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Research Article

Foliar-applied humic acid modulates antioxidant and mineral profiles in tomato fruit across maturity stages

Md. Dulal SARKAR[1,2], Auruna BEGUM[1], Md. Abdul GOFFAR[3],
Md Obyedul Kalam AZAD[4], Most Tahera NAZNIN[4*]

[1]*Sher-e-Bangla Agricultural University, Faculty of Agriculture, Department of Horticulture, Dhaka, 1207, Bangladesh; dulal@sau.edu.bd; aurusau73@yahoo.com*
[2]*King Abdulaziz University, Faculty of Environmental Sciences, Department of Agriculture, Jeddah, 21589, Saudi Arabia*
[3]*Bangladesh Agricultural Research Institute, Horticulture Research Centre, Vegetable Division, Gazipur, 1701, Bangladesh; drmgoffar@bari.gov.bd*
[4]*University of Nevada, Reno, UNR Extension and Experiment Station, College of Agriculture, Biotechnology & Natural Resources, Department of Agriculture, Veterinary and Rangeland Sciences, Las Vegas, NV 89123, USA; azadokalam@gmail.com; mnaznin@unr.edu (*corresponding author)*

Abstract

Berry quality can further be modulated by applying foliar biostimulants to cherry tomatoes depending on their maturity stage. This study aimed to evaluate the effects of foliar application of humic acid (HA) on berry quality at different maturity stages. Humic acid was applied at concentrations of 0, 25, 50, and 75 mg L^{-1} during the vegetative, flowering, and fruit-setting phases. Berries were harvested at four distinct maturity stages: mature green (MG), breaker (BK), pink (PK), and red (RD) stages. HA, particularly HA$_{75}$, significantly enhanced berry quality when harvested at the red stage and exhibited better antioxidant activity due to elevated levels of vitamin C (44.20 mg 100 g^{-1} FW), lycopene (0.25 mg 100 g^{-1} FW), and β-carotene (27.58 mg 100 g^{-1} FW). It also had higher total soluble solids (6.17 °Brix) and pH (4.07) values. The reducing sugar content increased from 0.84% in mature green berries to 1.39% in red berries with the application of HA$_{0}$ and HA$_{75}$, respectively. The shelf-life of berries was extended from 13.28 days (HA$_{0}$) to 15.58 days (HA$_{75}$) at the green mature stage. Dry matter content peaked at 8.82% in HA$_{75}$-treated mature green berries, whereas moisture content was highest (94.19%) in untreated (HA$_{0}$) red berries. Conversely, at the red stage, HA$_{50}$ and HA$_{75}$ treatments resulted in the highest redness (a* values of 13.21 and 10.20, respectively), compared to 12.60 for the control (HA$_{0}$). Lightness (L*) was highest in mature green berries treated with HA$_{50}$ (61.43), indicating brighter fruit surfaces, whereas red berries showed lower L* values (49.80-52.70), consistent with ripening progression. Mineral uptake also improved, with Mg (2.39%), P (0.71%), and Fe (213.54 ppm) being the highest in HA$_{75}$-treated red berries. These findings suggest that while humic acid can enhance certain quality traits, the maturity stage remains the dominant factor influencing berry quality traits.

Keywords: antioxidant compounds; berry quality; biostimulants; humic substances; mineral profile; post-harvest indicators; *Solanum lycopersicum*

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Introduction

Amid rising environmental concerns and the demand for nutrient-rich quality food, sustainable agricultural inputs, such as biostimulants, have gained significant attention for enhancing crop performance and stress resilience (de Moura *et al.*, 2023; Rouphael and Colla, 2020). These substances, comprising natural compounds or beneficial microorganisms, enhance plant physiological processes independently of nutrient content (Disciglio *et al.*, 2024), promoting growth and productivity through improving metabolic efficiency (Yakhin *et al.*, 2017). Humic acid (HA) is a natural compound derived from the decomposition of organic matter (Hayes and Swift, 2020). It is an ecologically safe biostimulant that has shown promise in improving plant growth (Vacelik *et al.*, 2022), nutrient uptake (Canellas *et al.*, 2015; Sheikha *et al.*, 2022; Massimi *et al.*, 2023;), and stress tolerance (Atero-Calvo *et al.*, 2024) through hormone-like activity and metabolic modulation (Ampong *et al.*, 2022; de Moura *et al.*, 2023; Souza *et al.*, 2022). HA influences physiological processes such as root architecture (Dang *et al.*, 2022; Vacelik *et al.*, 2022), photosynthetic efficiency (Canellas *et al.*, 2020; Nardi *et al.*, 2021; van Tol de Castro *et al.*, 2021), enzyme activity, and secondary metabolite synthesis (Tavares *et al.*, 2016; Romera *et al.*, 2021; Vacelik *et al.*, 2022). Compared to soil-based applications, its foliar application offers targeted delivery of bioactive compounds to plant tissues, enhancing antioxidant profiles and quality traits (Mosa *et al.*, 2022; Anandakumar *et al.*, 2024) due to quasi-hormonal attributes (Shahrajabian *et al.*, 2021). However, its efficacy varies with source (Sible *et al.*, 2021), concentration and timing (Nardi *et al.*, 2021; de Moura *et al.*, 2023). Given these variables, it is crucial to investigate how HA treatments influence the quality attributes of tomato fruits, particularly across different maturity stages during harvest.

Tomato is a widely popular vegetable for its important contribution to a health-promoting human diet (Zydlik and Zydlik, 2023). Its fruits are rich in minerals, vitamins, dietary fiber content, flavonoids and antioxidant properties (Tsouvaltzis *et al.*, 2023; Zhou *et al.*, 2024). Its carotenoids are the best dietary sources of lycopene and β-carotene (Tchonkouang *et al.*, 2022), which play a key role as precursors of vitamin A and give ripe fruits their distinctive hue (Mi *et al.*, 2022). Tomato, as a climacteric fruit, undergoes significant dietary biochemical changes with the advancement of maturity stages (Cheng *et al.*, 2023; Tsouvaltzis *et al.*, 2023). Maturity stage critically affects postharvest quality, shelf life, and nutritional value (Alenazi *et al.*, 2020; Gaweda and Jedrszczyk, 2020) due to progressive alteration in the amounts of antioxidant molecules and their activity (Loayza *et al.*, 2020). In the present study, 'BARI Tomato 11' (*Solanum lycopersicum* L.) was used as the test crop. It is commonly referred to as "Jhumka", which is a type of cherry tomato in Bangladesh. It is commonly harvested at the fully ripe stage for culinary use or the green or breaker stage for commercial purposes. However, critical questions remain regarding whether mature green tomatoes can match the nutritional and sensory qualities of fully ripened red tomatoes (Gaweda and Jedrszczyk, 2020). While tomato berries are extremely perishable, they are typically harvested at various stages of maturity, including green, half-ripe, and red (Baek *et al.*, 2020) for different uses. These maturity stages are critical determinants of tomato quality and yield, because harvesting at inappropriate or overripe stages can result in significant postharvest losses (Yang *et al.*, 2023). Generally, premature harvesting may result in reduced nutritional value, while delayed harvesting can lead to over ripeness, making them more susceptible to spoilage and mechanical damage during transportation (Tolasa *et al.*, 2021). Therefore, the precise identification of the optimal maturity stage is essential to maintain berry quality, resilience, and suitability for subsequent storage purposes.

