to the end of July, whereas in the current experiment it lasted from February to June.

Foliar application of 1 mg Se L^{-1} to tomato plants prior to fruit development resulted in an increased fruit selenium content of 0.6 mg Se kg^{-1}.[33] This content is much lower compared to what reported in the current experiment suggesting that the addition of Se to the nutrient solution is likely more effective than foliar application to produce Se-enriched tomatoes.

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In this study the addition of selenium to the nutrient solution did not affect either plant yield or the qualitative parameters of tomato, i.e. fruit dry matter content, soluble solid content, titratable acidity, maturity and taste index (Table 2). This is in agreement with previous studies conducted on tomato plants grown under similar experimental conditions.[11,12] The herein reported mean value of taste index was about 1, indicating that the tomatoes were of good quality. Tomatoes are considered as having little taste when the taste index is lower than 0.7.[24]

Ascorbate peroxidase (APX) and Catalase (CAT) activities were lower in red fruit of Se-enriched plants, 1.9 and 1.5 fold respectively, compared to control fruit. Instead, a significantly higher superoxide dismutase (SOD) activity was detected in fruit of treated plants. No differences were detected in glutathione peroxidase (GPXs) activity between control and treated plants (Table 3). This was an unexpected result that may have occurred because we analysed tomato fruit at red ripe stage. After the climacteric pick, the effect of ethylene on the oxidative status of fruit might have covered possible effects of selenium.

As far as the increase of SOD activity is concerned, plant might have perceived the increase of selenium content as a mild stress. Selenium may increase the antioxidant enzyme activity of SOD and GPXs[10] and decrease various reactive oxygen species, such as H_2O_2 and superoxide, during fruit storage.[8] Selenium has proven effective in increasing the oxidative status and improving the content of reduced glutathione and ascorbate of tomato fruit, thus delaying fruit ripening.[8,13]

Effects of selenium on post-harvest ripening

Delayed post-harvest ripening was detected in tomatoes harvested from Se-treated plants following the visual evaluation of colour development (Fig. 1). The timing of the delay in fruit ripening varied across trusses, and was more noticeable in fruit derived from the second and third truss compared to the first. In general, fruit from the first truss showed a more rapid development than fruit from the second and third truss. In fact, fruit from the first truss reached the red ripe stage earlier (7 days after harvest) compared to fruit from the second and third truss (9 days after harvest) (data not shown). The effect of selenium in delaying fruit post-harvest ripening, which was less evident in fruit from the first truss, may have been attenuated by the faster ripening.

The delay in fruit ripening was also confirmed by physiological parameters. Indeed, the addition of selenium to the nutrient solution was effective in postponing (2 days) the ethylene climacteric peak in fruit from all trusses (Fig. 2), and resulted in a reduced respiration rate at the climacteric peak, especially in fruit from the second and third truss (Table 4), as previously reported.[12] Zhu et al.[8] found that, in tomato fruit, Se suppressed the transcription of *ACO1, ACS2* and *ACS4*, genes involved in ethylene biosynthesis, with beneficial effects in terms of prolonged commercial life.The

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effects of Se on the postharvest ripening were also evident by data on fruit elasticity (Fig.3). Fruit elasticity is inversely related to fruit softening. Thus, the higher values of fruit elasticity detected in treated fruit compared to control may indicate that the ripening-related loss of firmness was indeed retarded by Se. A similar result was reported by Pezzarossa et al.[15] in flesh firmness of Se-enriched peaches.

Interestingly, the addition of selenium to the nutrient solution, either at 1 and 1.5 mg Se L^{-1}, reduced the fruit weight loss during the post-harvest ripening, especially after 12 days of storage (Fig. 4). The reduced weight loss could be related to delayed ripening or be the result of Se-induced changes in the cuticle thickness and composition. Golob et al.[34] reported that wheat plants treated with selenium had thicker cuticles for both the upper and lower leaf surface. Thicker cuticles represent a barrier to the water movement from inside the tissue/organ to the external atmosphere. The lower weight loss that occurred in Se-fortified fruit compared to control may be ascribed to the increase in water retention due to the increased selenium content, as observed in olive trees[35] and in Zea mays.[36] This effect can be attributable not only to an increase in cuticle thickness, but also to a gene expression regulation.[37]

