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Full length Research paper

The Role of Asprin in Delaying Postharvest Ripening of Tomato (*Solanum lycopersicum* L.) Fruits.

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Tomato is used as condiments for stew which is a regular feature of meals globally. Hence it is an important ingredient in the confectionary industry. Postharvest loss is a major challenge hampering tomatoes production in most developing countries. The present study was planned to investigate the role of acetylsalicylic acid in delaying post harvest ripening and enhancing shelf life of tomato fruits. The result indicated that there was significant difference between control and acetylsalicylic acid (ASA) treated groups during postharvest storage of tomato fruit samples for all measured fruit quality parameters including weight loss, vitamin C, chlorophyll, carotenoid, titratable acidity and sugar contents. It was also observed that mean values for weight loss, vitamin C, chlorophyll and total acidity for treatment groups were lower than for the control indicating that treatment with acetylsalicylic acid has slowed down reduction of these parameters during postharvest ripening of tomato fruits. Thus, ASA treatment delays role in fruit ripening and can increase shelf life of the fruits. The principal component analysis (PCA) was indicated that reducing sugars and carotenoid contents increase while weight loss, vitamin C, chlorophyll, and titrable acidity were decreasing during post harvest ripening of tomato fruits. Further studies are required on the effect of storage conditions, cultivar differences and environments on fruit quality parameters.

Keywords: Carotenoid content, fruit quality, Reducing sugars, shelf life, Total acidity, Vitamin C.

INTRODUCTION

Vegetables constitute a major part in terms of providing food and nutritional security in Ethiopia. Vegetables are important sources of minerals, vitamins and other nutrients. Solanaceous vegetables like tomato, potato and chilli have high demand in the country. Tomato (*Solanum lycopersicum* L.) is one of the important vegetable crops of the world and is widely cultivated throughout the tropical and subtropical countries. It is also among the most important vegetable crops in Ethiopia. The total production of this crop in the country has shown a marked increase since it became the most profitable crop providing a higher income for small to large scale farmers compared to other vegetable crops (Lemma *et al*., 1992). However, large portions of tomato would be lost worldwide after harvest, especially in sub-tropical countries (Javid, 2009). Postharvest loss is a major challenge hampering tomatoes production in most developing countries (Arah *et al.,* 2015). Postharvest loss is a major challenge hampering tomatoes production in most developing countries (Arah *et al.,* 2015).

Salicylic acid (SA) is a natural and safe chemical to maintain postharvest quality of horticultural crops and its application is adopting, although chemical treatments are banning in many countries (Supapvanich & Promyou 2013). SA is an active element of aspirin, and it regulates a number of processes in plants and mediate the defense in plant against pathogen (Javanmardi and Akbari, 2016). Tomato being a perishable crop as a result of its high moisture content has short shelf life of about 48 hours (Muhammed et al., 2011) under tropical conditions. Specialized postharvest handling practices and treatment methods are needed in order to extend the shelf life of the crop after harvest. In Ethiopia, limited studies are found on postharvest management of horticultural crops despite huge potential for production of horticultural crops like tomato. In light of this justification, the present study has planned to study the role of asprin (ASA) on

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postharvest management of tomato fruits in Eastern Ethiopia.

MATERIALS AND METHODS

The experiment was conducted in Biotechnology Laboratory, Haramaya University. Tomato fruits were collected from farm land in Erere district, Harari Regional State, Ethiopia. The mature green fruits were collected and immediately brought in a polystyrene bag to Biotechnology Laboratory of the university. Fruits were left for 22 hrs (after harvest) at room temperature in order to stabilize ethylene evolution caused by wounding. The experimental design was completely randomized design (RCBD) in two replications. Of the collected tomato fruit sample, 30 fruits of uniform size with no bruises or damage were selected. Fruit sample was surface sterilized with sodium hypochlorite solution (500 ppm) for 10 minutes so as to reduce fungal infection and air-dried for approximately 15 minutes. After surface sterilization, the fruit sample was immersed for five minutes in solutions of 0mM, 1mM, 2mM, and 4mM ASA solution, and was air–dried at room temperature for 1h following the method used by Dibbisa *et al.* (2016). The surface-dried fruit samples were individually packaged in perforated plastic bags to maintain relative humidity. Each bag was packaged in a 1L disposable container. All packages were sealed and stored at room temperature for 20 days. The treated groups were evaluated in each treatment at 1st, 10th and 20th days of treatment. Thereafter, fruits were assessed for different quality parameters such as physiological weight loss, vitamin C(ascorbic acid) content, titratable acidity, total carotenoids, total chlorophylls, reducing sugars at 1st, 10th and 20th days.