Nevertheless, despite the promising rapid functions of foliar-applied HA, particularly on leaves and fruits (de Moura *et al.*, 2023; Kohay *et al.*, 2025), limited studies have explored its role in modulating berry quality across distinct maturity stages. Given that fruit maturity involves significant physiological and biochemical transitions, the effectiveness of HA may vary depending on the fruit developmental stage. We hypothesized that tomatoes harvested at different ripening stages would exhibit distinct responses to foliar-applied HA, potentially enhancing or stabilizing postharvest quality, with green-mature fruit serving as a baseline for comparison. Therefore, this study aimed to evaluate the impact of foliar-applied humic acid on the antioxidant and mineral profiles of cherry tomatoes across multiple maturity stages under open-field conditions.

Materials and methods

Microenvironmental conditions

The study was conducted from mid-October 2019 to March 2020 in open-field conditions at the Horticulture Farm, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. The experimental site is situated in the Madhupur Tract- Agro-Ecological Zone 28 at 23°74" N latitude and 90°35" E longitude, and it is 8.2 meters above sea level. The climatic conditions in the subtropical region during the trial exhibited a gradual cooling trend, with mean air temperatures decreasing from 27.40 °C in October to 18.70 °C in January, followed by an increase to 24.30 °C in March. In October, the highest recorded rainfall was 177 mm, whereas the subsequent months were predominantly dry, with December and January showing no precipitation at all. The relative humidity (RH) ranged from 65% to 80%. During the trial, average monthly sunlight hours were 186 hours in October, 202 hours in November, 217 hours in December, 210 hours in January, 198 hours in February, and 204 hours in March (BMD, 2020).

Plant material and growth conditions

'BARI Tomato 11' (*S. lycopersicum* L.) is a locally recognized variety of cherry tomato named "Jhumka". It is introduced from Asian Vegetable Research and Development Center (AVRDC), developed by the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. It has indeterminate growth habit. The seeds were sown on 15 October 2019 in prepared seedbeds and lightly covered with a thin layer of soil to facilitate uniform germination. To prevent infestation by ants and soil-borne worms, Heptachlor 40 WP was applied at a rate of 4 kg ha^{-1} around each seedbed as a precautionary measure. To protect the emerging seedlings from excessive sunlight and heavy rainfall, temporary shading was provided using polythene sheets until the seedlings reached a stable growth stage. Healthy and uniform seedlings were transplanted in the main field at a spacing of 60 x 40 cm after reaching 28 days old. The recommended amount of NPK fertilizer was applied to the soil before planting and throughout the plant's development cycle, as per Bangladesh Agricultural Research Council (Ahamed *et al*., 2018). These suggested application rates are per hectare: 10 t of cow dung, 550 kg of urea, 450 kg of Mop, and 450 kg of TSP. In addition, all other cultural practices were implemented.

Humic acid application and treatments

Humic acid was applied as a foliar spray during the growth season at four concentrations: HA_0, 0 mg L^{-1} (tap water spraying); HA_{25}, 25 mg L^{-1}; HA_{50}, 50 mg L^{-1}; and HA_{75}, 75 mg L^{-1}. The solutions were prepared using commercially available humic acid powder (manufactured by Sigma-Aldrich, USA) dissolved in distilled water and applied using a hand-held sprayer until runoff. HA treatments were initiated at the vegetative (20 days), flowering (40 days), and fruit-setting (60 days) stages after transplantation. Care was also taken to ensure uniform application across all plants, and no other foliar inputs were used during the trial period to isolate the effects of humic acid. The berries were harvested at four distinct maturity stages (Figure 1): mature green (MG), breaker (BK), pink (PK), and red (RD) to assess stage-specific responses. The average harvest duration for the four stages of maturity was as follows: day 65 (mature green stage), day 70 (breaker stage), day 73 (pink stage), and day 76 (red stage).

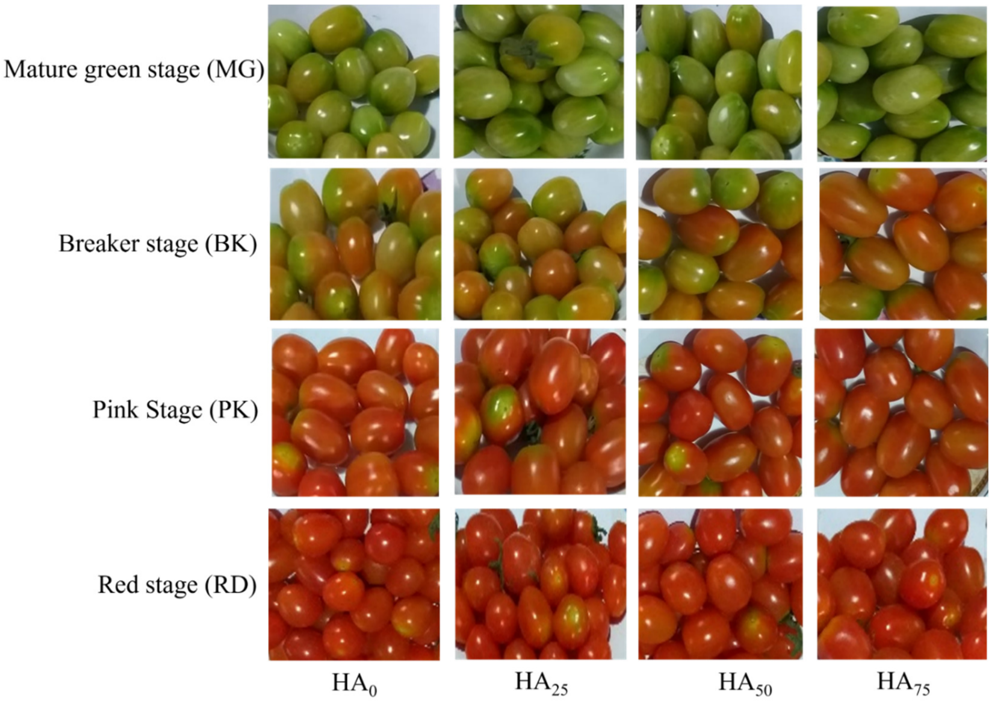


Figure 1. Visual representation of cherry tomato berries at four maturity stages under varying concentrations of foliar-applied humic acid
Abbreviations are as follows, HA$_0$, 0 mg L^{-1}; HA$_{25}$, 25 mg L^{-1}; HA$_{50}$, 50 mg L^{-1}; HA$_{75}$, 75 mg L^{-1}; MG, mature green stage; BK, breaker stage; PK, pink stage; and RD, red stage

Post-harvest indicators assessments

Post-harvest quality parameters were evaluated after harvest to determine the effects of humic acid treatments on fruit shelf-life and physical attributes. Assessments included berry color, dry matter content, moisture content, and shelf-life duration. The color of fresh berries was evaluated immediately after harvest using a Konica Minolta® CM-2002 spectrophotometer (Konica Minolta, Osaka, Japan), based on the CIE Lab color scale (L*, a*, b*) (Figure 1). The lightness parameter (L*) indicates brightness, ranging from 0 (black) to 100 (white). The a* value represents the red-green axis, with positive values indicating redness and negative values indicating greenness. Similarly, the b* value reflects the yellow-blue axis, where positive values denote yellowness and negative values denote blueness. Measurements were taken at three different points on each berry, and the average was calculated. For each treatment and ripeness stage, three fruits were randomly selected. Dry matter content was determined by drying approximately 100 g of berries at 70 °C for seven days, until a constant weight was achieved; the weight loss corresponded to moisture content using the formula, MC (%) = [(Fresh weight - Dry weight)/Fresh weight] × 100 (Gil-Ortiz *et al.*, 2020). Shelf-life was monitored by storing fruits in a room at ambient conditions (25 ± 2 °C, 60-75% RH) and recording the number of days until visible signs of deterioration (shrivelling, fungal growth, or softening) appeared.

