Research Article

Maturity Dependent Changes in Post-Harvest Physiological, Antioxidant and Anti-Microbial Attributes of Tomato

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Abstract | This study evaluates changes in physiological i.e., ash content, moisture content, pH, (total titratable acidity) TTA, (Total soluble solids) TSS, antioxidant activity using 2, 2-diphenyl, 1-picryl hydrazyl (DPPH) and antimicrobial activity in Roma VF tomato variety was assessed within three different mature levels viz., green, pink and red. Outcomes from recent study pointed out that maturity levels involved to change the quality of tomato. Moisture and ash increased with the maturity ranged from 89.38±0.54 to 93.34±0.48 and 0.35±0.02 to 0.41±0.02% respectively. pH increases 4.23±0.15 to 4.77±0.15 and % Titratable acidity decreased 0.77±0.03 to 0.62±0.01 with the increase in ripening. In addition, increase in TSS (5.1-6.0 °brix) and juice content with maturity stages (20.3-28.6 ml 500gm-1) from green to red tomatoes. Antioxidant activity (DPPH) continuously increased from green to red tomato level (37.5-46.3 %). Lycopene content is an antioxidant also increased from 22.1-33.5 mg/kg. Vitamin C content increase from green to pink (4 mg/100 g f.w to 17mg/100g f.w), then decrease till reached towards final stages red 14.5mg/100g f.w indicating decaying of fruits. Antimicrobial activity revealed that mature green has higher activity comparison to half and full ripe stages. Green stages showed ZOI of antimicrobial (10.7-11.6mm) and for antifungal (11.2-11.8mm) in methanol and acetone extracts. It was concluded from the results that ripening stage has effects on the nutritional values which point out that the ideal maturity stage to maintain optimal postharvest storage ability and nutritional value is breaking stage of fresh tomato which is the most suitable for storage.

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Introduction

Tomato (*Lycopersicon esculentum* Mill.) from the family of *Solanaceae* is one of the worldwide important vegetables has essential because it can consume as fresh and also in processed forms (Mutari and Debbie, 2011). Tomatoes are perishable in nature, in Pakistan during high temperature the production decreases and causes the loss of supply of tomato in summer season as compared to winter (Tahir *et al.*, 2021). Values of tomatoes are increasing on daily basis as tomatoes are rich in many nutritional components

like, antioxidants and fibers etc. (Hussain *et al*., 2001; Sgherrri *et al*., 2008).

Tomato is as an important economical crop for farmers also it can overcome the unemployment ratio in the processing and manufacturer industries by enhancing the employment rate (Barbara *et al*., 2005). In general, the tomato production has increased because of consumer's preference (Gupta *et al*., 2011).

Post-harvest maturity levels affect the shelf-life of tomato. Tomato is generally harvested at pinkish color maturity stage with maximum size by (Frary *et al*., 2000). However, at this pinkish edible maturity stage causes the post-harvest losses (Sankar *et al*., 2002) due to alteration in nutritional and physiological attributes as, fresh produces are alive at post harvest condition and physiological process are going on at this stage.

Several factors like ecological, pre- and postharvest attributes involved to cause the post harvest changes in tomato. Good post harvest practices retain the nutritional component also increase post harvest life of fruit as different mature levels (Suslow and Cantwell, 2006). Based on USDA colour chart table tomato has six mature levels. Full green, breakers, turning stage, pinkish, partial red and full red (Tadesse *et al*., 2012). Physical attributes like, colour, flavor, and texture also nutritional value is used to examine the eating quality. Colour is one of the main components as consumers prefer tomatoes with appealing color and indication for eating quality (Bhandari and Lee, 2016; Borji and Jafarpour, 2012).

Sugary flavor is due to total sugars like sucrose. Sucrose is depending on (TA) citric acid. Bitterness is related to the level of organic acid usually covers the sugary flavor and will be changed in mature level (Garcia and Barrett, 2005). Accumulation of sugars and acids during ripening can affect the post harvest performance of tomato (Adedeji *et al*., 2006).