Data Collection and Analysis

Physiological Loss of Weight

Weight loss was determined by using method indicated by Akbudak, (2007) periodically (on 1st, 10th, and 20th days of storage) by weighing tomato fruit samples in each treatment using digital balance (Denver Instrument XL-1810). Percentage weight loss was calculated using the following formula:

$$\%WL = \frac{Wi - Wf}{Wi} \times 100\%$$

Where: WL=Weight loss; Wi= Initial weight; Wf= Final weight

Ascorbic Acid Analysis

The ascorbic acid content was determined by the 2, 6- di-chlorophenol indophenol (DCPIP) dye method (AOAC, 2000). 5 ml of the standard ascorbic acid solution was pipetted into a 100 ml conical flask and 5ml of the 3% HPO_3 solution was added. The ascorbic acid solution was titrated with the dye solution to a pink colour, which should persist for 15sec. The titre value was recorded. The dye factor was calculated by dividing 5ml volume of ascorbic acid solution taken for titration by titrant volume of dye solution. Dye factor was expressed as mg of ascorbic acid per ml of the dye. Since 5ml of the standard ascorbic acid solution contains 0.5 mg ascorbic acid:

$$\text{Dye factor (mg ascorbic acid per dye)} = \frac{0.5\text{mg}}{\text{titrant volume}}$$

An aliquot of 5ml tomato fruit juice extract was diluted to 50 ml with 3% metaphosphoric acid in a 50 ml volumetric flask. The aliquot was then centrifuged (Model, Z300, 580W, 3052 Nm, German) for 15 minutes and titrated with the standard dye to a pink end point (persisting for 15 seconds). The ascorbic acid content was calculated from the titration value, dye factor, dilution and volume of the sample as

$$\%\ A.A = \frac{(ABRs)x\ \text{dye factor x volume of initial test solution}}{\text{volume of test solution titrated}} \times 100\%$$

Where: A.A=Ascorbic Acid; ABR= Average Burette reading of sample solution

Measurement of Total Acidity

The total acidity was determined by a standard titrimetric method. For the determination of total acidity, 5 grams of extracted tomato fruit juice was mixed with 100ml of distilled water. In the presence of phenolphthalein as an indicator, the mixture was titrated by adding 0.1 N NaOH until the break of light pink color (pH 8.2) observed for 15 seconds. The volume of NaOH added to the solution was multiplied by the correction factor of 0.064 for the calculation of titratable acidity as %age of citric acid. Titratable acidity was expressed as %age of citric acid (or total acidity) (AOAC, 2000).

$$\%\ \text{acid} = \frac{\text{titrant volume x 0.1N NaOH x acid factor x titration volume}}{\text{weight of the sample}} \times 100\%$$

Determination of Chlorophylls and Total Carotenoids

Chlorophylls and carotenoid contents were determined using spectrophotometric method following procedure of Nagata (1992) for the simultaneous determination of chlorophylls and total carotenoids in tomato fruit samples, 16ml of acetone–hexane (4:6) solvent was added to 1g of mandarin fruit juice homogenates. For this, the homogenous sample was prepared by Kenwood blender, (Model, BC311 P.R.C. China). The homogenate was centrifuged at 5000 rpm using centrifuge (Model, Z300, 580W, 3052 Nm, German) for 10 minutes at 20°C. Then after absorbance was measured at 663, 645 and 470nm in a Jenway model 6100 spectrophotometer. Total chlorophyll and carotenoid contents were calculated according to the equations indicated below, and the final results was expressed as µg/g for chlorophylls and mg/g for antioxidants.

$$\text{Chlorophyll a (µg/g)} = 0.999A_{663} - 0.989A_{645}$$

Chlorophyll b (µg/g) = $1.77A_{645}$- $0.328A_{663}$
Total Chlorophyll(a+b)(µg/g) = chla + chlb
TC (mg/g) = $\frac{1000A_{470}-2.27(\text{chla})-81.4(\text{chlb})}{227}$
Where: A = absorbance; TC=Total carotenoids

Sugar Analysis

Determination of standard glucose concentration
Total reducing sugar was estimated by using the technique used by Gros *et al* (2010) with some modifications. For extraction of total reducing sugars from the tomato fruit juice sample, 5g of homogenized juice sample was dissolved in 15ml of 80% ethanol, then mixed and heated in boiling water bath for sufficient time until the ethanol odor went off. Then, the solution was filtrated by adding 1ml of saturated Pb $(CH3COO)_2$ and 1.5ml of $NaHPO_4$ and the content was mixed by gentle shaking on Vortex Shaker.

