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Biochemical Composition and Antioxidant Activity of Different Types of Tomatoes Affected by Ethylene Treatment

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ABSTRACT
The effect of ethylene on biochemical composition and antioxidant activity in beefsteak, heirloom and cluster type of tomatoes were determined. For that purpose, tomato fruit were harvested at breaker maturity stage and divided into two groups one of which was applied with 150 µL L^{-1} ethylene while another remained untreated. Ethylene treated and untreated control fruit were stored at 12 °C and 90±5% relative humidity for 35 days with subsamples removed every 7 days for quality analysis. After each removal time, fruit were kept at 20 °C for additional 3 days to determine shelf life performance. Ethylene treatment enhanced the breakdown of total chlorophyll and accumulation of lycopene and carotenoid contents. At the end of cold storage and shelf life period, the maximum antioxidant activity, carotenoid and flavonoid contents were recorded in ethylene treated heirloom type tomatoes. It can be concluded that ethylene treated heirloom type tomatoes exhibited maximal postharvest quality as compared to beefsteak and cluster type of tomato in term of biochemical composition and antioxidant activity after 35 days of cold storage and shelf life.

Keywords: Ethylene, Antioxidant, Cold storage, Shelf life, Tomato quality

1. Introduction

Tomatoes are vital part of human nutrition around the world. Scientific studies have shown that tomatoes contain high amount of carotenoid, antioxidant, lycopene and are associated with dietary intake that reduces the risk of chronic diseases, cancer, osteoporosis and cardiovascular diseases in humans (Rao et al. 1998; Frusciante et al. 2007; Bhowmik et al. 2012). The presence of carotenoids, especially lycopene, ascorbic acid, vitamin E, phenolic compounds and different antioxidant properties in tomatoes affect the human health (Frusciante et al. 2007; Bhowmik et al. 2012).

Ripening is genetically programmed process which show substantial changes in color, texture, flavor and aroma (Alexander & Grierson 2002). Tomatoes being a climacteric fruit are sensitive to ripening hormone ethylene. Exogenous application of ethylene in climacteric fruit can trigger and enhance the ripening process (Tucker 1993). It is thought that ethylene regulates the formation of carotenoids present in the chloroplast through synthesis of new enzymes and influence mitochondria based organic acid concentrations (Mcglasson 1970). Ethylene does not only affect biochemical composition but also increases respiration rate and aging of fruit and vegetables (Prasanna et al. 2007).

The higher antioxidant activity of tomato could mitigate the effect of ethylene as ripening process involved series of physiological and biochemical changes in which antioxidant properties play fundamental role (Jimenez et al. 2002). The signal transduction by ethylene in important for secondary metabolites synthase. The biosynthesis of flavanol is modulated by ethylene through transcription factor (Lewis et al. 2011). During stress the polyphenol oxidase (PPO) activity is increased through ethylene signalling which supports its involvement in the defence resistance of plant (Bosch et al. 2014). In tea, the phenolic compounds, flavonoids and antioxidant activity were increased by ethylene signalling (Ke et al. 2018).

The nutritional value, color and flavor of tomatoes are mainly dependent on the ratios of lycopene, β-carotene, ascorbic acid and sugars (Nguyen & Schwartz 1999). Epidemiological studies have shown that lycopene and β-carotene serve as an antioxidant and functional food (Tonucci et al. 1995). The assessment of the effects of different tomato varieties on the synthesis of carotenoid and other phenolic compounds are important to enhance the concentration of these antioxidant compounds. The studies conducted previously confirmed that amount of carotenoid and other antioxidant compounds in tomato fruit exhibit differences among genotypes (George et al. 2004). According to Viskelis et al. (2007) the Lithuanian cultivar 'Rutuliai' displayed the highest lycopene content (over 10 mg 100 g^{-1}) which was 1.6-fold more than hybrid 'Admiro' and 2-fold higher than hybrid

'Kassa'. Radzevicius et al. (2013) reported that the different cultivars of tomato have wide variations in term of ascorbic acid contents. The increasing economic importance of tomato throughout the world as a functional food have necessitated to find out the effect of ethylene on the biochemical composition and antioxidant capacity of different types of tomato and therefore this experiment was conducted.