Same as nutritional quality, antioxidant activity is also an important parameter in term of post harvest attributes. Oxidation processes are essential for living systems, but during stress condition ROS are produced. Higher production of ROS (reactive oxygen species) can lead to tissue injury. So, a defense system based on antioxidant that helps to protect the cells from the dangerous molecules (Sies, 1997). Antioxidant protects the cells from free radicals that can injure the cells and may play a key role in several human diseases related to heart, cancer and others. Tomatoes have numerous natural antioxidants compounds for instance: lycopene (bio active compound), phenolic compounds and ascorbic acid etc., that showed high antioxidant properties (Martinez *et al*., 2010; Valverde *et al*., 2002; Shi and Le Maguer, 2000; Umair *et al*., 2020). Oxidative stress cause diseases in human, although by the use of antioxidants that are naturally present in fruits such as vitamin C can fight the radicals and prevent the cell to damage (Giovannucci,1999). Tomato fruit has a lot of antioxidants which linked to health benefits. Ascorbic Acid helps the plant for survival in both biotic and abiotic stress (Kuzniak and Sklodowska, 2005) and protect the cells from damage by improving the fresh produces shelf life (Malacrida *et al*., 2006).

Intake of several forms of tomatoes contains a huge amount of phytochemicals that keeps check on unstable molecules (Borguini and Torres, 2009). Consumption of tomatoes as fresh or processed have been shown to be prevented against cardiovascular diseases (Rao and Rao, 2007). The objectives of the recent experiments included to evaluate the physiological, antimicrobial and antioxidant parameters of fruit at different stages of maturity.

Materials and Methods

Collection of samples

The trial was conducted to assess the physico-chemical quality of three different mature levels of tomato variety Roma VF (Green, Pink and Red). The tomatoes were collected from the fruit market of Karachi. On the basis of color the samples put in sterile bag with proper labeled and were transported to the lab for further analysis.

Sample preparation

After collection, Samples were washed with 0.2% sodium hypochlorite (NaOCl) to remove contamination on the surface and dried; fruits from each mature level 1500gm were used and samples were subjected to ambient environmental condition of a normal temperature (21+2°C) and relative humidity of (75-78%) for analysis.

Physico-chemical analysis

Juice content: Tomato juice was extracted from

selected maturity level (Green, Pink and Red) by using a juice extractor. For analysis, a glass cylinder was used and expressed in milliliter (ml) of juice per kilogram fruit weight (ml/kg) (Gharezi *et al*., 2012).

Moisture content: Ten gram of tomato was chopped into a pre-weighed petri-dish and dried in an oven at 105oC for 4hrs and then cooled. Weigh the petri dish (Owusu *et al*., 2012).

$$Moisture\% = W1-W2/W1-W0 \times 100$$

Where; The weight of empty crucible = W0; Weight of crucible plus samples = W1; Weight of crucible plus oven-dried sample = W2.

Ash content: It was determined following methodology described by (AOAC, 2010). Two gram of the chopped tomato sample was placed in a crucible and put in a muffle furnace at 600°C for 3-4 hrs. After that the crucible was cooled and the weight was taken. The percentage of ash was calculated by:

$$Ash\ \% = Weight\ of\ Ash / Weight\ of\ original\ sample \times 100$$

Total soluble solids (TSS)

TSS of tomatoes was find out by Digital refractometer (Hanna's, HI 96801) was used for total soluble solids in Brix (Umair *et al*., 2019).

pH values

The pH values were measured with bench top pH meter (HANNA, HI 3512, USA) (Umair *et al*., 2019).

Total titratable acidity (TTA)

Take 10 ml of a tomato juice, and dilute untill 50 mL distilled water, titrate it against 0.1% NaOH solution at a pH of 8.17 (Adubofuor *et al*., 2010) and the result was calculated as g/L.