After filtration, the extract was made to 1: 10 dilution with distilled water. From this solution 0.8ml and 1.2ml of sample solution was taken and made up to 2ml with distilled water in labeled test tubes. Thereafter 1ml of copper reagent was added to both solutions and heated for 20 minutes in a boiling water bath. After heating, the contents were cooled under running tap water without shaking. Then, 1ml of sodium acetate was added (as a color reagent), mixed well, and left for about 10 minutes to allow color development. Then after, the absorbance was read using spectrophotometer at 540 nm.

Finally, the content of sugars in the unknown and standard solution (in mg/ml and mg %) was estimated by using the standard curve.

The calculation of the concentration on the sample was done using the linear regression equation of standard solution obtained y = ax + b where Y is the absorbance of the measured sample solution, b is the y-intercept and X is the concentration of the sample solution (mg/ml):

$X=\frac{Y-b}{a}$

Conc of sugar in sample soln $=\frac{\text{OD of test sample}-\text{OD of blank}}{\text{OD of std}-\text{OD of blank}}$ x conc of std

Mg of reducing sugar = $\frac{\text{OD of test sample}-\text{OD of blank}}{\text{OD of std}-\text{OD of blank}} * \text{conc of std glucose} * \text{df}$

Dilution factor = $\frac{\text{final dilution volume}}{\text{original volume of substance being diluted(aliquote vol)}}$

% reducing sugar = $\frac{\text{mg of reducing sugar}}{\text{mg of original sample}}$ x 100%

mg std glucose = concentration of working std solution x volume of standard solution
Data were subjected to ANOVA (Analysis of Variance) and mean separation based on DMRT (Duncan's Multiple Range Test) using SAS 9.1.2 statistical software. All significance tests were made at (P ≤ 0.01 and/or 0.05) levels.

RESULT AND DISCUSSION

Weight Loss and Vitamin C content

Weight loss was measured as the difference between the initial weight (weight before treatment) and final weight (weight taken after 20 days of storage). Statistical analysis showed that there was significant difference in weight loss between control and acetylsalicylic acid (ASA) treated groups (Table 1) during postharvest storage of tomato fruit samples. There was also significance difference between initial weight and final weight (measured on first and 20th days respectively) during postharvest ripening of tomato fruit samples. This finding was in agreement with Kazemi (2014) who suggested SA treatments were generally effective on vegetative growth, photosynthetic pigments, minerals, yield and tomato fruit quality. SA inhibit grey mould growth, significantly decrease weight loss, increase storage life, maintain total soluble solids, titratable acidity, antioxidant, ascorbic acid and pH value in Kiwi fruits (Fatemi *et al.,* 2013). Islam *et al.* (2018) found that a significant lower fresh weight loss was observed in SA-treated tomato fruit due to lower transpiration (moisture loss). Reducing the fresh weight loss of tomato fruits in storage helps maintain fruit quality. The control and 0.50 mM treatment presented marketable visual quality (≥3) for 16 and 25 days of storage, accordingly. As a similar maturity stage (light red) was selected for this experiment, there were no significant differences in color, lycopene and titratable acidity at harvest time, but the tomato fruit showed significant differences in firmness, vitamin C and soluble solids.

In tomato, the SA influenced the defense pathway which induces resistance to *Botrytis cinerea* (fungus) but not to *O. neolycopersici* and in tobacco SA defense pathway induces resistance to *Oidium neolycopersici* (tomato powdery mildew) but not to *B. cinerea* (Achuo *et al.,* 2004). SA concentration, light, temperature, plant growing stages and plant species influenced the action on plants (Javanmardi and Akbari, 2016). SA delayed fungal incidence, prolonged the storage life and maintained the valuable attributes of postharvest tomatoes by inhibiting the ripening and senescence processes (Pila *et al.,* 2010). Moreover, SA delayed the apple fruit ripening processes during postharvest storage (Mo *et al*., 2008).