Biochemical properties analysis

The quantification of sugar contents was done using the Fehling reagent technique (AOAC, 1990). The modified HPLC technique was used to analyze tomato berries for carotenoids (Hart and Scott, 1995). Fresh samples of 10 mg were extracted in 5 ml of a chloroform-MeOH (1:1) solution for 30 minutes. The resulting precipitate was centrifuged, filtered using a 0.45 μm syringe filter, and then placed in a 1.5 mL amber vial for later use. The lights in the room were turned down to prevent carotenoids from degrading too quickly during sample processing. The 10 μL sample was then run through a 1260 Infinity HPLC system fitted with a Nova-

Pak C18 4 μm (3.9 ×150 mm) column and a diode array detector set to 470 nm. Carotenoids were separated using an isocratic mobile phase of 100% methanol flowing at a rate of 1.5 ml min^{-1}. The peaks were identified and quantified using lycopene and β-carotene standards ranging in concentration from 0 to 50 ppm, and results were represented as mg100 g^{-1} fresh weight. TSS was measured directly in the berry juice with a digital hand refractometer (ERMA, Tokyo, Japan) at room temperature with a 58-92% range. The pH of the berry juice was measured using the technique described by Ranganna *et al.* (1977). The fruit was thoroughly cut and crushed in a blender to make juice. After calibrating the pH meter with standards (pH 4.0 and 7.0), the pH of tomato juice was measured using a Microcomputer pH meter fitted with a glass electrode. The vitamin C content of tomato berries was measured using a modified version of the HPLC technique described by Spínola *et al.* (2012). Extraction with a metaphosphoric acid solution of 5% was performed on 5 g of a paste made from ground fresh fruit. Following centrifugation, a 0.20 μm syringe filter was used to remove any remaining debris. Subsequently, the sample aliquot (10 μL) was analyzed at 254 nm using a 1260 Infinity HPLC system outfitted with an Acquity UPLCHSS T3 (2.1 × 100 mm, 1.8 μm, Waters) column and diode array detector. The ascorbic acid peak was separated using a mobile phase of an isocratic aqueous 0.1 % (v/v) formic acid solution flowing at 0.3 mL min^{-1}. Vitamin C levels were determined using the calibration curve (mg 100 g^{-1} fresh weight).

Mineral composition analysis

In accordance with the method suggested by Miyazawa (1984), the Ca and Mg contents were determined using an atomic absorption spectrophotometer. Spectrophotometry was used to ascertain the digest's overall K and P content. A combination of sulfuric and perchloric acids at a ratio of 7:3 was used to digest the 0.2 g dried berry sample (Soil Survey Staff, 2014). The quantification of iron concentration in berries was determined by converting iron to its ferric form using an oxidizing agent, namely potassium persulfate. This was treated with potassium thiocyanate, forming a red-colored compound known as ferric thiocyanate, as described by Ranganna (1986). A total of 5 mL of the fruit extraction was mixed with 0.5 mL of concentrated sulfuric acid (H_2SO_4), 1.0 mL of saturated potassium persulfate ($K_2S_2O_8$), and 2 mL of 3 N potassium thiocyanate (KSCN). The mixture was then diluted with deionized water to a final volume of 15 mL. The solution's absorbance was promptly measured at a wavelength of 480 nm after mixing. The calibration curve was prepared using iron standards with 5 to 25 mg L^{-1} concentrations.

Experimental design and statistical analysis

The experiment followed a randomized complete block design (RCBD), with tomato maturity stages (mature green, breaker, pink, and red) serving as temporal blocks representing distinct harvest timings. Within each block, four humic acid treatments (0 mg L^{-1}; 25 mg L^{-1}; 50 mg L^{-1} and 75 mg L^{-1}) were randomly assigned to plots of 15 plants each. This design allowed for comparison of treatment effects within uniform physiological stages. Statistical analysis was performed using SPSS, version 25.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to evaluate treatment effects, and Turkey's HSD test was applied for post post-hoc comparisons at a 5% significance level ($p \leq 0.05$).

Results

Color assessment of berries

Humic acid at different times during tomato maturation led to statistically significant changes in the fruit exocarp's lightness (L*) and green-red chromaticity (a*). At the same time, it did not affect the exocarp's blue-yellow chromaticity (b*). The berry exocarp showed the highest L* values (59.80 to 61.43) in mature green berries with the control (HA$_0$) treatment, while red berries showed lower L values (49.80-52.70), consistent with ripening progression (Table 1). The results (L* > 50) suggested that the fruits did not undergo severe darkening. The redness indicator (a*) of exocarp color was most positively correlated with pink and red berries. HA_{50} and HA_{75} treatments resulted in the highest redness (a* values of 13.21 and 10.20, respectively), compared to 12.60 in the control; HA_0 (Figure 1). In contrast, negative values indicated no redness was present in fruits collected at the mature green or, in some instances, the breaker stage. It is probable that applying humic acid at the breaker stage to onward its ripening phases attributed all the fruit to have a yellowish criterion, shown by the positive values of the yellowness (b*) parameter in Table 1.

Table 1. Effect of foliar-applied HA on the tomato berries' lightness (L*), green-red chromaticity (a*), and blue-yellow chromaticity (b*) at different stages of maturity

Humic acid	Maturity stage	L*	a*	b*
0 mg L^{-1}	MG	59.80 ± 1.62^{ab}	-4.94 ± 0.88^{ef}	$23.90 \pm 1.43^{a\text{-}e}$
	BK	55.43 ± 0.29^{cd}	-5.22 ± 0.47^{ef}	26.34 ± 4.06^{abc}
	PK	$53.72 \pm 0.53^{c\text{-}f}$	7.85 ± 1.23^{cd}	$23.69 \pm 2.05^{b\text{-}e}$
	RD	52.70 ± 0.19^{ef}	12.60 ± 1.36^{a}	23.38 ± 1.12^{cde}
25 mg L^{-1}	MG	58.34 ± 0.54^{b}	-4.25 ± 0.43^{f}	21.00 ± 0.53^{de}
	BK	55.07 ± 0.29^{cde}	4.10 ± 0.39^{f}	$24.41 \pm 1.15^{a\text{-}e}$
	PK	53.02 ± 0.48^{def}	10.11 ± 0.48^{b}	28.17 ± 0.21^{ab}
	RD	52.37 ± 0.68^{fg}	12.27 ± 0.74^{a}	27.24 ± 1.14^{abc}
50 mg L^{-1}	MG	61.43 ± 0.60^{a}	-4.10 ± 0.38^{f}	20.57 ± 0.96^{e}
	BK	$54.77 \pm 0.18^{c\text{-}f}$	6.34 ± 0.52^{de}	21.70 ± 1.91^{de}
	PK	50.20 ± 0.53^{gh}	8.23 ± 0.58^{bcd}	22.80 ± 1.12^{cde}
	RD	49.80 ± 1.81^{h}	13.21 ± 0.26^{a}	22.88 ± 1.34^{cde}
75 mg L^{-1}	MG	60.64 ± 0.68^{ab}	-4.60 ± 0.28^{ef}	20.58 ± 1.07^{e}
	BK	55.73 ± 0.64^{c}	5.52 ± 0.28^{ef}	$25.11 \pm 1.57^{a\text{-}d}$
	PK	$53.33 \pm 0.44^{c\text{-}f}$	8.51 ± 1.07^{bc}	$23.75 \pm 0.14^{b\text{-}e}$
	RD	49.90 ± 1.68^{gh}	10.20 ± 0.67^{b}	28.29 ± 1.12^{a}
$LSD_{(0.05)}$		2.49	2.04	4.52
P-value		0.03	0.05	0.15
CV (%)		2.73	16.08	11.34