Se-enriched fruit showed also a delayed accumulation of reducing sugars (glucose and fructose) during the first week of postharvest phase (Table 5). Zhu and colleagues[33] reported that Se-enriched tomato exhibited higher reducing sugars contents compared to the control fruit at harvest. However, the data of the present work investigated the evolution of ripening during postharvest. Once the ripening fruit is detached from the plant, starch breakdown represents the only source for sugars (sucrose and reducing sugars) accumulation.[38] The herein reducing sugars trends in Se-enriched fruit might therefore relate with a slower (or postponed) starch degradation process due to Se-induced ripening delay. This hypothesis seemed supported also by the fruit elasticity data, which showed a quicker softening in control fruit (Fig. 3).

As above reported, the visual evaluation of fruit after harvest indicated a different colour development in Se-treated fruit (Fig. 1). Indeed, the analysis of pigments content confirmed this data: lycopene and beta-carotene accumulations were delayed in Se-treated fruit through postharvest, even if no differences were detected between Se-treated and control fruit at the end of the experiment (Table 6). In addition, chlorophylls degradation rates were slower in Se-enriched fruit compared to control (Table 6).

In the current experiment, the activity of antioxidant enzymes and the lipid peroxidation level detected in red fruit were in agreement with the delayed ripening of Se-treated fruit. During ripening, the antioxidant capacity decreases, and the lipid peroxidation consistently increases. [39] The antioxidant enzymes behave differently during fruit ripening: SOD activity decreases whereas

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CAT [40] and APX [41] activity increase. Therefore, a lower lipid peroxidation, CAT and APX activities and a higher SOD activity, as detected in Se-treated fruit, may indicate a delay in fruit ripening that is not possible to discern only from the fruit color, since from the outside all fruit were at the red ripe stage. Se has an antioxidant function as a cofactor of glutathione peroxidase. In the present experiment differences in GPXs activity between treatments were not detected, probably because the Se concentration in fruit was too low. In fact, even though in plants Se has not an antioxidant function as cofactor of glutathione peroxidase as in mammalians, Castillo-Godina[42] found an increase in GPXs activity in tomato fruit treated with 5 mg Se L^{-1}, but only when fruit accumulated about 30 mg Se kg^{-1}.

The reduction in respiration rate has been already detected in tomato fruit treated with Se,[8] and can induce a delay of fruit ripening, as found in banana by Srivastava and Dwivedi.[43] A lower respiration during storage has a positive effect in improving post-harvest shelf-life of fruit.

The evaluation of tomato fruit ripening on the basis of the fruit color showed a slower color change in fruit of plants treated with Se compared to the control. This was associated with a delayed lycopene and b-carotene synthesis and chlorophylls degradation during postharvest. This could be the result of a delayed ripening, since lycopene and b-carotene concentrations increase, whereas the concentration of chlorophylls decreases during tomato ripening.[44,45]

As the final issue, the effect of the consumption of Se-enriched tomatoes on health was taken into consideration. Since the estimated values for Se daily intake are 60 µg for women and 70 µg for men,[46] the consumption of 100 g of the Se-enriched tomatoes obtained in this experiment, with an average total Se content of 23.7 µg, could provide a good Se diet supplementation. The consumption of 200 g of fresh Se-enriched tomato could provide the recommended daily intake of Se. On the basis of the results obtained in the present experiment, and in accordance to previous studies,[12] the hydroponic cultivation of tomato plants using a nutrient solution enriched in Se could represent a viable way to produce Se-enriched tomatoes.

Conclusions

The results of the present experiment support the hypothesis that Se affects specific ripening-related processes and may have a positive effect in delaying fruit ripening, thus positively affecting the postharvest shelf life of tomatoes. Selenium-treated fruit showed in fact lower respiration rate and ethylene production that slowed the postharvest ripening process. This was associated with a delayed lycopene and b-carotene synthesis, and chlorophylls degradation. The consumption of Se-enriched fruit may contribute to the daily supplementation of selenium, thus improving human

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health. Further studies are needed to fully understand how Se affects the molecular and biochemical processes involved in fruit ripening and post-harvest storage, in order to optimize the Se-enrichment technique and the combination with post-harvest storage conditions.