The ascorbic acid (vitamin C) content of tomato fruit during postharvest storage was indicated in Table 2. Significance difference between ASA treated and control groups was observed for ascorbic acid content during 10th and 20th days of storage of tomato fruit. However, no significance differences were observed among treatments. It was also demonstrated that ascorbic acid content was decreasing in control group during postharvest fruit ripening of tomato fruit. The highest

Table 1: Percentage weight loss during post harvest ripening of tomato fruit as treated by different concentrations of ASA solution

Treatment	Wi	Wf	WL
0mM	421.00±5.66aA	314.50±10.61cB	25.31±1.52a
1mM	434.00±5.66aA	358.25±8,84bB	17.46±0.96b
2mM	421.50±2.12aA	389.40±3.39aB	7.61±1.27c
4mM	430.00±7.07aA	397.75±3.04aB	7.48±2.23c

Wi: initial weight; Wf: final weight. Means followed by same letter within a column were not significantly different at 0.05. Probability level based on DMRT (Duncan's Multiple Range Test). Small letters: significance within column; capital letters: significance within row.

Table 2: Vitamin C content during postharvest ripening of tomato fruit as treated by different concentrations of ASA solution

Treatment	1st day	10th day	20th day
0mM	5.77±0.33aA	4.46±0.29bA	1.85±0.10bB
1mM	6.04±0.26aA	5.33±0.32aA	2.94±0.55aB
2mM	5.71±0.10aA	5.60±0.25aA	3.59±0.26aB
4mM	5.98±0.03aA	5.55±0.32aA	3.70±0.42aB

Means followed by same letter within a column were not significantly different at 0.05. Probability level based on DMRT (Duncan's Multiple Range Test). Small letters: significance within column; capital letters: significance within row.

mean vitamin C content was observed for control than treated groups indicating that ASA treatment has slowed down change in ascorbic acid content of the fruit sample. This finding was in accordance with Wang *et al.,* (2006) who suggested that tomato fruit treated with SA had higher vitamin C at harvest time as well as final storage day than the control. Among the treatments, the 0.50 mM SA resulted in the highest vitamin C content.

Chlorophyll and Carotenoid Contents

Mean values for total chlorophyll and carotenoid content was shown in Table 3.

There was significance difference in total chlorophyll and carotenoid contents between control and aloe gel treated groups during 10th and 20th days of storage tomoato fruits. It was also observed from mean values in Table 3 that carotenoid content was increasing while chlorophyll content was decreasing during postharvest ripening of the fruits.

The highest mean carotenoid content was observed for control group showing that treatment with ASA solution can delay ripening of tomato fruit. Finally, carotenoid content was decreasing during 20th day of storage showing deterioration in fruit quality.

This finding was supported by Kumar *et al* (2018) who reported progressive increase in physiological lycopene content, β-carotene content, total sugars, reducing sugars and non-reducing sugars contents during postharvest ripening of tomato fruit.

Titratable Acidity

Mean values for total acidity as indicated in Table 4. It was observed that there was no significance difference in total acidity (predominantly citric acid) between control and ASA treated groups during first day treatment tomato fruit.

However, significance differences between control and ASA treated groups were observed during 10th day of treatment. It was also observed from mean values in Table 4 that total titratable acidity was decreasing during postharvest ripening of tomato fruit.

This finding was in agreement with Islam *et al*. (2018) who reported that the highest mean total acidity was observed for control group showing that treatment with ASA solution slowing increase of total acidity in tomato fruit. The titratable acidity showed higher in SA treated tomato fruits at after storage.

The SA increased ascorbate peroxidase, which activated antioxidant abilities and vitamin C in peach fruit. The antioxidant ability of SA can prevent vitamin C destruction of tomato fruits during storage. Pila *et al.* (2010) similarly found in tomato fruits that SA retards degradation of ascorbic acid content during storage. A reduced metabolic rate has implications for better vitamin C content in tomatoes (Tigist *et al.* 2013).

Perhaps the SA-treated tomato fruits have higher titratable acidity because of fewer metabolic changes in organic acid related to respiration after storage. This relates to Han and Li (1997) who reported higher titratable acidity after storage in apple fruit treated with SA.