2. Material and Methods

Beefsteak (cv. Tybif), heirloom (cv. Yuksel Koy) and cluster (cv. Merkur) types of tomato were harvested at 'breaker stage'. There was definite break in color from green to tannish yellow, pink or red on not more than 10% of the surface. All fruit were picked from a commercial greenhouse in Antalya, Turkey (36°59'57.3" N and 30°51'20.4" E). During the entire vegetation period, uniform irrigation and fertigation management procedures were applied to the tested tomato types. All fruit were harvested on the same day and immediately brought to the postharvest physiology laboratory at Akdeniz University, Antalya, Turkey. Fruit with any defects i.e. decayed, bruised and non-uniform, were discarded and the remainder were split into two groups. The first group of tomato fruit were applied with 150 µL L^{-1} of ethylene at 20 °C in a 20 m^3 room and the second group were left untreated (control). Both groups of fruit samples were stored at 12 °C and 90±5% relative humidity for 35 days. Fruit samples for different quality analysis were removed from cold room at 7 days intervals and they were also kept at 20 ºC and 60±5% relative humidity for additional 3 days to simulate shelf life performance.

Tomato puree was utilized for analysis of total chlorophyll, lycopene, total phenolic, carotenoid, flavonoid, ascorbic acid contents and antioxidant activity. Homogenization of tomato samples for all quality analysis ultra turrax homogenizer (IKA-Labortechnique Typ T 25 JANKE & KUNKEL GMBH & CO.KG) was used. The samples absorbances for all quality analysis were read through Analytik Jena AG Specord 40 ST spectrophotometer.

The total chlorophyll contents were determined according to the method of Lichtenthaler & Wellburn (1983). Tomato puree of 3 g was homogenized with 80% acetone through ultra turrax homogenizer. The centrifuge of homogenized samples was performed at 8600 x g for 5 min under 4 °C. After centrifuge, sample supernatant was used for determination of chlorophyll content. The supernatant was read through Specord 40 ST spectrophotometer against blank 80% acetone solvent at the wavelengths of 646 and 663 nm. The total chlorophyll contents of tomato fruit were computed through equation (1) given below and given as g kg^{-1} fresh weight (fw).

$$\text{Chlorophyll a} = 12.21 \times A_{663} - 2.81 \times A_{646}$$
$$\text{Chlorophyll b} = 20.13 \times A_{646} - 5.03 \times A_{663}$$
$$\text{Total chlorophyll} = (C_a + C_b) \quad (1)$$

The method explained by Fish et al. (2002) was used for the determination of lycopene contents in different types of tomato. For that purpose, the tomato samples were homogenized through homogenizer and 0.5 g of samples were weighed and put in 50 mL test tubes. 5 mL butylated hydroxytoluene (BHT) prepared with acetone (0.05% w/v), 5 mL ethanol (95%) and hexane at 10 mL concentration were added to sample. Prepared samples were shaken at 4 °C for 5 min at 180 rpm through shaker. After, 3 mL distilled water was added to sample and shaken again for 5 min. Then, the samples were left to separate the phase for 5 min at room temperature to obtain colored layer of hexane at the top surface. The supernatant containing hexane layer was read in spectrophotometer at 563 nm of absorbance. Data obtained from the measurements were calculated by equation (2) below and reported as mg kg^{-1} fw.

$$\text{Lycopene (mg kg}^{-1}) = A_{503} \text{ x } 0.0312/\text{kg, sample} \quad (2)$$

A_{503} = The absorbance value at 503 nm
0.0312 ($\mathcal{E}$) = Extinction coefficient of lycopene.

The extraction of fruit samples was done with 80% methanol for antioxidant activity, total phenolic and total flavonoid contents analysis. For this purpose, tomato puree of 20 g and 80% methanol of 20 mL was homogenized with the help of Ultra-Turrax homogenizer. The samples were centrifuged at 8600 x g for 20 min at 4 °C after homogenization. The antioxidant activity of tomato was determined according to DPPH method described by Benvenuti et al. (2004). For that purpose, 1 m*M* DPPH* radical solution of 600 µL was taken in 4 test tubes and 1, 2, 3 and 4 mL of extracted samples were added into test tubes. After, 80% methanol was used to bring total volume in each tube to 6 mL. The mixture in tubes were vortexed and left to incubate in dark at room temperature for 15 min. Additionally, the control sample was prepared by taking 600 µL of 1 m*M* DPPH* radical solution and 5.4 mL of methanol in a tube and allowed to incubate in a dark place at room temperature for 15 min. After incubation samples absorbance were read at 517 nm wavelength by using spectrophotometer against blank solvent of 80% methanol and control sample. Percent inhibition values proportionate to each sample volume were computed by using equation (3).

$$\% \text{ inhibition} = A_{DPPH} - A_{Extract} / A_{DPPH} \text{ x } 100 \quad (3)$$

A_{DPPH}: DPPH control sample absorbance value; $A_{Extract}$: Test sample absorbance value

The inhibition values and sample volumes were used to obtain a graph. Linear regression analysis was applied to the graph, sample curve and equation explaining the curve was acquired. Equation was used for calculation of EC_{50} (effective concentration) value of the sample. The antioxidant activity by DPPH method is determined through EC_{50} value. The EC_{50} value reflects amount of antioxidant substances present in fruit and vegetables sample that inhibit 50% of DPPH radical. Decrease in EC_{50} value exhibit increase in the antioxidant activity (Cemeroglu 2010). The EC_{50} value was reported in g kg^{-1} fw EC_{50}.