TSS and acidity ratio of tomato

The TSS and acidity ratio of fruit pulp was analyzed using the formula:

$$TSS\ and\ acidity\ ratio\ of\ fruit\ pulp = \%\ TSS\ content / \%\ Acidity$$

Antioxidant activity

Antioxidant activity was measured by DPPH radical scavenging activity reported by (Coklar and Akbulut, 2017) with slight modifications. One-gram samples were added to 10 mL of water and centrifuged 10,000 rpm for 10 min and filtered. 2.0 mL extract was mixed with 2.0 mL of 0.1 mM DPPH radical solution. The absorbance of the sample at 517nm was read after 30 min incubation in the dark at room temperature. The DPPH radical-scavenging activity (RSA) was calculated by the following formula:

$$DPPH\ radical\text{-}scavenging\ activity = [1-(A_{517}\ sample / A_{517}\ Blank)] \times 100\%$$

Ascorbic acid content

The ascorbic acid content was analyzed by titration method using a 2, 6-dichlorophenolindophenol sodium salt solution (AOAC, 1990).

Lycopene content

Tomato powder (1.0 g) of each sample was accurately weighed into 200 ml flask, then 100 ml of hexane: acetone: Ethanol in ratio 2:1:1 was added. The above mixture was centrifuged for 15 min at 4000 rpm. Then, 3mL D.W was added. The tube was then shaken for 5 min and rest for 2 min at room temperature to allow phase separation. The upper n-hexane layer was used to determine the absorbance by using a spectrophotometer BMS 1602 at 503 nm against the blank (Obadina *et al*., 2018; Umair *et al*., 2021).

$$Lycopene\ (mg/kg) = (A_{503} \times 171.7) / W$$

Where; A_{503} = absorbance at 503 nm; W = weight of sample.

Antimicrobial and antifungal activity

Antimicrobial and antifungal activity of extracts was assessed by well diffusion method as proposed by (Gavade *et al*., 2015; Umair *et al*., 2020). For this study both Gram positive (*S. aureus*) and Gram negative (*E. coli*) food-borne pathogenic bacteria and fungi (*A. niger* and *A. flavus*) were used. This was performed by determining ZOI (zone of inhibition), which is rapid and easy method. Pure cultures of bacteria were sub cultured into nutrient broth and incubated at 37°C for 24 hours, while fungi in Potato dextrose agar at 35°C for 5-7 days. Petri plates containing 15-20 ml Muller Hinton medium (for bacteria) and potato dextrose agar (for fungi) were seeded with bacterial and fungal strains. For analysis of extracts, six wells (9 mm in diameter) were prepared by using sterile cork borer. Using sterile micropipette tips (100 µL) of the extracts of each maturity stage of tomato was pipette

out into the wells in all the plates. The plates were pre-incubated for 1hr at room temperature and incubated at 37°C for 24 hr for antibacterial and antifungal activities respectively. After incubation the plates were determined by measuring the diameter of ZOI as indicated by clear area which was free from growth of microbes was measured (Balouiri *et al*., 2016).

Results and Discussion

Tomato fruits with different mature levels (Green, Pink and Red) were studied for postharvest physiological attributes. The characteristics such as; fruit juice content, pH, TA, TSS, moisture content, ash content, antioxidant activity, lycopene content, antimicrobial and antifungal activity were determined.

Moisture

As tomato fruits ripening progressed the MC was increased. Table 1 shows the variation in MC with three maturity stages. The range of MC was recorded (89.38-93.55 %) Green, Pink and Red tomatoes. Variation in moisture content level for tomatoes regards the ripening stages previously published by (Hossain *et al*., 2010; Sulieman *et al*., 2011) range of MC (88.19-90.67%), John *et al*., 2020 (91-95%). While, the food with low moisture content level has longer shelf life results from Table 1 shows that green tomatoes with low MC compared to pink and red tomatoes.