Total Reducing Sugars

Table 3. Mean comparison for chlorophyll and carotenoid content during post harvest ripening of tomato fruit as treated by different concentrations of ASA solution

Treatment	Chlorophyll			Carotenoid		
	1st day	10th day	20th day	1st day	10th day	12th day
0mM	0.72±0.05aA	0.18±0.01dB	0.10±0.01cB	1.31±0.07aC	2.22±0.01aA	1.76±0.01aB
1mM	0.76±0.01aA	0.21±0.01cB	0.17±0.04bB	0.95±0.02aC	2.09±0.03bA	1.54±0.02bB
2mM	0.73±0.01aA	0.24±0.05bB	0.21±0.04aB	0.89±0.01aC	1.99±0.02cA	1.36±0.05cB
4mM	0.71±0.03aA	0.27±0.01aB	0.20±0.03aB	0.82±0.03aC	1.75±0.02dA	1.30±0.06cB

Means followed by same letter within a column were not significantly different at 0.05. Probability level based on DMRT (Duncan's Multiple Range Test). Small letters: significance within column; capital letters: significance within row.

Table 4. Mean comparison for total acidity during post harvest ripening of tomato fruit as treated by different concentrations of ASA solution

Treatment	1st day	10th day	20th day
control	72.03±7.11aA	11.98±2.37cB	6.44±1.90bC
1mM	67.00±4.74aA	21.78±2.37aB	14.41±0.47aC
2mM	68.68±7.11aA	18.26±1.42bB	13.07±0.47aB
4mM	78.73±2.37aA	17.76±0.24bB	12.23±0.23aB

Means followed by same letter within a column were not significantly different at 0.05. Probability level based on DMRT (Duncan's Multiple Range Test). Small letters: significance within column; capital letters: significance within row.

Table 5. Percentage reducing sugar during post harvest ripening of tomato fruit as treated by different concentrations of ASA solution

Treatment	1st day	10th day	20th day
0mM	1.88±0.27aB	7.89±0.24aA	1.99±0.21cB
1mM	2.27±0.50aB	6.56±0.11bA	2.98±0.29bB
2mM	2.18±0.43aB	5.96±0.27bA	3.81±0.29aB
4mM	2.14±0.69aB	4.93±0.24cA	3.30±0.05abB

Means followed by same letter within a column were not significantly different at 0.05. Probability level based on DMRT (Duncan's Multiple Range Test). Small letters: significance within column; capital letters: significance within row.

It was observed in Table 5 that there was no significance difference in total reducing sugars between control and ASA treated groups in freshly harvested (1st day treatment) tomato fruit. However, significance differences, in sugar content between control and ASA treated groups, were observed during 10th and 20th days of treatment. It was also observed from mean values in Table 5 that total reducing sugars was increasing during postharvest ripening of tomato fruit. The highest mean reducing sugar was observed for control group showing that treatment with ASA solution total slowed down increase in reducing sugar in tomato fruit. The low sugar content in ASA treated group showing that ASA delays postharvest ripening and increases shelf life of tomato fruit. Titrable acidity was slightly declined during different experimental periods up to 45 days of storage; however either SA or ASA treatments had no significant influence on TA of fruits over the storage time in mandarin fruits (Orabi *et al*., 2018). Alejandra *et al*., (2017) and Chanikan *et al*., (2015) illustrated that the slight decline in TA was probably due to the slow rate of respiration and metabolic processes converting citric acid into sugars as a function of applied SA.

SA may take part in regulation of ethylene formation also by restraining the increase in superoxide free radical production and cell membrane deterioration and tissue senescence induced by superoxide free radicals, rather than, or in addition to, its action on enzymes directly participating in ethylene synthesis. Research on tobacco (Hennig *et al*. 1993) suggested that once the free SA content of plant tissue exceeds certain levels, dependent on growing and environmental conditions, excess