The total carotenoid content analysis was performed by using the method of Witham et al. (1971). For that purpose, tomato puree of 0.25 g was homogenized with 10 mL 80% acetone for 3-4 min through ultra-turrax homogenizer and 80% acetone solvent was used to bring the total volume of sample to 15 mL. After, the samples were centrifuged at 8600 x g for 10 min at 4 °C after homogenization. The carotenoids content was determined by using the supernatant fraction. The samples absorbance used for chlorophyll a, chlorophyll b and carotenoid were 663 nm, 645 nm and 440 nm respectively. The samples absorbance was read through spectrophotometer against a blank solvent of 80% acetone. The total carotenoid content was calculated through equation (4) and expressed as g kg^{-1} fw.

Chlorophyll a (g kg^{-1}) = [12.7 (D663) – 2.69 (D645)] x V/1000 x W Chlorophyll b (g kg^{-1}) = [22.9 (D645) – 4.68 (D663)]×V/1000×W
Carotenoids (g kg^{-1}) = [4.69 (D440) – (chlorophyll a + chlorophyll b)×0.286]×V/1000×W (4)

V = Extract volume
W = Sample quantity

The total flavonoid content of tomatoes was analysed through the procedure explained by Karadeniz et al. (2005). In 50 mL tube, 1 g of tomato puree, distilled water of 5 mL and 5% $NaNO_2$ (Merck) of 0.3 mL were added, respectively. The test tubes were closed and strongly mixed. 5 min later 0.6 mL of 10% $AlCl_3.6H_2O$ (Merck) was added and after 5 min 2 mL of 1 mol L^{-1} NaOH was added. The distilled water was used to bring total volume in tube to 10 mL. Tubes were then vortexed and samples absorbance was measured at wavelength of 510 nm using spectrophotometer against solvent of blank 80% methanol. The standard calibration curve prepared with catechin were used to compute the total flavonoid content of tomatoes and given as mg kg^{-1} fw.

The total phenolic contents analysis was performed according to the Folin-Ciocalteu method explained by Spanos and Wrolstad (1990). Extract of 0.1 mL was blended with distilled water of 0.9 mL and 0.2 mol L^{-1} N Foline-Ciocalteu reagent of 5 mL. After 3 min, aqueous solution of Na_2CO_3 (75 g L^{-1}) at 4 mL concentration was added into blend and samples were kept for 2 h in the dark at room temperature. The samples absorbance was recorded at the wavelength of 765 nm against blank 80% methanol solvent through spectrophotometer. The total phenolic contents calculated were reported as mg of gallic acid equivalent per kg (mg kg^{-1} GAE) fw.

The total ascorbic acid contents analysis was conducted according to Cemeroglu (2010). For that purpose, the tomato samples were extracted with 6% metaphosphoric acid and in 50 mL tube, extract of 5 mL, acetate buffer solution (pH 4.0) of 5 mL, 2,6 dichlorophenolindophenol dye solution of 1 mL, and xylene of 10 mL were added. After, the tubes were stirred for 10 s and centrifugation was performed at 8600 x g for 10 min at 4 °C. The control sample was prepared in a test tube containing 6% metaphosphoric acid of 5 mL, acetate buffer solution (pH 4.0) of 5 mL, 1 mL of 2,6 dichlorophenolindophenol dye solution and 10 mL of xylene. Samples absorbance was recorded at wavelength of 500 nm through spectrophotometer against xylene and control sample. The ascorbic acid calibration curve equation was $y = 0.0123x + 0.0134$ and coefficient of determination was $R^2 = 0.9557$. The equation (5) was used to determine the total ascorbic acid contents and expressed as mg 100 g^{-1} fw.

Ascorbic acid (mg $100g^{-1}$) = $A_2 – A_1/a$ x DF (5)

A_1: Extract sample absorbance value; A_2: Control sample absorbance value; DF: Dilution factor; a: Ascorbic acid standard curve slope

The experiment was designed according to completely randomized design (CRD) with three replications. Each replication contained ten fruit. Duncan's multiple range test was used to determine significant differences among means. Mean values obtained were analysed with SAS program.