HA_0, 0 mg L^{-1}; HA_{25}, 25 mg L^{-1}; HA_{50}, 50 mg L^{-1}; HA_{75}, 75 mg L^{-1}; MG, mature green stage; BK, breaker stage; PK, pink stage; and RD, red stage. Mean values in each bar followed by the same letter are not significantly different according to the Duncan test ($p \le 0.05$). Values are mean ± SE

Dry matter content of berries

The dry matter content of tomato fruit showed a notable impact (with a significance level of $P \le 0.05$) in response to foliar applications of humic acid at different stages of fruit maturity (Figure 2). The pulp obtained from tomatoes picked during the red stage of fruit ripening without HA had the lowest dry matter, reaching 5.81%. In contrast, the fruits collected at the mature green stages of fruit ripening and treated with HA had a notably elevated dry matter content (8.82%) in their pulp compared to those harvested during the later phases of fruit ripening. The dry matter content of tomatoes collected at the mature-green stage was about 1.52 times more than that harvested at the red stage (Figure 2).

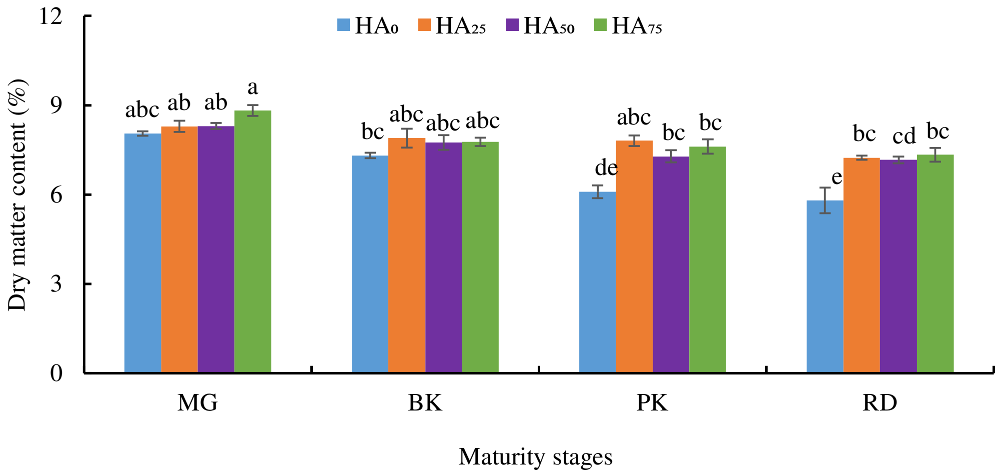


Figure 2. Effect of foliar-applied humic acid on fruit dry matter content (%) of cherry tomato berries at four maturity stages

HA_0, 0 mg L^{-1}; HA_{25}, 25 mg L^{-1}; HA_{50}, 50 mg L^{-1}; HA_{75}, 75 mg L^{-1}; MG, mature green stage; BK, breaker stage; PK, pink stage; and RD, red stage. Mean values in each bar followed by the same letter are not significantly different according to the Duncan test ($p \leq 0.05$). Vertical bars indicate standard errors of means

Moisture content of berries

The moisture level of tomato berries is an essential indicator in determining their quality standards, as it prevents early shriveling and promotes ripening till the senescence stage (Figure 3). The present research found a statistically significant variation ($p < 0.05$) in moisture content throughout several development stages and with the application of humic acid. The highest moisture content (94.19%) was recorded in red tomato samples, while the lowest (91.18%) was found in mature green tomato samples obtained from the control group and the HA_{75} treatment group, respectively (Figure 3). The results indicate a reduction in moisture content across all instances of humic acid treatment throughout the mature green stages.

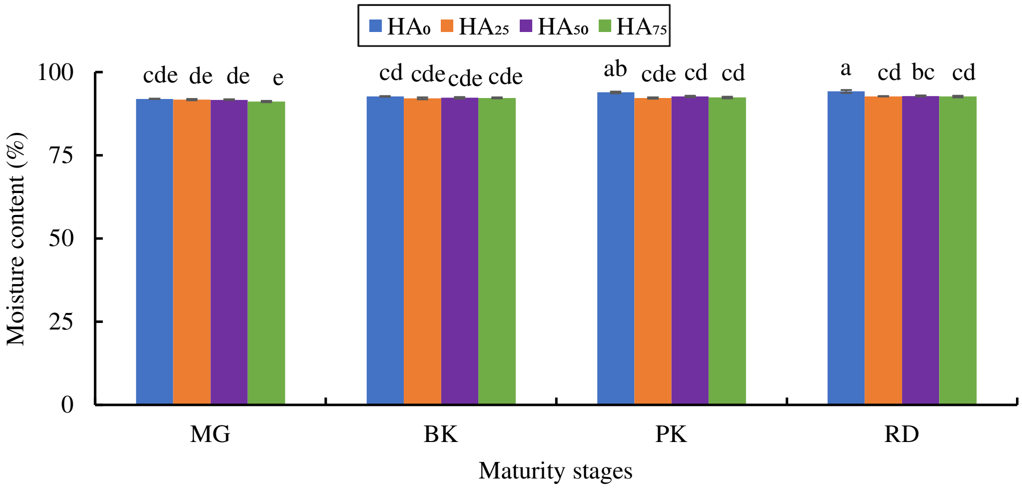


Figure 3. Influence of foliar-applied humic acid on fruit moisture content (%) of cherry tomato berries at four maturity stages

HA_0, 0 mg L^{-1}; HA_{25}, 25 mg L^{-1}; HA_{50}, 50 mg L^{-1}; HA_{75}, 75 mg L^{-1}; MG, mature green stage; BK, breaker stage; PK, pink stage; and RD, red stage. Mean values in each bar followed by the same letter are not significantly different according to the Duncan test ($p \leq 0.05$). Vertical bars indicate standard errors of means

Shelf-life of berries

A statistically significant difference ($p \leq 0.05$) was found in relation to the shelf-life of berries subjected to humic acid treatment. Humic acid application, specifically HA_{75}, extended shelf-life in all maturity stages, with the most pronounced effect observed at the mature green stage (Figure 4). mature green berries exhibited the longest shelf-life of 15.58 days (HA_{75}), compared to 13.28 days that was noticed in the control (HA_0). Similarly, breaker stage berries showed an increase from 13.11 days (HA_0) to 15.40 days (HA_{75}). However, at the red stage, the effect was less pronounced, with shelf-life ranging from 10.15 days (HA_0) to 10.49 days (HA_{75}). These results suggest that while the maturity stage remains the dominant factor, humic acid application, particularly at higher concentrations (HA_{75}), can enhance postharvest longevity, especially at earlier ripening stages.