Acknowledgments
Authors wish to thank Marco Landi, Department of Agriculture, Food and Environment at University of Pisa, for his helpful comments.

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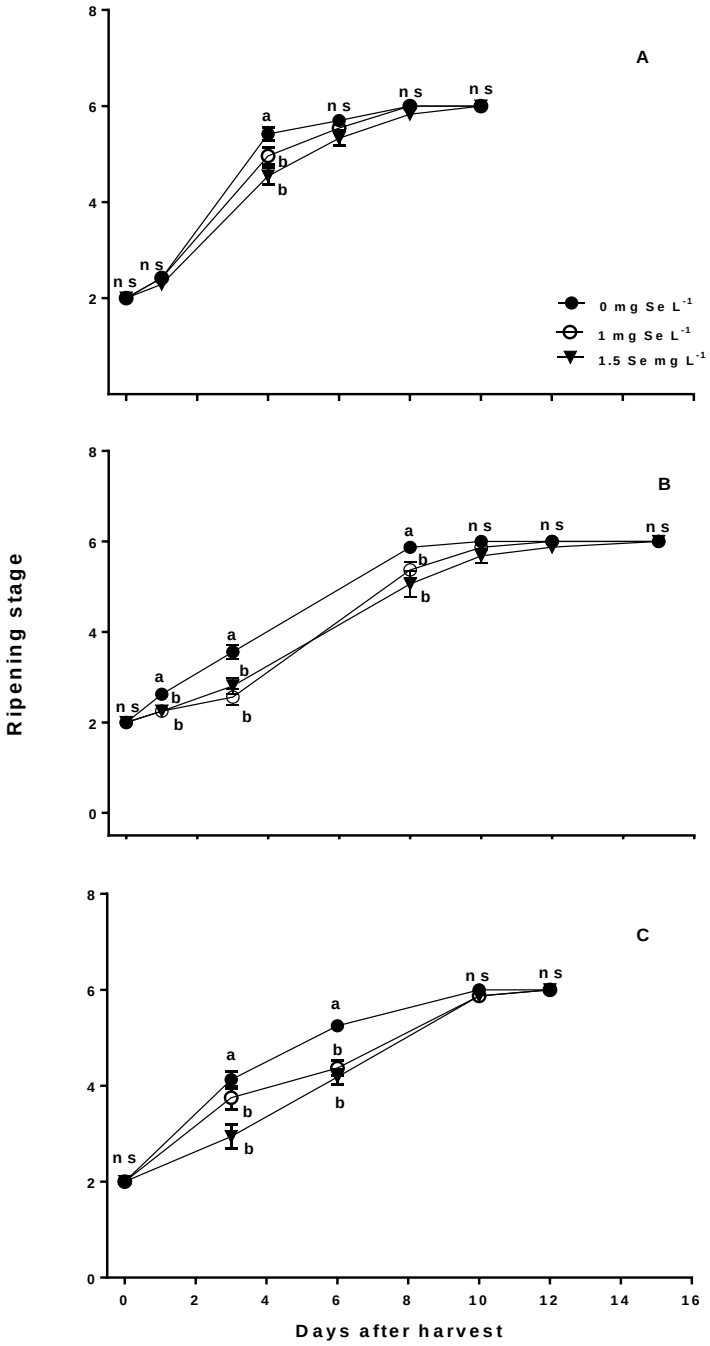


Figure 1. Post-harvest ripening process in fruit of the first (A), second (B) and third (C) truss of tomato plants (cv. Red Bunch) grown in nutrient solution supplied with 0, 1 and 1.5 mg Se L^{-1}, on the basis of visual color appearance. Data are means ± SE (n=4). Values with the same letter are not statistically different for p≤0.05%; ns=not significant