3. Results and Discussion

3.1. Total chlorophyll content

Ethylene treated tomatoes had less chlorophyll content and different types of tomatoes exhibited decrease in total chlorophyll content by the end of storage period. After cold storage, the maximum amount of total chlorophyll content (0.0030 g kg^{-1}) was found in untreated heirloom type while the minimum total chlorophyll content (0.0001 g kg^{-1}) was obtained in ethylene treated cluster type of tomatoes (Table 1). There were no significant differences between ethylene treated heirloom and cluster type of

tomatoes by the end of cold storage. After shelf life period, the highest total chlorophyll content (0.0016 g kg^{-1}) was noted in control beefsteak type while lowest total chlorophyll content (0.0004 g kg^{-1}) was obtained in ethylene treated cluster type of tomatoes (Table 2). There were no significant differences between heirloom and cluster type of tomatoes at the end of shelf life period.

Table 1- Effect of ethylene on the total chlorophyll, lycopene, total phenolic contents, antioxidant activity, carotenoid, flavonoid, and ascorbic acid contents of different types of tomatoes under cold storage at 12 °C

		Storage duration (Days)					
Parameters	**Treatments**	**0**	**7**	**14**	**21**	**28**	**35**
Total chlorophyll content (g kg^{-1} fw)	**BS**†	0.050ab*	0.0042ac	0.0024cj	0.0017dj	0.0011gj	0.0010gj
	BS+Ethyl.	0.0038af	0.0030bi	0.0021cj	0.0018di	0.0014fj	0.0007hj
	HL	0.0044ac	0.0039ae	0.0038ae	0.0038af	0.0032bg	0.0030bi
	HL+Ethyl.	0.0040ad	0.0037bf	0.0031bh	0.0029bi	0.0012gj	0.0005j
	CL	0.0061a	0.0042ac	0.0033bg	0.0033bg	0.0030bi	0.0012gj
	CL+Ethyl.	0.0053ab	0.0021cj	0.0016ej	0.0008hj	0.0007ij	0.0001j
	$LSD_{5\%}$: St. Dur.: 0.0008 **St. Dur. × Trt.:** 0.0019 **Trt.:** 0.0008						
Lycopene content (mg kg^{-1} fw)	**BS**	1.93k	6.47ik	9.96gk	17.50eh	26.12de	25.84de
	BS+Ethyl.	5.10ik	6.62ik	12.63fk	18.11eg	22.84df	26.65de
	HL	2.98jk	5.131ik	14.26fj	18.59eg	39.08ac	45.25a
	HL+Ethyl.	4.07ik	5.14ik	10.15gk	31.06bd	42.95a	47.37a
	CL	2.31k	2.46k	6.76hk	25.57de	38.18ac	40.30ac
	CL+Ethyl.	2.98jk	4.87ik	14.81fi	26.59de	30.02cd	41.46ab
	$LSD_{5\%}$: St. Dur.: 3.825 **St. Dur. × Trt.:** 9.3694 **Trt.:** 3.825						