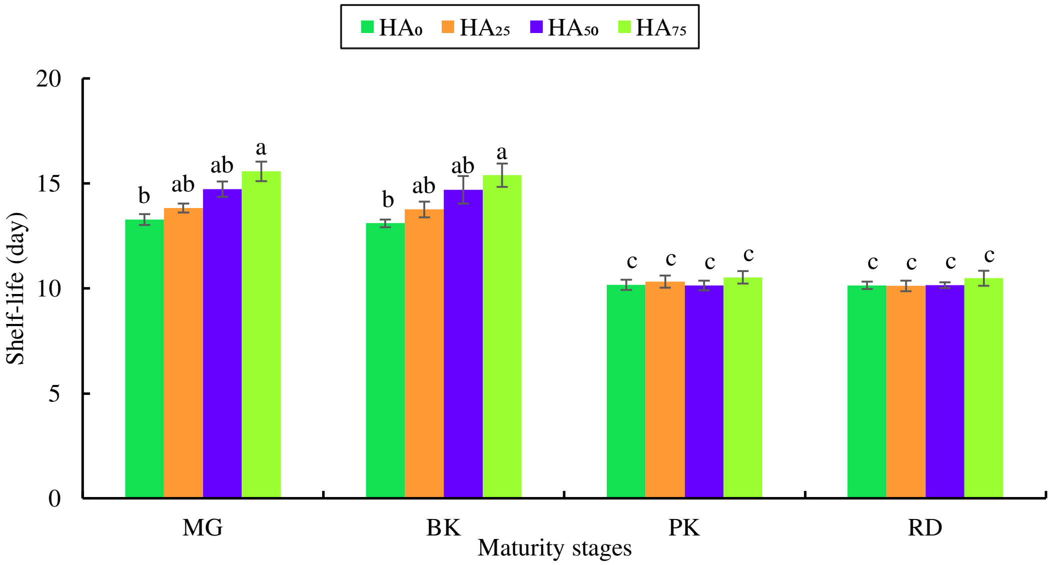
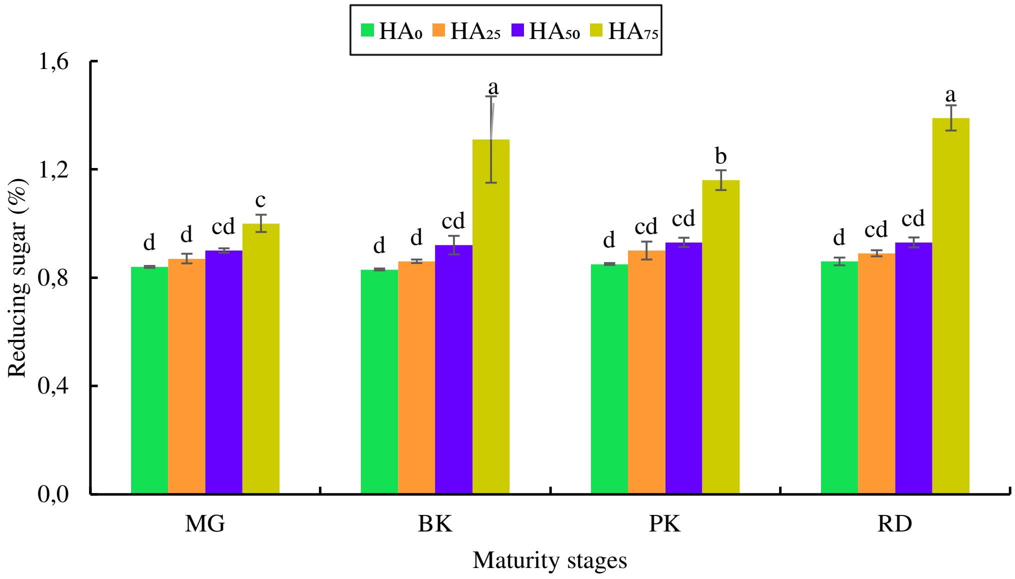


Figure 4. Effect of foliar-applied humic acid on shelf-life (days) of cherry tomato berries at four ripening stages

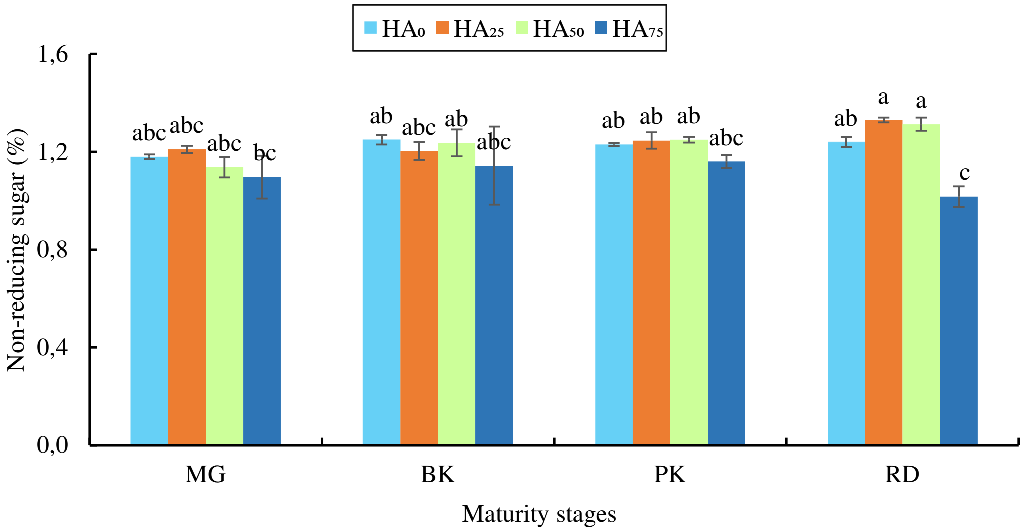
HA_0, 0 mg L^{-1}; HA_{25}, 25 mg L^{-1}; HA_{50}, 50 mg L^{-1}; HA_{75}, 75 mg L^{-1}; MG, mature green stage; BK, breaker stage; PK, pink stage; and RD, red stage. Mean values in each bar followed by the same letter are not significantly different according to the Duncan test ($p \leq 0.05$). Vertical bars indicate standard errors of means

Comparative biochemical study of berries

The metabolic compositional attributes of tomato berries were considerably influenced by maturity stage, particularly in the presence of humic acid (Figure 5-7). Significant differences in non-reducing sugar content were observed at the red maturity stage, particularly with the HA_{75} treatment, which showed a slight decline compared to other treatments (Figure 5B). Although pH values increased slightly with fruit maturity, the overall range remained (3.95-4.07), and humic acid treatments did not significantly alter pH levels of the fruits (Figure A1). The study revealed that reducing sugar, beta-carotene, lycopene, total soluble solids (TSS), and vitamin C increased as the fruit matured. The biochemical constituents were modulated by the utilization of humic acid throughout all harvesting phases, with the fruits collected at the red stage of maturity exhibiting the highest levels of metabolic components. The levels of reducing sugars in the tomatoes obtained from the red and mature green stages exhibited variability, ranging from 1.39% at HA_{75} to 0.84% at HA_0 (Figure 5A). The antioxidant concentration decreases when the fruit is harvested at a mature green stage (Figure 6). The levels of beta-carotene (Figure 6A) and lycopene (Figure 6B) in the tomatoes collected at the red stage with the treatment of HA_{75} were found to be 13.68% and 0.14% higher, respectively, compared to the tomatoes harvested at the green stage without HA application (13.90% and 0.11%). The tomatoes that were collected in the green stage without the application of HA exhibited the lowest vitamin C content, 22.90% (Figure 6C) and total soluble solids (TSS), 5.17% (Figure 7). In contrast, tomatoes harvested at the red stage with the application of HA_{75} showed an increase of 21.30% in vitamin C and 1% in TSS. The berry juice pH increased with the advancement of the maturity stage but did not vary significantly even with the application of humic acid (Figure A1).