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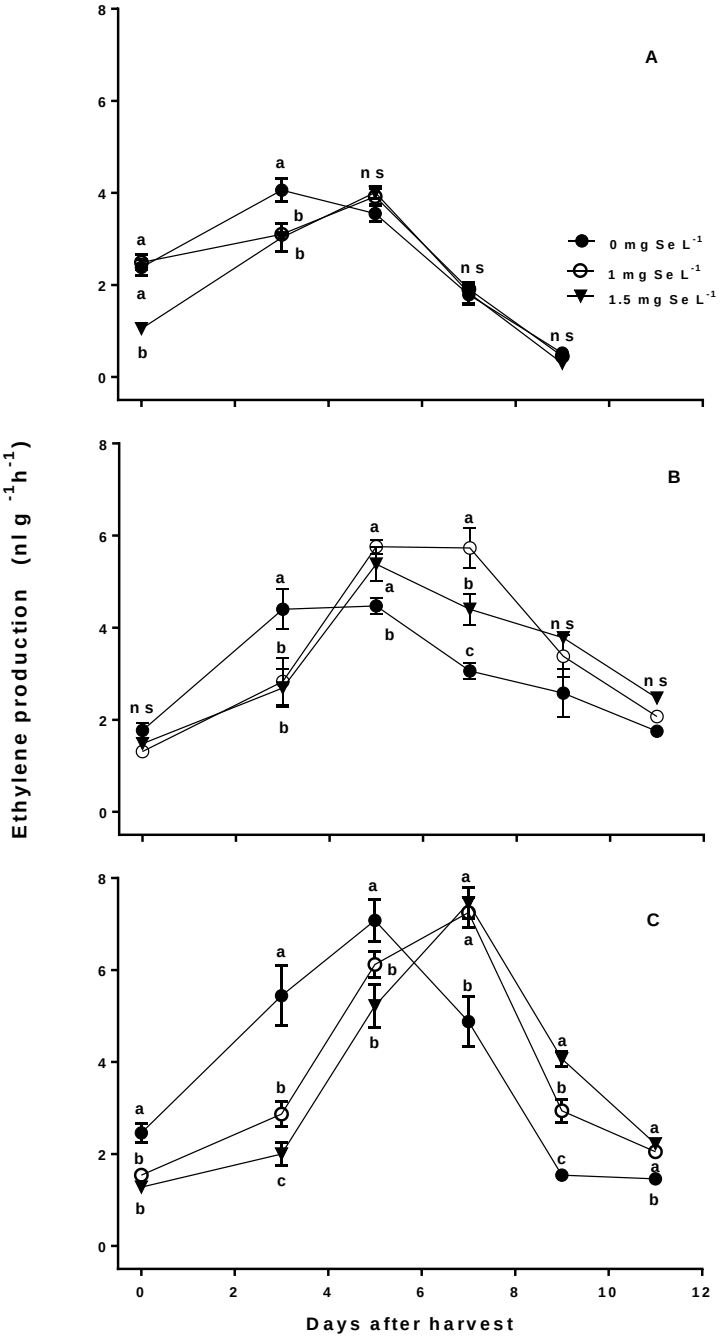


Figure 2. Ethylene production during post-harvest ripening in fruit of the first (A), second (B) and third (C) truss in tomato plants (cv. Red Bunch) grown in nutrient solution supplied with 0, 1 and 1.5 mg Se L^{-1}. Data are means ± SE (n=4). Values with the same letter are not statistically different for p≤0.05%; ns=not significant.