Antioxidant Activity (g kg^{-1} fw EC_{50})	**BS**	1.53a	0.75di	1.09bd	0.56fl	0.66el	0.75di
	BS+Ethyl.	1.40ab	1.14bc	0.72dj	0.72dj	0.85cg	0.89cf
	HL	0.44gl	0.36il	0.25l	0.27kl	0.47gl	0.43hl
	HL+Ethyl.	0.30kl	0.27kl	0.66ek	0.32jl	0.42hl	0.33jl
	CL	1.02ce	0.79ch	0.52fl	0.62el	0.83ch	0.65el
	CL+Ethyl.	1.50a	0.77ci	0.59fl	0.67ek	0.72dj	0.65el
	$LSD_{5\%}$: St. Dur.: 0.1369 **St. Dur. × Trt.:** 0.3353 **Trt.:** 0.1369						
Carotenoid content (g kg^{-1} fw)	**BS**	0.0046jk	0.0062ik	0.0084hk	0.0113gk	0.0201eg	0.0287de
	BS+Ethyl.	0.0042jk	0.0116gk	0.0149fj	0.0155fj	0.0231ef	0.0680b
	HL	0.0048jk	0.0049jk	0.0050jk	0.0112gk	0.0156fj	0.0172fi
	HL+Ethyl.	0.0045jk	0.0058ik	0.0062ik	0.080hk	0.0142fj	0.1182a
	CL	0.0020k	0.0046jk	0.0051jk	0.0068ik	0.0149fj	0.0250df
	CL+Ethyl.	0.0051jk	0.0099gk	0.0148fj	0.0185eh	0.0340d	0.0545c
	$LSD_{5\%}$: St. Dur.: 0.0039 **St. Dur. × Trt.:** 0.0095 **Trt.:** 0.0039						
Flavonoid content (mg kg^{-1} fw)	**BS**	252.7a	709.7cd	57.6cd	56.3cd	25.2d	9.8d
	BS+Ethyl.	237.2ab	123.9ad	65.8cd	61.9cd	37.2cd	22.6d
	HL	113.5bd	72.7cd	40.0cd	34.7cd	29.4d	23.0d
	HL+Ethyl.	106.0bd	77.6cd	59.1cd	47.4cd	45.5cd	42.2cd
	CL	133.2ad	60.8cd	52.0cd	33.0d	30.1d	12.3d
	CL+Ethyl.	183.5ac	101.6bd	55.5cd	41.7cd	32.7d	27.5d
	$LSD_{5\%}$: St. Dur.: 49.4 **St. Dur. × Trt.:** 121 **Trt.:** 49.4						
Total phenolic contents (mg kg^{-1} GAE fw)	**BS**	26.0be	20.6cg	20.0cg	16.3dg	16.2dg	13.7fg
	BS+Ethyl.	24.2bf	22.4bf	21.6cf	19.7cg	17.1dg	12.8fg
	HL	33.4ab	30.1ac	21.1cf	20.0cg	18.7cg	16.2dg
	HL+Ethyl.	37.2a	26.2bd	23.4bf	23.4bf	17.1dg	17.1dg
	CL	21.0cf	19.7cg	19.3cg	14.7dg	13.3fg	9.0g
	CL+Ethyl.	23.1bf	21.8cf	21.2cf	18.9cg	16.8dg	14.4eg
	$LSD_{5\%}$: St. Dur.: 3.9 **St. Dur. × Trt.:** 9.5 **Trt.**: 3.9						
Ascorbic acid (mg $100g^{-1}$ fw)	**BS**	32.17a	22.90dj	23.57cg	19.5.fk	19.52fk	17.18k
	BS+Ethyl.	29.55ab	23.41ch	19.46gk	17.17k	19.56fk	18.92ik
	HL	30.31ab	25.34cd	25.23cd	19.84fk	19.07ik	20.32ek
	HL+Ethyl.	25.43cd	27.42bc	22.33dj	19.91ek	18.65jk	17.69k
	CL	33.23a	23.82cf	23.12di	19.90ek	19.71fk	16.18k
	CL+Ethyl.	30.20ab	23.53ch	24.10ce	19.35gk	19.23hk	16.07k
	$LSD_{5\%}$: St. Dur.: 1.45 **St. Dur. × Trt.:** 3.55 **Trt**.: 1.45						