(A)



(B)

Figure 5. Effect of foliar-applied humic acid on reducing sugar (A) and non-reducing sugar (B) content in cherry tomato berries at different ripening stages

HA_0, 0 mg L^{-1}; HA_{25}, 25 mg L^{-1}; HA_{50}, 50 mg L^{-1}; HA_{75}, 75 mg L^{-1}; MG, mature green stage; BK, breaker stage; PK, pink stage; and RD, red stage. Mean values in each bar followed by the same letter are not significantly different according to the Duncan test ($p \leq 0.05$). Vertical bars indicate standard errors of means

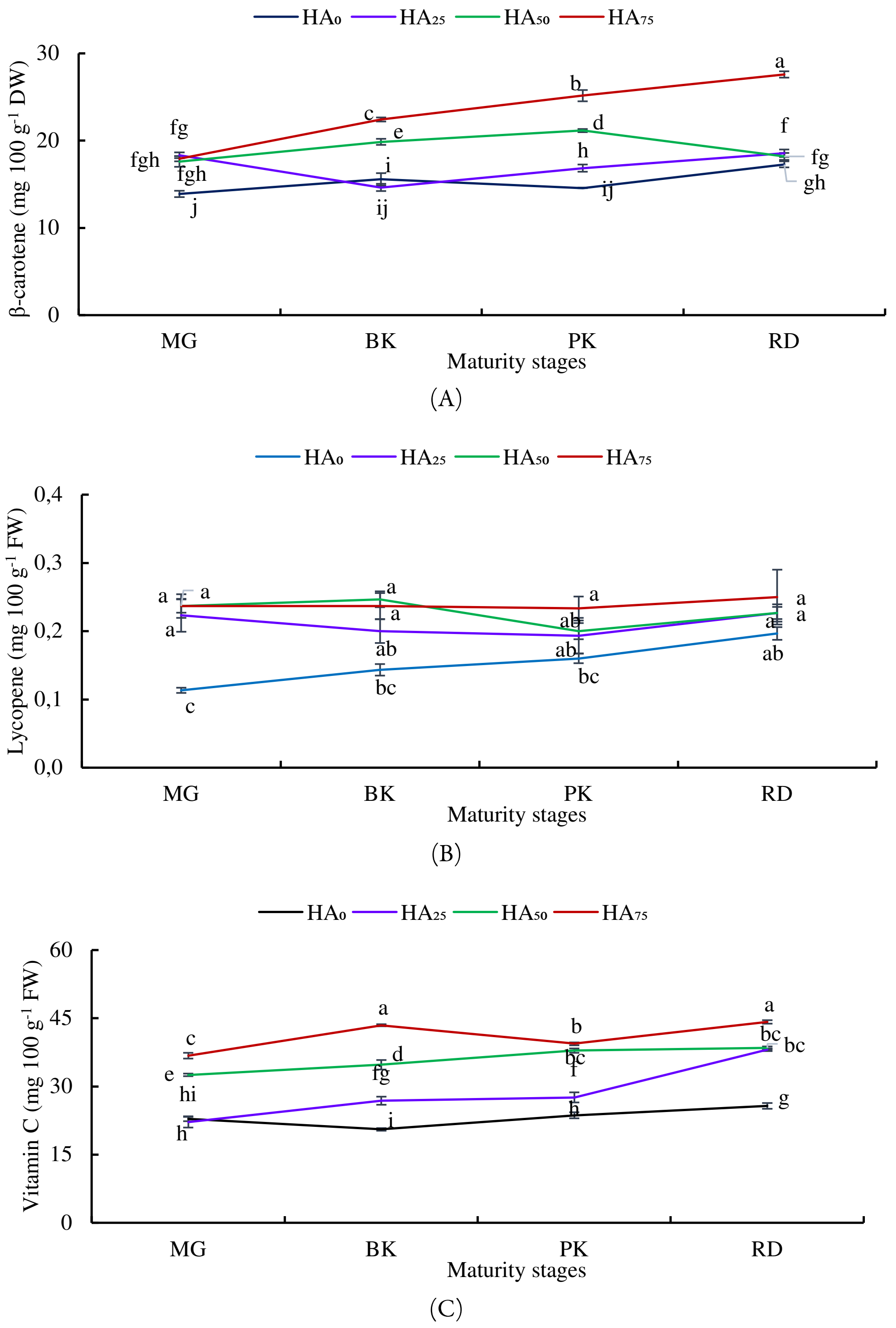


Figure 6. Effect of foliar-applied humic acid on antioxidant properties: β-carotene (A), Lycopene (B) and Vitamin C (C) content in cherry tomato berries at different ripening stages

HA_0, 0 mg L^{-1}; HA_{25}, 25 mg L^{-1}; HA_{50}, 50 mg L^{-1}; HA_{75}, 75 mg L^{-1}; MG, mature green stage; BK, breaker stage; PK, pink stage; and RD, red stage. Mean values in each bar followed by the same letter are not significantly different according to the Duncan test ($p \leq 0.05$). Vertical bars indicate standard errors of means

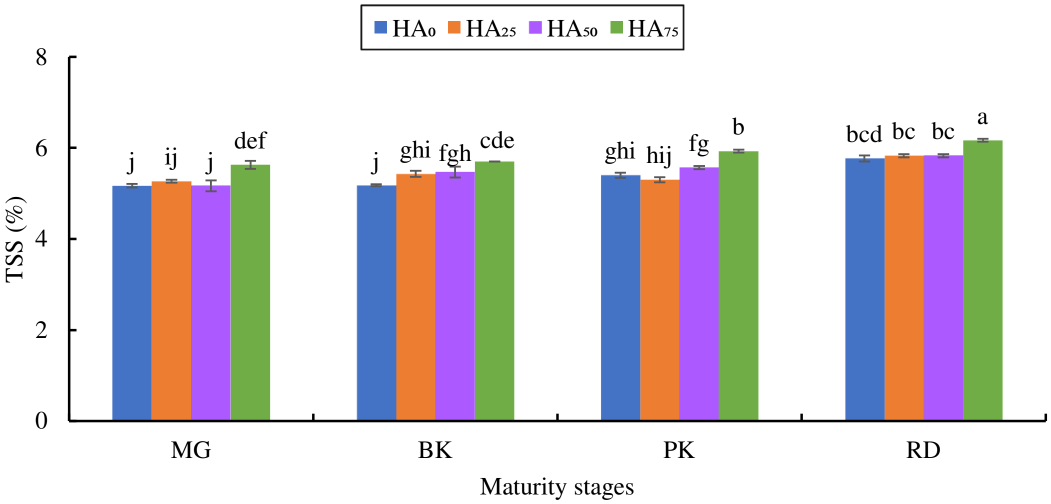


Figure 7. Effect of foliar-applied humic acid on total soluble solids (TSS, %) in cherry tomato berries at four ripening stages

0 mg L^{-1}; HA$_{25}$, 25 mg L^{-1}; HA$_{50}$, 50 mg L^{-1}; HA$_{75}$, 75 mg L^{-1}; MG, mature green stage; BK, breaker stage; PK, pink stage; and RD, red stage. Mean values in each bar followed by the same letter are not significantly different according to the Duncan test ($p \leq 0.05$). Vertical bars indicate standard errors of means

Comparative study of berries' mineral content

The levels of magnesium (Mg), phosphorus (P), and iron (Fe) in tomato berries were considerably influenced by the foliar application of humic acid at different stages of maturity (Table 2). The interaction between the maturity stages and the application of humic acid had the most pronounced effect on the nutritional characteristics of fruits. However, the levels of calcium (Ca) and potassium (K) were found to be insignificant (Table 2). Regarding the nutritional composition of tomato pulp at various stages of maturity, it was observed that the application of HA$_{75}$ increased the levels of magnesium (2.39%), phosphorus (0.71%), and iron (213.54 ppm. However, it should be noted that the interaction between the maturity stage and humic acid mostly influenced the variations in Mg, P, and Fe content. Additionally, these elements were present in higher rates in fruits picked at the red stage (Table 2).