Table 1: *Moisture and ash % of tomato fruit at different maturity stages.*

Maturity stages	Moisture (%)	Ash (%)
G	89.38±0.54	0.35±0.02
P	92.79±0.23	0.38±0.01
R	93.55±0.48	0.41±0.02

G: Green; P: Pink; R: Red

Ash

The average ash contents of Green, Pink and Red tomatoes as 0.35, 0.38, and 0.41% with variation among the stages reported in Table 1. Present results was close to previous results 0.2-0.4% studied by (Hossain *et al*., 2010; Suleiman *et al*., 2011; John *et al*., 2020) and 0.34-0.43% (Tilahun, 2013).

From the results it was observed that red tomatoes, has more ash content and has more mineral than green and pink stage. Difference in Ash % content in each maturity stage due to absorption of minerals by root in water medium.

Juice content

The juice content in stages was varying, lower juice content (20.3 ml 500gm-1) in green and higher (28.6 ml 500gm-1) juice content was recorded in red tomato (Figure 1). Results was agreed with previous reported by (Beckles, 2012) juice content was recorded in from (31.66 -38.66 ml kg-1) juice content in green to red tomatoes. The difference in juice content could be due variation during harvest in maturity stage.

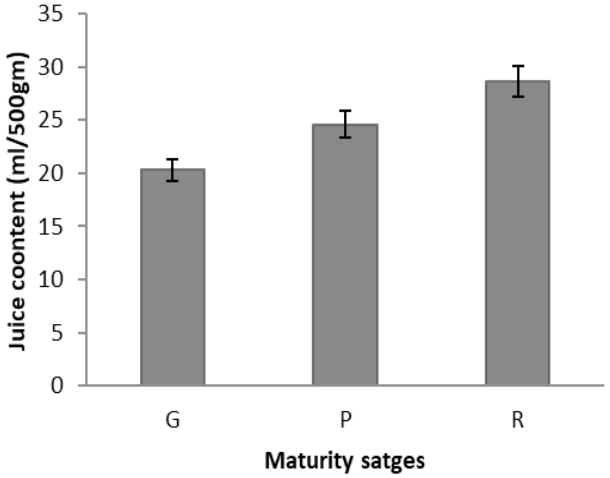

Figure 1: *Juice content of tomato fruit at different maturity stages (G: green, P: pink, R: red).*

TSS

The total soluble solids in a solution show tomato quality. variation in TSS was observed in present results from different stages maximum TSS was shown in red tomatoes 6.00 brix while, lowest in green 5.1 brix and 5.3 was recorded in pink tomatoes Figure 2.

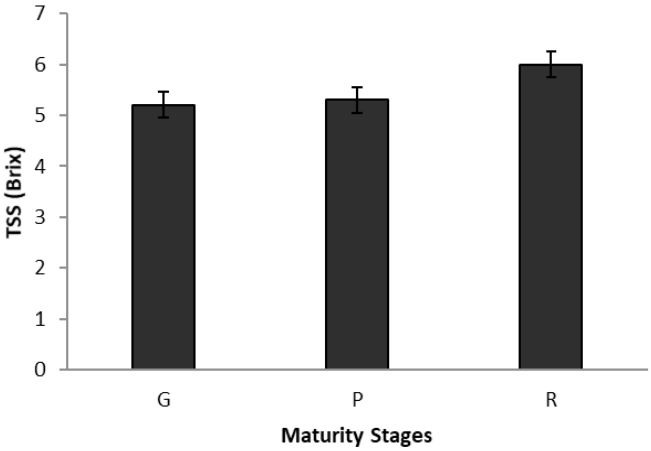

Figure 2: *Total soluble solids of tomato fruit at different maturity stages (G: green, P: pink, R: red).*

TSS of fruits is the sum of sugars, acid and other components (Pinho *et al*., 2011). The total soluble solids (TSS) values at green and red (Figure 2) agree

with (Getinet *et al*., 2008) that was between 4.0 -6.00 Brix from mature to full ripe in tomatoes.