*: Means with different letters are statistically significant at P≤0.05 according to Duncan's multiple range test; †BS: Beefsteak; BS+Ethyl.: Beefsteak+Ethylene, HL: Heirloom; HL+Ethyl.: Heirloom+Ethylene; CL: Cluster; CL+Ethyl.: Cluster+Ethylene; LSD: Least significant difference; St. Dur.: Storage duration, St. Dur. × Trt.: Storage duration × Treatments; Trt: Treatments

Table 2- Effect of ethylene on the total chlorophyll, lycopene, total phenolic contents, antioxidant activity, carotenoid, flavonoid, and ascorbic acid contents of different types of tomatoes under shelf life conditions at 20 °C

		Storage duration (Days)					
Parameters	**Treatments**	**0**	**7+3**	**14+3**	**21+3**	**28+3**	**35+3**
Total chlorophyll content (g kg^{-1} fw)	**BS**†	0.0050ad	0.0041af	0.0036ag	0.0034ci	0.0018ej	0.0016fj
	BS+Ethyl.	0.0038ag	0.0036ag	0.0025dj	0.0020ej	0.0020ej	0.0015fj
	HL	0.0044ae	0.0044ae	0.0044ae	0.0016fj	0.0007j	0.0005j
	HL+Ethyl.	0.0040af	0.0043ae	0.0036ag	0.0024ej	0.0006j	0.0006j
	CL	0.0061ab	0.0061a	0.0035bh	0.0013il	0.0008ij	0.0006j
	CL+Ethyl.	0.0053ac	0.0015fj	0.0014fj	0.0009hj	0.0007j	0.0004j
	LSD$_{5\%}$: St. Dur.: 0.0009 **St. Dur. × Trt.:** 0.0022 **Trt.:** 0.0009						
Lycopene content (mg kg^{-1} fw)	**BS**	1.93p	5.88np	16.87kn	19.81km	25.16gl	37.18dg
	BS+Ethyl.	5.10op	9.72mp	21.99il	25.33gl	32.40ej	37.31dg
	HL	2.98p	23.93hl	37.05dg	37.72df	38.85df	54.94ab
	HL+Ethyl.	4.07op	21.69jl	28.37fk	42.75ce	42.83ce	56.36a
	CL	2.31p	21.75jl	35.29eh	39.51df	44.11be	48.71ad
	CL+Ethyl.	2.98p	14.98lo	33.83ei	34.50eh	39.90df	53.85ac
	LSD$_{5\%}$: St. Dur.: 4.2219 **St. Dur. × Trt.:** 10.342 **Trt.:** 4.2219						
Antioxidant Activity (g kg^{-1} fw EC$_{50}$)	**BS**	1.53a	0.96bd	0.41jn	0.87be	0.72dh	0.60fl
	BS+Ethyl.	1.39a	0.98bc	0.80bf	0.89be	0.67ej	0.69ei
	HL	0.44in	0.26n	0.34ln	0.36ln	0.38kn	0.47hn
	HL+Ethyl.	0.30mn	0.30mn	0.47hn	0.39kn	0.51gn	0.41jn
	CL	1.02b	0.69ei	0.55fm	0.66ej	0.63ek	0.81bf
	CL+Ethyl.	1.50a	0.56fm	0.46in	0.74cg	0.81bf	0.76cg
	LSD$_{5\%}$: St. Dur.: 0.0888 **St. Dur. × Trt.:** 0.2175 **Trt.:** 0.0888						
Carotenoid content (g kg^{-1} fw)	**BS**	0.0046e	0.0072de	0.0078de	0.0118de	0.0051e	0.0279be
	BS+Ethyl.	0.0042e	0.0048e	0.0055e	0.0149ce	0.0384ad	0.0449ac
	HL	0.0048e	0.0061e	0.0133de	0.0192be	0.0240be	0.0290be
	HL+Ethyl.	0.0045e	0.0094de	0.0128de	0.0122de	0.0230be	0.0660a
	CL	0.0020e	0.0020e	0.0041e	0.0087de	0.0098de	0.0149ce
	CL+Ethyl.	0.0051e	0.0048e	0.0087de	0.0099de	0.0315ad	0.0454ab
	LSD$_{5\%}$: St. Dur.: 0.0105 **St. Dur. × Trt.:** 0.0256 **Trt.:** 0.0105						
Flavonoid content (mg kg^{-1} fw)	**BS**	252.7a	190.8ac	112.7be	87.3ce	30.5e	24.1e
	BS+Ethyl.	237.2ab	55.7ce	31.1e	45.7de	41.6de	8.9e
	HL	113.5be	96.9ce	82.7ce	43.7de	26.1e	19.3e
	HL+Ethyl.	106.0be	51.6ce	50.7ce	49.9ce	47.4ce	44.3de
	CL	133.2ae	128.5ae	66.0ce	41.8de	21.4e	11.4e
	CL+Ethyl.	183.5ad	68.7ce	43.2de	42.0de	22.4e	12.3e
	LSD$_{5\%}$: St. Dur.: 48.2 **St. Dur. × Trt.:** 118.1 **Trt.:** 48.2						
Total phenolic contents (mg kg^{-1} GAE fw)	**BS**	26.0be	23.4cf	25.6be	19.3ej	24.8ce	12.6ij
	BS+Ethyl.	24.2cf	19.0ej	23.2cf	21.0dh	22.5cf	11.6j
	HL	33.4ab	33.4ab	28.2bd	22.7bd	20.1di	12.5ij
	HL+Ethyl.	37.2a	30.0ac	37.2a	22.3cf	16.2fj	12.3ij
	CL	21.0dh	18.5ej	18.5ej	16.1fj	13.5gj	13.3hj
	CL+Ethyl.	23.1cf	21.7cg	17.5ej	23.1cf	13.7gj	12.4ij
	LSD$_{5\%}$: St. Dur.: 2.8 **St. Dur. × Trt.:** 6.9 **Trt.**: 2.8						
Ascorbic acid (mg 100 g^{-1} fw)	**BS**	32.17a	29.16ac	25.31be	19.57fj	18.18hk	13.96kl
	BS+Ethyl.	29.55ab	25.48bd	23.43ch	19.56fj	17.22il	16.19il
	HL	30.31ab	29.71ab	23.83cg	19.84ej	21.25di	17.05il
	HL+Ethyl.	25.43bd	23.96cf	21.17di	20.37di	19.91ej	17.25il
	CL	33.23a	25.56bd	18.76fk	19.86ej	14.67jl	17.35il
	CL+Ethyl.	30.20ab	23.51ch	20.97di	18.49gk	19.35fj	12.54l
	LSD$_{5\%}$: St. Dur.: 1.82 **St. Dur. × Trt.:** 4.45 **Trt.**: 1.82						