Table 2. Effect of foliar-applied humic acid on mineral content (Ca, Mg, K, P, Fe) of cherry tomato berries across four ripening stages

Humic acid	Maturity stage	Ca	Mg	K	P	Fe
0 mg L^{-1}	MG	1.83 ± 0.03^{a}	0.79 ± 0.06^{h}	1.15 ± 0.01^{a}	0.31 ± 0.01^{i}	119.61 ± 0.97^{k}
	BK	1.96 ± 0.05^{a}	0.84 ± 0.01^{gh}	1.26 ± 0.08^{a}	0.33 ± 0.01^{hi}	125.93 ± 0.73^{jk}
	PK	1.88 ± 0.04^{a}	0.93 ± 0.07^{gh}	1.27 ± 0.09^{a}	0.34 ± 0.01^{hi}	129.08 ± 1.80^{ijk}
	RD	2.08 ± 0.10^{a}	1.74 ± 0.14^{ef}	1.41 ± 0.08^{a}	0.36 ± 0.00^{gh}	135.39 ± 2.84^{hij}
25 mg L^{-1}	MG	2.07 ± 0.04^{a}	0.83 ± 0.09^{gh}	1.54 ± 0.00^{a}	0.38 ± 0.01^{fg}	136.69 ± 3.04^{hij}
	BK	2.00 ± 0.11^{a}	0.86 ± 0.18^{gh}	1.56 ± 0.10^{a}	0.38 ± 0.01^{fg}	138.96 ± 3.33^{hi}
	PK	2.23 ± 0.04^{a}	0.98 ± 0.08^{gh}	1.70 ± 0.04^{a}	0.40 ± 0.01^{ef}	142.32 ± 4.09^{gh}
	RD	1.94 ± 0.42^{a}	1.05 ± 0.11^{g}	1.77 ± 0.04^{a}	0.43 ± 0.01^{e}	144.01 ± 2.27^{gh}
50 mg L^{-1}	MG	2.50 ± 0.28^{a}	1.94 ± 0.03^{de}	1.78 ± 0.02^{a}	0.45 ± 0.02^{d}	152.44 ± 4.40^{fg}
	BK	1.96 ± 0.06^{a}	1.70 ± 0.12^{f}	1.81 ± 0.04^{a}	0.46 ± 0.01^{d}	158.76 ± 5.61^{ef}
	PK	2.01 ± 0.05^{a}	1.99 ± 0.01^{cd}	1.85 ± 0.02^{a}	0.54 ± 0.02^{c}	166.90 ± 3.14^{de}
	RD	2.22 ± 0.03^{a}	2.11 ± 0.06^{bcd}	1.89 ± 0.01^{a}	0.56 ± 0.02^{c}	172.95 ± 4.57^{cd}
75 mg L^{-1}	MG	2.70 ± 0.12^{ab}	2.26 ± 0.03^{ab}	2.16 ± 0.13^{a}	0.61 ± 0.01^{b}	176.74 ± 4.32^{bcd}

	BK	2.59 ± 0.04^{a}	2.19 ± 0.05^{abc}	2.29 ± 0.07^{a}	0.63 ± 0.00^{b}	181.74 ± 4.89^{bc}
	PK	2.95 ± 0.03^{a}	2.33 ± 0.01^{ab}	2.35 ± 0.04^{a}	0.64 ± 0.01^{b}	185.86 ± 6.18^{b}
	RD	2.96 ± 0.06^{a}	2.39 ± 0.01^{a}	2.44 ± 0.01^{a}	0.71 ± 0.01^{a}	213.54 ± 7.53^{a}
$LSD_{(0.05)}$		0.41	0.24	0.17	0.03	11.92
P-value		0.16	0.00	0.88	0.02	0.05
CV (%)		10.88	9.15	5.88	4.37	4.62

Ca, Mg, K, and P are expressed as % and Fe as ppm. Abbreviations are as follows, HA_0, 0 mg L^{-1}; HA_{25}, 25 mg L^{-1}; HA_{50}, 50 mg L^{-1}; HA_{75}, 75 mg L^{-1}; MG, mature green stage; BK, breaker stage; PK, pink stage; and RD, red stage. Mean values in each bar followed by the same letter are not significantly different according to the Duncan test ($p \leq 0.05$). Values are mean ± SE

Discussion

The maturity stage predominantly influenced the tomato berry quality, with foliar-applied HA serving as a modulatory factor. Exocarp color development is considered the most significant external feature determining the ripening stage (Sharma *et al.*, 2020). Hence, in our study, color changes in tomato berries (Table 1), particularly redness (a*) and lightness (L*), increased with ripening (Figure 1) due to notable accumulation of lycopene and β-carotene (Figure 6). The present findings are also aligned with the earlier research on tomato, which indicated that color transitioning from green to orange and eventually red is attributed to the synthesis of carotenoids (Suliman *et al.*, 2020; Tsouvaltzis *et al.*, 2023; Disciglio *et al.*, 2024). Foliar-applied HA treatments, especially HA_{75}, also enhanced these pigments, likely by stimulating biosynthetic pathways through hormone-like activity (Canellas *et al.*, 2020; Souza *et al.*, 2022) and sugar composition (Sarkar *et al.*, 2019). Additionally, these color changes happened due to chlorophyll's breakdown, resulting from the conversion of chloroplasts into chromoplasts (Gonzali and Perata, 2021; Kasampalis *et al.*, 2021).

Dry matter content peaked in mature green berries, and it gradually declined during the transition from the light red to red phases of fruit maturation. This is because fruit harvesting in its early stages promotes transpiration, decreasing fruit water content and indirectly increasing dry matter content (Tsouvaltzis *et al.*, 2023). While ripening berries consistent with increased moisture content might be due to metabolic shifts (Figure 5 and Figure 7) as a result of the timing of fruit harvesting (Alenazi *et al.*, 2020; Gaweda and Jedrszczyk, 2020). On the other hand, HA (HA_{75}) application improved dry matter retention, possibly by enhancing nutrient uptake (Table 2) and root cell membrane permeability (Bhardwaj *et al.*, 2020; de Moura *et al.*, 2023; Jing *et al.*, 2022), which led to an increase in tomato berries' fresh and dry matter content (Pillajo *et al.*, 2024).

Shelf-life was longest in mature green berries and decreased with progress in maturity, confirming that early harvest stages confer greater postharvest resilience (Lufu *et al.*, 2020; Prasad *et al.*, 2023). The current results validated that the red or pink stages exhibited a lower shelf-life because of faster water loss through transpiration and evaporation (Kasampalis *et al.*, 2021; Prasad *et al.*, 2023). This research revealed that an extended harvesting time resulted in the initiation of metabolic processes in tomatoes immediately after harvest. Although the maturity stage remains the dominant factor, foliar-applied HA has a partial impact on these characteristics. HA_{75} extended shelf-life across all stages, particularly at mature green, as shown in Figure 4. This extended shelf-life may be attributed to delayed ripening physiology, potentially associated with reduced moisture loss (Figure 3), as suggested in previous studies on humic acid-treated fruits (Abdelkader *et al.*, 2019; Suliman *et al.*, 2020).