TSS is an important parameter for maturity indicator as it increases with the maturity increase (Tilahun *et al*., 2017a) also, several factors that are responsible for variation in TSS level during different maturity stages are postharvest storage time and temperature (Tilahun *et al*., 2017b; Rai *et al*., 2012).

pH and TTA

pH content of tomato fruits varied according to ripening stages, present results showed the results that pH increase from green to red (Table 2). Lowest pH value is (4.3) was recorded in green compared by pink (4.5) and red stage (4.8). Same observations also reported by (Tilahun *et al*., 2019) in tomato fruit during storage. Tolesa and Workneh (2017) selected four cultivars of tomato and observed variation in pH from 3.43 to 4.63. Increase in pH value as maturity increase also reported by (Gautier *et al*., 2008). During storage several enzymatic reactions causes' pectin breakdown and leads minor changes in pH level that ultimately undergoes changes in physiological process and spoilage of fresh produces occur.

Table 2: *pH, acidity and pH-acidity ratio of tomato fruit at different maturity stages.*

Maturity stages	pH	TTA (%)	TSS and acid ratio
G	4.23±0.15	0.77±0.03	6.8
P	4.50±0.10	0.69±0.01	7.6
R	4.77±0.15	0.62±0.01	9.6

TTA: Total Titratable Acidity, G: Green; P: Pink; R: Red

The variation in TTA (%) are reported in (Table 2). The TA decreased as the maturity stage proceeded. It was found that TTA decrease from green to red ripe tomato samples. The green tomatoes contain 0.77% followed by pink 0.69% and red 0.62%.

The TTA content of tomatoes was also supported by previously results that TTA decreases with ripening of tomato fruit. The minimum TTA was showed in red, while the maximum showed in the breaker level (Tolesa and Workneh, 2017; Pila *et al*., 2010). Physiological processes (ripening and respiration etc) are involved that reduces the organic acids in different mature levels (Tolesa and Workneh, 2017; Moneruzzaman, *et al*., 2008; Singleton and Gortner, 1965). Respiration is main process that causes conversion of acid into sugars during maturity proceeds (Pinho *et al*., 2011).

TSS and acidity ratio

Current results assessed to increased sugar to acid ratio as ripening proceed. Full red ripening stage tomato observed the maximum ratio is 9.6 and green stage showed lowest ratio 6.7 (Table 2). Similar results reported by (Singleton and Gortner, 1965), during storage of tomatoes with different treatments application and (Sammi and Masud, 2007) reported increase of TSS to acid ratio with the ripening in pineapple. Taste is related to the bitterness and sweetness of fruit fructose and citric acid are essentially components. Sugar to acid ratio between sugar and acid is an important aspect for flavor of the fruits.

(DPPH) radical scavenging activity

Antiradical activity by free radical (DPPH) scavenging assay was assessed. The DPPH assay is simple method for evaluating the antiradical activity.

Radical scavenging percentage increases from green to red tomatoes in term of percent (37.5-43.6 %) Figure 3. same results previously reported by (Bhandari and Lee, 2016) among tomato ripening stages. The antioxidant activity was addressed to analyze the capability of the antioxidants that slow down the oxidation process in the fruit and help to prevent from spoiling. Also, antioxidant compound were mainly responsible for the phamacological activities (Umair *et al*., 2018).

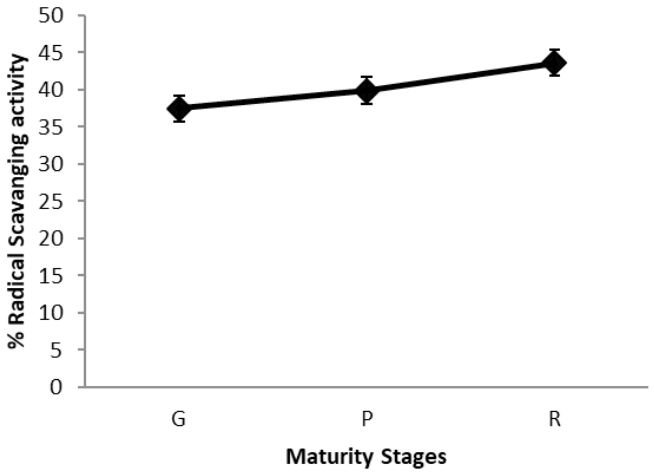

Figure 3: *DPPH radical scavenging activity of tomato fruit at different maturity stages (G: green, Pink: P R: Red).*