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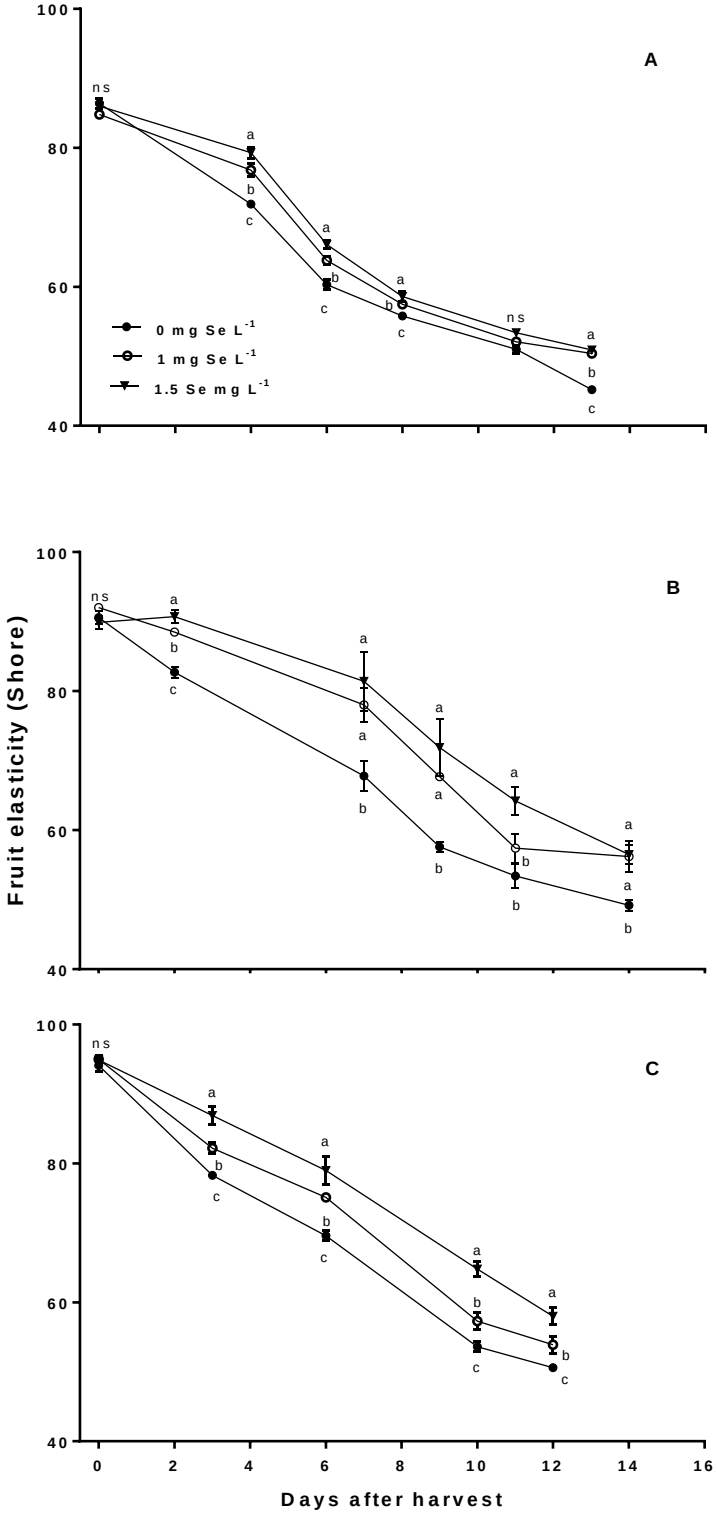


Figure 3. Fruit elasticity (Shore) during post-harvest ripening of fruit from the first (A), second (B) and third (C) truss of tomato plants (cv. Red Bunch) grown in nutrient solution supplied with 0, 1 and 1.5 mg Se L^{-1}. Data are means ± SE (n=6). Values with the same letter are not statistically different for p≤0.05%; ns=not significant.