*: Means with different letters are statistically significant at P≤0.05 according to Duncan's multiple range test; †BS; Beefsteak, BS+Ethyl.; Beefsteak+Ethylene; HL: Heirloom; HL+Ethyl.: Heirloom+Ethylene; CL: Cluster; CL+Ethyl.: Cluster+Ethylene; LSD: Least significant difference, St. Dur.: Storage duration; St. Dur. × Trt.: Storage duration × Treatments; Trt: Treatments.

During this study, the decline in chlorophyll content may be because of progress in ripening that caused color change in fruit from green to red maturity stage with the transformation of chloroplast into chromoplasts, breakdown of chlorophyll and synthesis of carotenoids occurred as reported by Alexander & Grierson (2002). Watada et al. (1986) expressed that ethylene treatment enhances the degradation of chlorophyll in citrus fruit; turns it to yellow and then to orange color from green that agrees with the finding of less chlorophyll content obtained in this study in ethylene treated tomatoes.

3.2. Lycopene content

Extending storage time had considerably increased the lycopene content in tomatoes. At the end of cold storage, the highest lycopene content (47.37 mg kg^{-1}) was recorded in ethylene treated heirloom type while lowest lycopene content (25.84 mg kg^{-1}) was observed in control beefsteak type of tomatoes (Table 1). There were no significant differences between ethylene treated and control heirloom type of tomatoes at the end of cold storage. At the end of shelf life, the maximum lycopene content (56.36 mg kg^{-1}) was also recorded in ethylene treated heirloom type whereas the minimum lycopene content (37.18 mg kg^{-1}) was noted in control beefsteak type of tomatoes (Table 2). However, there were no significant differences between ethylene treated and control heirloom type of tomatoes at the end of shelf life period.

Increase in lycopene concentration of tomato with extension in storage of tomato was reported by Khairi et al. (2015) which agreed with the findings in this study. Tadesse et al. (2016) reported as the maturity of tomato fruit at green mature stage enhances it converts chloroplast into chromoplast where the lycopene is present in membrane bound crystals. Dhall & Singh (2013) reported higher lycopene content in ethylene treated tomatoes as obtained in this study. The lycopene content determined in the shelf life period were higher than cold storage in our experiment which was in confirmation with Tadesse et al. (2015) who expressed higher lycopene content in tomato kept at 20 and 30 °C than at 4 °C.

3.3. Antioxidant activity

Antioxidant activity showed an increase in beefsteak and cluster types of tomatoes whereas in heirloom type of tomatoes it had decreased. There was no significant difference between control and ethylene treated cluster type of tomatoes on 35th day of cold storage. The minimum EC_{50} value indicate the maximum antioxidant activity. After cold storage, the highest antioxidant activity (0.33 g kg^{-1} EC_{50}) was recorded in ethylene treated heirloom type while the lowest antioxidant activity (0.89 g kg^{-1} EC_{50}) was noticed in ethylene treated beefsteak type of tomatoes (Table 1). After shelf life period, the highest antioxidant activity (0.41 g kg^{-1} EC_{50}) was recorded in ethylene treated heirloom type while the lowest antioxidant activity (0.81 g kg^{-1} EC_{50}) was observed in control cluster type of tomatoes (Table 2).

Tilahun et al. (2017) reported that antioxidant activity in tomatoes was higher at red maturity stage than those of harvested at green maturity stage which can be because of increase in lycopene concentration. This result confirmed our outcomes of ethylene treatment increased lycopene concentration and antioxidant activity.

3.4. Carotenoid content

Extending storage time had increased the carotenoid contents. Ethylene treated tomatoes had more carotenoid content than untreated ones. After cold storage, ethylene treated heirloom type tomatoes had maximum carotenoid content (0.1182 g kg^{-1}) while minimum carotenoid content (0.0172 g kg^{-1}) was noted in control heirloom type of tomatoes (Table 1). At the end of shelf life period, maximum carotenoid content (0.0660 g kg^{-1}) were also noted in ethylene treated heirloom type whereas minimum total carotenoid content (0.0149 g kg^{-1}) was observed in control cluster type of tomatoes (Table 2).