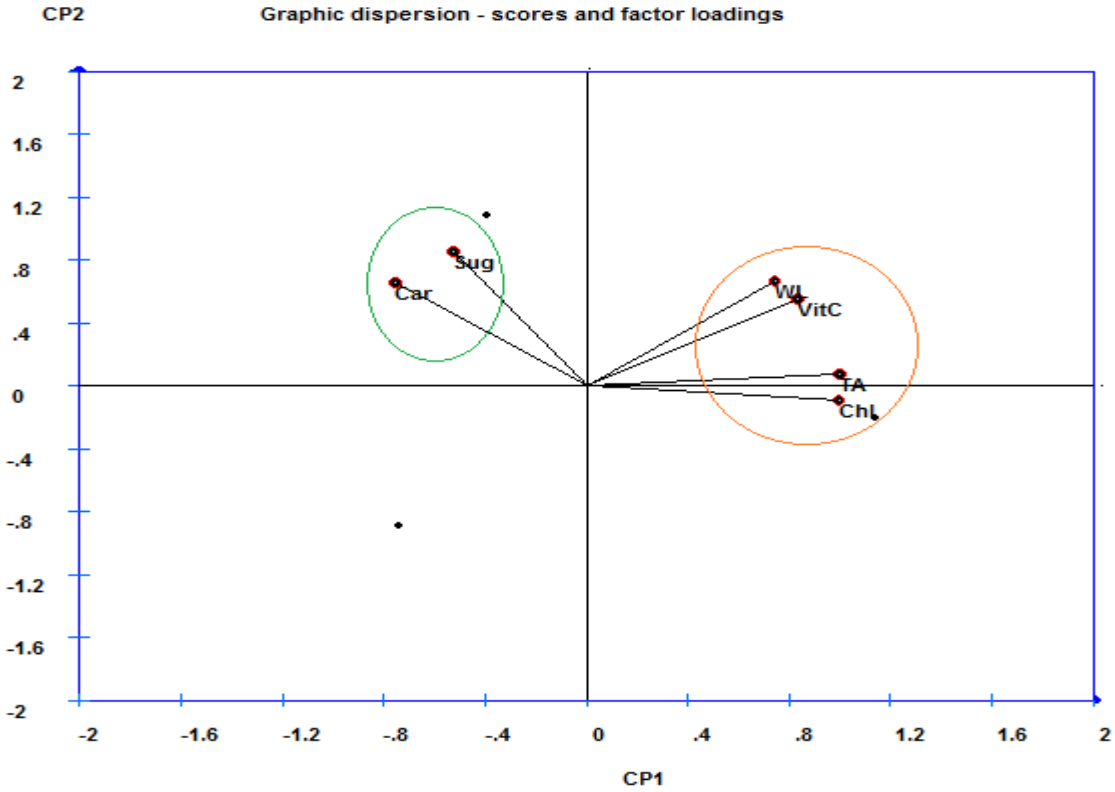

Figure 1: PCA scores for six fruit quality parameters measured for tomato fruit. RS: total reducing sugars; Car: carotenoid content; TA: total titratable acidity; VitC: vitamin C content; WL: weight loss; and Chl: total chlorophyll content.

amounts are converted into conjugated SA, considered to be the storage form of this compound in plants. Exogenous SA is reportedly able to induce salicylic acid glucosyltransferase (SA-Gtase) activity, and consequently promote the conjugation of SA into salicylic acid glucoside (SAG) (Yalpani *et al*., 1992). Zhang *et al*. (2003) demonstrated that ASA treatment resulted in an increase in total SA content of banana fruit.

Accumulation of conjugated SA was accompanied by a decrease in free SA content during the course of ripening in ASA-treated fruit.

Based on the plot for PC2 vs PC1 for D statistics (Figure.1), reducing sugars and carotenoid having close PC1 and PC2 scores (with vector angle $<90^0$) showing similar/correlated effects while reducing sugars and/or carotenoids with chlorophyll content, weight loss and vitamin C contents have vector angle greater than 90^0 showing opposite effects or more divergence. Furthermore, vitamin C, chlorophyll content and weight loss have similar effect since their vector angle $<90^0$. That is reducing sugars and carotenoid contents increase while weight loss, vitamin C, chlorophyll, and titrable acidity were decreasing during post harvest ripening of tomato fruits.

CONCLUSION

Acetylsalicylic acid treatment during postharvest storage of tomato fruits has beneficial effects in retarding the ripening process. This treatment was effective as a physical barrier and thus reduced the weight loss, vitamin C, chlorophyll and totrable acidity during postharvest storage. The results of this paper show that *acetylsalicylic acid* could be applied as a postharvest treatment to enhance quality and shelf life during postharvest storage of tomato fruits.

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