Ascorbic acid content

Current study showed changes in the vitamin C content in three maturity levels. Green stage has

minimum amount is about 5.4 mg/100 g f.w. was recorded and the higher 17 mg/100 g f.w. in pink while 14.5 mg/100 g f.w. in red stage Figure 4. Similar results were reported by (Soare *et al.*, 2019) observed 4.8-20.7 from green phase to full ripe phase. Tigist *et al.* (2013) reported values from 14.6 and 21.7 mg/100 g f.w. in ripened tomato. Also, Sima *et al.* (2009) showed from 20.42–24.28 mg/100 g f.w. Several enzymatic oxidation processes are involved that enhance and decline the AA content. Increased in ascorbic acid level showed ripening while decrease indicate the decaying (Pila *et al.*, 2010; Dragan *et al.*, 2010).

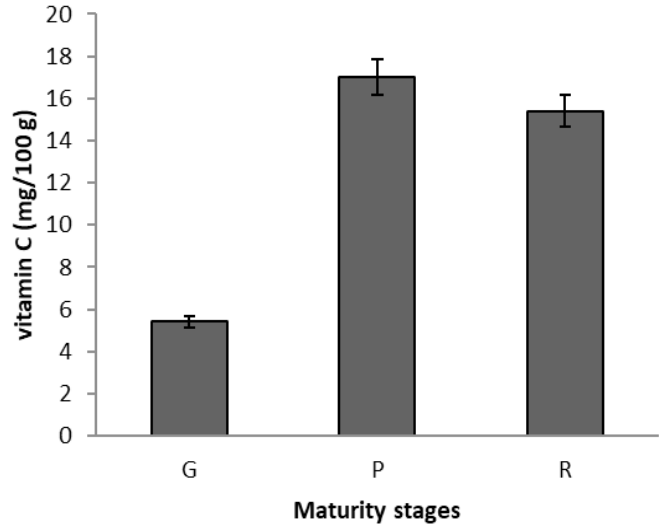

Figure 4: *Vitamin C content of tomato fruit at different maturity stages (G: green, Pink: P, R: Red).*

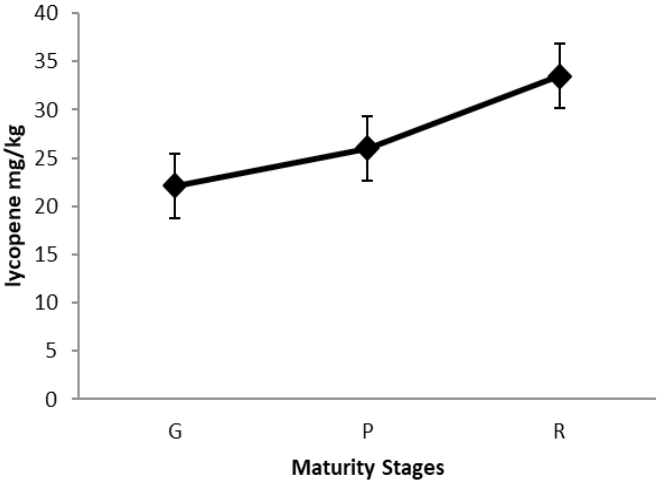

Figure 5: *Lycopene content of tomato fruit at different maturity stages (G: green, Pink: P, R: Red).*

Lycopene content

Current results showed changes in lycopene content Figure 5. The lowest lycopene content was observed in green tomato 22.1mg/kg while highest in red tomatoes as 33 mg.kg and pink tomatoes showed 26 mg/kg. Lycopene content increased as ripening proceeds reported by different authors (George *et al.*, 2004; Brandt *et al.*, 2006; Dumas *et al.*, 2003; Helyes *et al.*, 2006). Luna-Guevar *et al.* (2014) reported 24.5 mg/kg lycopene content in light red tomatoes while, (Toor and Savage, 2005) obtained 28 mg/100gm. Fraser *et al.* (1994) stated that the changing in color from green to red due to breakdown of pigments from xanthophylls to lycopene, β, and γ carotenes in mature fruits.