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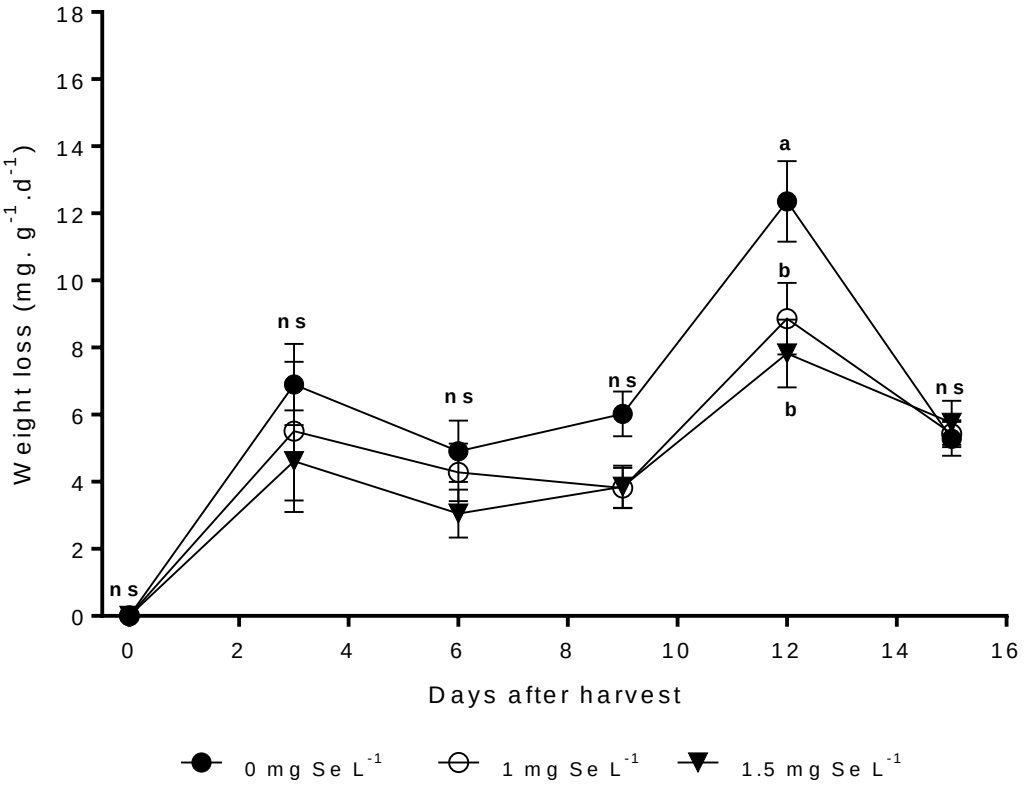


Figure 4. Post-harvest weight loss, expressed as mg/g per day, in fruit of tomato plants (cv. Red Bunch) grown in nutrient solution supplied with 0, 1 and 1.5 mg Se L^{-1} and stored for 15 days. Data are the means of the three trusses ± SE (n=18). Values with the same letter are not statistically different for p≤0.05%; ns=not significant.

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Table 1
Se concentration in red fruit of tomato plants (cv. Red Bunch) grown in nutrient solution supplied with 0, 1 and 1.5 mg Se L^{-1}.

Se (mg L^{-1})	Se concentration (mg kg^{-1} DW)			Se concentration (µg kg^{-1} FW)		
	Truss			Truss		
	1st	2nd	3rd	1st	2nd	3rd
0	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}
1	2.76 ± 0.18^{b}	1.82 ± 0.02^{b}	0.94 ± 0.05^{b}	220 ± 18^{b}	146 ± 3^{b}	75 ± 5^{b}
1.5	3.54 ± 0.07^{a}	3.14 ± 0.02^{a}	2.08 ± 0.09^{a}	282 ± 16^{a}	257 ± 16^{a}	170 ± 13^{a}
Significance						
Se (mg L^{-1})	***	***	***	***	***	***

*Values followed by different letters in the same column differ significantly at 5% level by the LSD test. Significance level: *** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05; ns = not significant.*

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Table 2
Yield and qualitative characteristics of red fruit of tomato plants (cv. Red Bunch) grown in nutrient solution supplied with 0, 1 and 1.5 mg Se L^{-1}. Data are the means of 3 replicates.

Parameters		Se added (mg L^{-1})			
		0	1	1.5	Significance
Total yield	g FW m^{-2}	2471 ± 310	2517 ± 290	2522 ± 204	ns
Commercial yield	g FW m^{-2}	1768 ± 155	1739 ± 124	1753 ± 139	ns
Solid content	%	6.78 ± 0.8	6.72 ± 0.72	6.61 ± 0.53	ns
Solid soluble content (SSC)	°Brix	8.30 ± 0.74	8.11 ± 0.86	8.17 ± 0.79	ns
Titrable acidity	g citric acid 100 ml^{-1}	0.70 ± 0.06	0.69 ± 0.04	0.71 ± 0.08	ns
Maturity index		12	11.8	11.5	ns
Taste index		0.98	0.97	1.00	ns