In this study, ethylene treated tomatoes had higher carotenoid content at the end of storage which was in confirmation with the outcomes of Cruz et al. (2018). These researches expressed that ethylene regulates the carotenoid synthesis during ripening of tomatoes. Increase in carotenoid content in this study can be because of advancement in maturity that change color of tomato from green to red with conversion of chloroplast to chromoplast, resulting degradation of chlorophyll and accumulation of carotenoid as explained by Alexander & Greirson (2002).

3.5. Flavonoid content

Flavonoid content decreased during storage. There was a significant interaction ($P \leq 0.05$) between storage duration and treatments. After cold storage, maximum flavonoid content (42.2 mg kg^{-1}) occurred in ethylene treated heirloom fruit with minimum (9.8 mg kg^{-1}) flavonoid content was in control beefsteak tomatoes (Table 1). During shelf life period, the highest flavonoid content (44.3 mg kg^{-1}) was recorded in ethylene treated heirloom type with the lowest flavonoid content (8.9 mg kg^{-1}) was in ethylene treated beefsteak tomatoes (Table 2).

Riadh et al. (2016) stated that different cultivars of tomatoes significantly affected flavonoid content which agreed with significant effect obtained between different types of tomatoes treated with ethylene in our study. Flavonoid content showed decrease with increase in storage duration during our experiment which was supported by outcomes of Howard et al. (2000) who described decrease of flavonoid content during maturation of peppers. The losses in flavonoid content may be because of metabolic transformation to secondary phenolic compounds as reported by Barz & Hoesel (1979).

3.6. Total phenolic contents

Prolonging storage duration decreased the total phenolic contents. At the end of cold storage, the maximum total phenolic content (17.1 mg kg^{-1} GAE) was exhibited by ethylene treated heirloom type whereas the minimum total phenolic contents (9.0 mg kg^{-1} GAE) was found in control cluster type of tomatoes (Table 1). There were no significant differences between ethylene treated and control heirloom type of tomatoes by the end of cold storage. At the end of shelf life period, highest total phenolic content (13.3 mg kg^{-1} GAE) was reported in control cluster type while lowest total phenolic content (11.6 mg kg^{-1} GAE) was recorded in ethylene treated beefsteak type of tomatoes (Table 2).

Extension in storage showed decrease in total phenolic contents of different types of tomatoes in our experiment. According to Day (2001) the higher respiration rate can be the reason of degradation of phenolic compounds**.** Control beefsteak type of tomatoes during cold storage had resulted more total phenolic contents which agreed with Dominguez et al. (2016) who demonstrated reduction in ethylene had increased the total phenolic contents in 'Delizia' tomato cultivar. The higher amount of total phenolic contents in heirloom type of tomatoes in this study during cold storage may be attributed to higher content of lycopene as described by Riadh et al. (2016).

3.7. Ascorbic acid content

Ascorbic acid displayed declining trend with extension in storage duration. At the end of cold storage, highest ascorbic acid content (20.32 mg 100 g^{-1}) was found in control heirloom type whereas lowest ascorbic acid content (16.07 mg 100 g^{-1}) was reported in ethylene treated cluster type of tomatoes (Table 1). There were no significant differences between control beefsteak, ethylene treated heirloom, control cluster and ethylene treated cluster type of tomatoes by the end of cold storage. At the end of shelf life period, untreated cluster type of tomatoes had the maximum ascorbic acid contents (17.35 mg 100 g^{-1}) while the minimum ascorbic acid content (12.54 mg 100 g^{-1}) was displayed by ethylene treated cluster type of tomatoes (Table 2). There were no significant differences between both ethylene treated and untreated heirloom and cluster type of tomatoes.

Declining trend in ascorbic acid content were exhibited by different types of tomatoes with extension in storage duration during this study which agreed with the findings of Tudor-Rado et al. (2016) who stated that decrease in ascorbic acid content of various tomato cultivars and the reason for decline in ascorbic acid may be because of oxidation caused by oxidizing enzymes. Our findings were in contradiction with Dhall & Singh (2013) who reported ethylene treated tomatoes had more ascorbic acid content when comparison was made with control.

4. Conclusions

In conclusion, ethylene treatment resulted in higher lycopene and carotenoid contents with lower total chlorophyll contents in all tested tomato types. After cold storage and shelf life period, the maximum antioxidant activity, carotenoid and flavonoid content were recorded in ethylene treated heirloom type tomatoes. Furthermore, heirloom type tomatoes retained better postharvest quality as compared to beefsteak and cluster type of tomatoes at the end of 35 days of cold storage.

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