Antimicrobial and antifungal activity

Total of three extracts of tomato stages Green, Pink and Red (acetone and methanol) were used to examine the antimicrobial and antifungal potential. In agar well diffusion assay for antimicrobial activity, Mature green stage gave 10.7-11.6mm MIZD, O. half ripe showed 09.3-10.4 mm and full ripe 09-9.3mm ZOI in methanol and acetone. It also reveals that the bacterial culture (*E. coli* and *S.aureus*) used for the activity were fully active. Results of different maturity stages extract against bacterial strains Table 3.

Table 3: *Zone of inhibition (mm) of bacteria.*

Maturity stages	*E. coli*		*S. aureus*	
	Methanol	Acetone	Methanol	Acetone
G	11.3 ±0.5	10.7±0.6	11.6±0.3	11.4±0.5
P	10.1± 0.3	9.3±0.3	10.4±0.1	9.7±0.1
R	9.3 ±0.3	9.0±00	9.2 ±0.4	9.1±0.1

G: Green; P: Pink; R: Red

Antifungal activity, green level gave 11.2-11.8mm ZOI while, pink and red ripe 09.7-10.8mm and full ripe 09.3-09.8 mm ZOI in methanol and acetone against *A.niger* and *A.flavus* represented in Table 4.

Table 4: *Zone of inhibition (mm) of fungi.*

Maturity stages	*A. niger*		*A. flavus*	
	Methanol	Acetone	Methanol	Acetone
G	11.6 ±0.2	11.2±0.3	11.8±0.4	11.7±0.2
P	10.8± 0.2	9.7±0.6	10.5±0.5	10.0±0.0
R	9.5 ±0.8	9.4±0.4	9.8 ±0.8	9.3±0.6

G: Green; P: Pink; R: Red

The tomato maturity stage extracts possess antibacterial and antifungal activity due to phytochemical and secondary metabolites. Earlier research provided information that phenolic compounds have antibacterial activity, Antimicrobial and antifungal activity of tomato paste also reported by (Murali *et al.*,

2013) within different extracts ether and chloroform showed higher activity.

Conclusions and Recommendations

Tomato is a profit crop as it is cost-effective and high nutritional importance. Maturity levels are main aspects that linked with the physiological characteristic of tomato fruit in term of fresh and processed form. The results form study showed that TTA is inversely correlated with pH and TSS. While, antioxidant properties lycopene and DPPH scavenging activity increase with the increase in maturity level. Enhancement in maturity can increase in various antioxidant compounds that help against cardiovascular and other diseases. From the results it can be concluded that for maintaining physiological quality and marketability value of fruit, selection of maturity levels is most important parameters for fresh used ripened red is used and for marketing in distance area green stage necessary to be selected.

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Novelty Statement

Physiological, antioxidant and antimicrobial attributes of tomato variety Roma VF (Green, Pink and Red) was not evaluated before.

Author's Contribution

Hafiza Mehwish Iqbal: Designed, analysed physiologically and wrote manuscript.
Qurrat Ul Ain Akbar: Gave technical support and conceptualization.
Saqib Arif: Gave technically input at every step and reviewed the manuscript.
Shahid Yousaf: Statistical application and analysed.
Salman Khurshid: Provided assistance throughout analysis.
Saqib Jabbar: Managed the article.
Neelofar Hamid: Reviewed the manuscript.
Uzma Sitara: Data analysis and wrote the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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