Values followed by different letters in the same column differ significantly at 5% level by the LSD test.
*Significance level: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; ns = not significant.*

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Table 3

Activity of antioxidant enzymes [Ascorbate peroxidase (APX), Catalase (CAT), Superoxide dismutase (SOD), Glutathione peroxidase (GPXs)] and lipid peroxidation expressed as malondialdehyde (MDA)

Se (mg L^{-1})	Respiration rate (µmol CO_2 g^{-1} h^{-1})				
	Days after harvest				
	First truss				
	0	3	5	7	9
0	2.70 ± 0.36	2.31 ± 0.05	2.40 ± 0.40	3.89 ± 0.29	2.36 ± 0.17
1	2.66 ± 0.37	2.29 ± 0.14	2.36 ± 0.15	3.43 ± 0.25	2.22 ± 0.20
1.5	2.23 ± 0.35	2.35 ± 0.13	2.40 ± 0.13	3.41 ± 0.20	2.26 ± 0.12
Significance	ns	ns	ns	ns	ns

	Second truss					
	0	3	5	7	9	11
0	1.36 ± 0.14	1.15 ± 0.11	1.20 ± 0.09	1.52 ± 0.12[a]	2.13 ± 0.14[a]	1.12 ± 0.10
1	1.22 ± 0.10	1.05 ± 0.11	1.17 ± 0.07	1.56 ± 0.15[a]	1.46 ± 0.10[b]	0.83 ± 0.07
1.5	1.19 ± 0.12	1.16 ± 0.08	1.19 ± 0.07	1.25 ± 0.10[b]	1.66 ± 0.13[b]	0.90 ± 0.09
Significance	ns	ns	ns	*	*	ns

	Third truss					
	0	3	5	7	9	11
0	2.01 ± 0.08	2.05 ± 0.12	3.57 ± 0.32[a]	0.97 ± 0.06[b]	1.51 ± 0.13	0.94 ± 0.07[b]

content in red fruit of the first truss of tomato plants (cv. Red Bunch) grown in nutrient solution supplied with 0, 1 and 1.5 mg Se L^{-1}.

Se added mg L^{-1}	APX activity µmoli AsA g^{-1} FW min^{-1}	CAT activity µmol H_2O_2 g^{-1} FW min^{-1}	SOD activity SOD Units g^{-1} FW	GPXs activity µmoli GSH g^{-1} FW min^{-1}	MDA content mmol MDA EQ g^{-1} FW
0	0.634±0.090[a]	0.412±0.037[a]	59.4±0.5[c]	0.094±0.004	156±6[a]
1	0.316±0.023[b]	0.270±0.026[b]	67.1±1.8[b]	0.090±0.008	138±4[b]
1.5	0.348±0.040[b]	0.279±0.016[b]	83.6±1.6[a]	0.089±0.009	126±4[b]
Significance	**	**	***	ns	*

*Values followed by different letters in the same column differ significantly at 5% level by the LSD test. Significance level: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; ns = not significant.*

Table 4. Respiration rate during post-harvest ripening in fruit of first, second and third truss of tomato plants (cv. Red Bunch) subjected to different Se treatments. Values are means with standard errors (n=4).

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1	1.81 ± 0.20	1.89 ± 0.12	2.78 ± 0.09^{b}	1.49 ± 0.08^{a}	1.33 ± 0.10	1.38 ± 0.16^{a}
1.5	1.92 ± 0.05	1.63 ± 0.14	2.78 ± 0.22^{b}	1.11 ± 0.09^{b}	1.57 ± 0.12	1.57 ± 0.14^{a}
Significance	ns	ns	*	*	ns	*
Se	Days after harvest					
(mg L^{-1})	Glucose (mg g^{-1} FW)					
	0		4	8	12	
0	13.6 ± 1.3		16.0 ± 0.8^{a}	17.2 ± 0.5^{a}	17.1 ± 0.3	
1	13.4 ± 1.2		14.3 ± 0.6^{b}	15.5 ± 0.5^{b}	16.6 ± 0.6	
1.5	12.5 ± 0.8		14.3 ± 0.6^{b}	15.1 ± 0.4^{b}	17.2 ± 0.8	
Significance	ns		*	*	ns	
	Fructose (mg g^{-1} FW)					
	0		4	8	12	
0	14.6 ± 1.2		18.9 ± 0.7^{a}	20.2 ± 0.7^{a}	20.6 ± 0.6	
1	13.9 ± 1.3		16.9 ± 0.6^{b}	18.4 ± 0.2^{b}	20.2 ± 1.1	
1.5	13.5 ± 0.5		16.8 ± 0.2^{b}	17.4 ± 0.4^{b}	19.8 ± 0.7	
Significance	ns		*	**	ns	