Biochemical traits, including reducing sugars, vitamin C, lycopene, β-carotene, and total soluble solids (TSS), increased with ripening and were further elevated by foliar-applied HA treatments. Red berries had an extended time frame for harvesting compared to the stages from mature green to onward, consistently showed the highest values, suggesting enhanced ascorbic acid (Abdelkader *et al.*, 2019) and metabolic activity (Jiang *et* *al.*, 2022) during late maturation. Consequently, leading to a series of physiological and biochemical transformations throughout the harvesting period that significantly change the series of quality features (Ngcobo *et al.*, 2020; Bai *et al.*, 2023). These enhancements are supported by **Figures 5-7**, which show significant increases in antioxidant compounds and TSS with HA_{75} treatment. Generally, sugar compositions start to break down at breaker stages of maturity, largely responsible for their distinctive sweetness and acidity (Huang *et al.*, 2020), flavor intensity (Nicolas *et al.*, 2023) and hydrophilic antioxidant properties in tomato fruits (Cheng *et al.*, 2023), linked to applied HA (Bhardwaj *et al.*, 2020; de Moura *et al.*, 2023). This is because HA has stimulatory effects primarily on auxin and cytokinin activity in plants (Puglisi *et al.*, 2018), enhances root morphology, nutrient uptake, crop performance and fruit quality (Vujinović *et al.*, 2020). The pH of tomato berries showed minimal variation across humic acid treatments and maturity stages, remaining within a narrow acidic range. This stability suggests that while HA influences metabolic and antioxidant profiles, it does not significantly alter the fruit's acid-base balance. The slight increase in pH with ripening is consistent with previous findings in climacteric fruits, where organic acid degradation contributes to reduced acidity (Priyankara *et al.*, 2017; Huang *et al.*, 2020; Tsouvaltzis *et al.*, 2023). However, no significant changes across the treatments imply that HA does not interfere with this natural progression.

Mineral uptake, particularly of Mg, P, and Fe, was significantly improved by foliar-applied HA across the different maturity stages of berries. Red stage berries showing the highest concentrations of these macro elements with foliar-applied HA due to its symbolizes effect (de Moura *et al.*, 2023). These enhancements may result from HA's chelating properties and its role in stimulating plant growth morphological characteristics and root development (Vacelik *et al.*, 2022; Zhao *et al.*, 2020) and foliar nutrient absorption (Vujinović *et al.*, 2020; Pillajo *et al.*, 2024). The mineral composition data in **Table 2** confirm that HA_{75}-treated red berries had the highest levels of these nutrients. Similarly, earlier studies have been reported as humic substances leading to an increased rate of essential micro and macronutrients (Abdelkader *et al.*, 2019; Pizzeghello *et al.*, 2020; Massimi *et al.*, 2023; Pillajo *et al.*, 2024) under diverse abiotic conditions (de Moura *et al.*, 2023). The present study indicates that HA boosted P uptake, and its consequences may be direct and indirect (Karimzadeh *et al.*, 2021; Hemati *et al.*, 2022; Massimi *et al.*, 2023). The observed improvements might be due to quasi-hormonal substances belonging to hormonal groups inside the plant influenced by HA's source, quantity, receptor type, pH, and application method (de Moura *et al.*, 2023).

Overall, HA application positively influenced tomato berry quality, but its effects were stage dependent. Harvesting at mature green or breaker stages combined with HA treatment offers a promising strategy to balance quality, shelf life, and marketability.

Conclusions

This study demonstrates that foliar application of humic acid, particularly at 75 mg L^{-1}, significantly influences the physiological, biochemical, and nutritional quality of tomato berries across maturity stages. Color development, particularly in terms of lightness (L*) and redness (a*), was primarily driven by fruit ripeness, while HA enhanced these visual attributes, likely by promoting carotenoid synthesis. Dry matter content was highest in fruits harvested at the mature green stage and decreased as ripening progressed, with HA application amplifying dry matter retention and modulating moisture levels. Biochemical parameters, such as reducing sugars, lycopene, beta-carotene, vitamin C, and total soluble solids (TSS), increased with fruit maturity and were further elevated by HA treatment, especially at higher concentrations (HA_{75}). Additionally, HA positively influenced mineral uptake, notably increasing the magnesium, phosphorus, and iron content in ripened berries. Shelf-life analysis confirmed that tomatoes harvested at the mature green stage exhibited the greatest postharvest longevity, and HA application, particularly at 75 mg L^{-1}, further extended their storage potential. These findings underscore the critical role of harvest timing and HA in determining tomato quality, storability, and nutritional value. Harvesting tomatoes at the mature green or breaker stage, combined with targeted HA treatments, can enhance fruit quality, extend shelf-life, and reduce post-harvest losses, making it a viable strategy for long-distance transportation and market sustainability. However, these findings are preliminary and based on a single-season open-field trial conducted under subtropical conditions. Further multi-location and multi-season studies are recommended to validate the consistency of these responses in diverse agroecological settings. Additionally, the results specifically pertain to the 'BARI Tomato 11' cultivar (Jhumka), a cherry-type tomato grown in Bangladesh. Given the known phenotypic variability in ripening behaviour, antioxidant capacity, and postharvest traits among tomato genotypes, further comparative studies are needed to evaluate whether similar responses to foliar-applied humic acid across maturity stages are observed in other commercially popular cultivars, commonly used in processing and fresh markets.

Authors' Contributions

Conceptualization: AB and MDS; Execution: AB; Data curation: AB; Formal analysis: MDS; Funding acquisition: MDS; Investigation: AB and MDS; Methodology: AB, MDS and MAG; Project administration: MDS; Resources: MDS and MAG; Software: MDS and MOKA; Supervision: MDS and MAG; Validation: MDS; Visualization: MDS; Writing- original draft: MDS; Writing - review and editing: MAG, MTN and MOKA.

All authors read and approved the final manuscript

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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Appendix

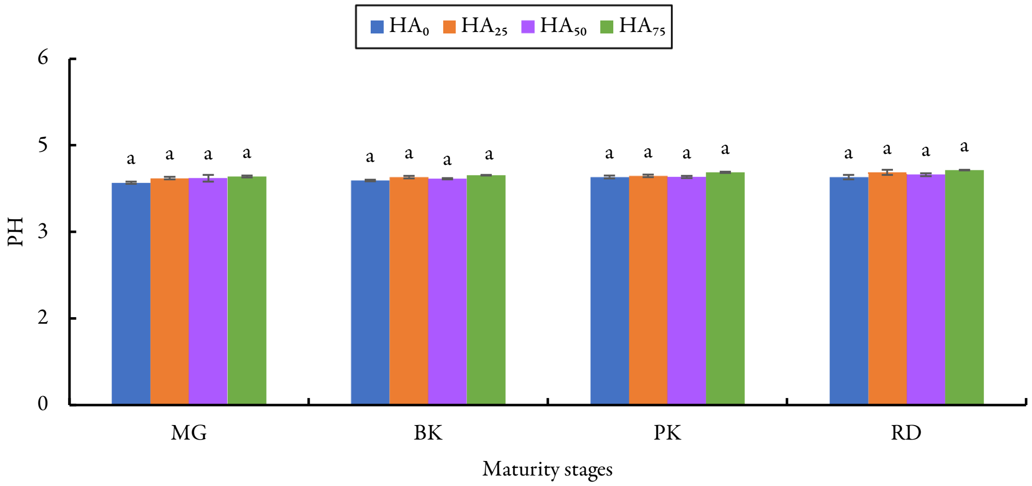

Figure A1. Effect of foliar-applied humic acid on pH in cherry tomato berries at four ripening stages
Abbreviations are as follows, HA_0, 0 mg L^{-1}; HA_{25}, 25 mg L^{-1}; HA_{50}, 50 mg L^{-1}; HA_{75}, 75 mg L^{-1}; MS, mature green stage; BK, breaker stage; PK, pink stage; and RD, red stage. Mean values in each bar followed by the same letter are not significantly different according to the Duncan test ($p \leq 0.05$). Vertical bars indicate standard errors of means

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