Values followed by different letters in the same column differ significantly at 5% level by the LSD test.
*Significance level: *** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05; ns = not significant.*

Table 5. Non-structural carbohydrates concentration in fruit of tomato plants (cv. Red Bunch) subjected to different Se treatments. Values are means with standard errors (n=4).

Values followed by different letters in the same column differ significantly at 5% level by the LSD test
*Significance level: *** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05; ns = not significant.*

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Se (mg L^{-1})	Days after harvest			
	Lycopene (µg g^{-1} FW)			
	0	4	8	12
0	2.97 ± 0.09	84.8 ± 4.43[a]	174.4 ± 6.23[a]	195.5 ± 18.58
1	3.25 ± 0.26	48.6 ± 6.03[b]	110.8 ± 8.45[b]	210.3 ± 18.17
1.5	3.38 ± 0.23	46.3 ± 4.17[b]	90.3 ± 7.96[c]	199.7 ± 4.90
Significance	ns	**	***	ns
	β-carotene (µg g^{-1} FW)			
	0	4	8	12
0	3.59 ± 0.30	5.79 ± 0.30[a]	6.67 ± 0.34[a]	6.99 ± 0.57
1	3.49 ± 0.28	4.62 ± 0.12[b]	5.43 ± 0.19[b]	6.89 ± 0.53
1.5	3.63 ± 0.19	4.05 ± 0.20[c]	4.68 ± 0.19[c]	6.50 ± 0.23

Table 6. Lycopene, β-carotene, chlorophyll a and chlorophyll b concentration in fruit of tomato plants (cv. Red Bunch) subjected to different Se treatments during post-harvest.

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Significance	ns	***	**	ns
	Chlorophyll a (µg g^{-1} FW)			
	0	4	8	12
0	1.72 ± 0.008	1.29 ± 0.12	0 ± 0[b]	0 ± 0
1	1.59 ± 0.11	1.50 ± 0.08	1.03 ± 0.03[a]	0 ± 0
1.5	1.76 ± 0.06	1.41 ± 0.08	0.93 ± 0.03[a]	0 ± 0
Significance	ns	ns	***	ns
	Chlorophyll b (µg g^{-1} FW)			
	0	4	8	12
0	1.57 ± 0.02	0.64 ± 0.05	0.00 ± 0.00[b]	0 ± 0
1	1.40 ± 0.11	0.93 ± 0.08	0.53 ± 0.02[a]	0 ± 0
1.5	1.56 ± 0.03	0.79 ± 0.09	0.48 ± 0.01[a]	0 ± 0
Significance	ns	ns	***	ns

*Values followed by different letters in the same column differ significantly at 5% level by the LSD test. Significance level: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; ns = not significant.*

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Effect of selenium enrichment on metabolism of tomato (Solanum lycopersicum) fruit during postharvest ripening

Puccinelli, Martina; Malorgio, Fernando; Terry, Leon A.; Tosetti, Roberta; Rosellini, Irene ...

2018-10-27
Wiley
https://doi.org/10.1002/jsfa.9455
Puccinelli M, Malorgio F, Terry LA, Tosetti R, Rosellini I & Pezzarossa B. Effect of selenium enrichment on metabolism of tomato (Solanum lycopersicum) fruit during postharvest ripening. Journal of the Science of Food and Agriculture, Volume 99, Issue 5 2019, pp. 2463-2472

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