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Variations in Non-enzymatic Antioxidants in *Hisar Arun* (Local) and *Kashi Vishesh* (Hybrid) Cultivars of Tomato Fruits Treated with H_2O_2 during storage periods

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ABSTRACT

Alterations in non-enzymatic antioxidants of tomato fruits of two cultivars (*Kashi Vishesh*: a local & *Hisar Arun*: a hybrid) during their storage were studied. Tomatoes, harvested at mature green, breaker, and mature red stages were treated with 1% and 4% solution of H_2O_2 and then kept at storage temperatures 5°C,10°C, and 15°C. Antioxidant activity, ascorbic acid, carotenoids, Lycopene and total phenol content were measured after every 7-day interval up to a total storage duration of 21 days. The recorded non-enzymatic characteristics shown an increase upto 14 days and then started declining irrespective of storage temperature and concentration of H_2O_2 treatment and maximum change was seen at15°C and 1% H_2O_2. On the other hand, lycopene content increased asymptotically at all maturity stages and at all storage temperatures which is suggestive of slowing ripening process. In conclusion, local cultivar, harvested at mature green stage, showed slowest increase rate in antioxidants activities when treated with 1% H_2O_2 and stored at 5°C.

Keywords: Antioxidant Activity, Carotenoids Lycopene, Total Phenol and Tomato.

INTRODUCTION

Tomato *(Lycopersicum esculentum)* belongs to a night shade family which is an adaptable vegetable and considered to be extensively consumed produce (Ahmed *et al.,* 2012)[1]. On one hand, tomato fruits are consumed in fresh state while processed products are also commonly produced. Additionally, the nutritional content offered by tomatoes appeals for its induction in dietary habit leading to healthy life style (Uthairatanakij *et al.,* 2017)[2]. Further, functional characteristics of tomato fruits undeniably allow for the devastating epidemiological sign leading to reduction in the risk of chronic disease for instance cancer and cardiovascular disease (Sgherri *et al.,* 2008)[3].

The defensive act of tomato fruits is characteristically ascribed to antioxidant compounds ascorbic acid, carotenoids including lycopene and

beta- carotene, pro-vitamin A, flavonoids, phenolics and vitamin E (Odriozola-Serrano *et al.,* 2008; Mehdizadeh *et al.,* 2013)[4,5]. Tomato is a climacteric fruit, in which, the process of respiration can occur even after harvesting. In the course of ripening fruits experiences a sequences of structural, physiological and biochemical changes which are characterized by depletion of chlorophyll, softening of fruit, and rise in respiration rate, ethylene production and synthesis of sugars, lycopene and acids (Joshi *et al.,* 2017)[6] which are subject to be controlled during pre- and post-harvest by means of chemical treatment as well as developing customized storage conditions with the aim to enhance their shelf life.

The physical and chemical processes in the course of ripening of tomatoes have been extensively studies for extending shelf life of tomato fruits. The specific information on the actual stage of harvest and consequence of ripening action on antioxidant capacity and antioxidant content were not available (Bayoumi, 2008)[7]. In this context, Hydrogen peroxide (H_2O_2) has been approved as a GRAS (Generally regarded as safe) treatment by USA, as an alternative to currently used chemicals in postharvest treatments (Rodrigues *et al.,* 2012)[8]. Owing to the less toxicity and safe decomposition products; hydrogen peroxide which is a strong oxidizing agent recommended as a substitute for decontamination of fruits and vegetables (Alexandre *et al.,* 2012; Loredo *et al.,* 2013)[9,10]. An extensive variation in transmittable biological agents ranging as of spores of bacteria, vegetative cells, protozoa and their cysts, fungi, viruses and even prions have been inactivated by hydrogen peroxide. (Malik *et al.,* 2012; Delgado *et al.,* 2012; Loredo *et al.,* 2013)[11,12,10]. Hydrogen peroxide (H_2O_2) can have a lethal or inhibitory effect on microorganisms, depending on the pH, temperature and other environmental factors (Augspole *et al.,* 2017)[13]. Therefore, in this paper, we present an in-depth investigation of the evolution of non-enzymatic characteristics of tomatoes when treated with H_2O_2.

MATERIAL AND METHODS

Sample Preparation

The experiment was conducted in the research laboratory of SHUATS, Allahabad. Two cultivars of tomato fruit (*L. esculentum*) namely *Hisar arun*, (a Local variety) and *Kashi Vishesh*, (a hybrid variety) were harvested from the experimental field at different maturity stages i.e. Mature Green, Breaker and Mature red; fruits were then graded according to shape, size, color and appearance. Fruits were then rinsed with tap water and dipped in an aqueous solution of 1% sodium hypochlorite for 1 min. for surface sterilization. After surface sterilization, fruits were dipped in 1% and 4% Hydrogen peroxide (H_2O_2) solution for 20 min. next, fruits were stored in suitable plastic box for 21 days at a temperature of 5°C, 10°C and 15°C. The analysis was done for an interval of every 7 days. Over-ripened tomatoes of different treatments with the passage of time during storage were excluded from the trial. As following, we present the specific examinations made in the present investigation.

Antioxidant activity

Total antioxidant activity was assayed by % scavenging of the DPPH free radicals as the method mentioned by Yen and Duh (1994)14. DPPH solution (0.004% w/v) was prepared in 95% methanol. The crude extracts were mixed with 95% methanol to prepare solution of known concentration as 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml respectively in five test tubes. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes and after 10 min. the absorbance was taken at 517 nm wavelength. Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (10mg/100ml or 100µg/ml) of extracts. Control sample was prepared containing the same volume without any extract.

Ascorbic Acid

Ascorbic acid was estimated using the method described in AOAC (1984)[15]. The 1.0 g dried and finely powdered sample were dissolved with 10 ml of 0.4% oxalic acid in water and centrifuged at 8000 rpm. Supernatant was used to test the content of Ascorbic Acid. 1 ml aliquots of the supernatant were maintained to 3.0 ml by 0.4% oxalic acid followed by the addition of 7.0 ml of 2, 6-dichlorophenol indophenol dye solution. The test mixture was properly mixed and its absorbance was recorded immediately at 518 nm. The amount of ascorbic acid was estimated by comparing with a standard curve drawn under identical experimental conditions.

Carotenoids

Total carotenoids in the plant tissues were estimated according to the method by Jensen (1978)16. One gram of each sample were extracted with 80% methanol and centrifuged. The supernatants were concentrated to dryness. The residues thus obtained were dissolved in 15 ml of diethyl ether and after addition of 15 ml of 10% methanolic KOH, the mixture was washed with 5% ice-cold saline water to remove alkali. The collective saline washings were extracted with ether (3:15 v/v). The ether extract from both were mixed together followed by washing with cold water till alkali free. The alkali free ether extract was dried over anhydrous Na_2SO_4 for two hours in the dark. The ether extracts were filtered and its absorbance was measured at wavelength 450 nm (λmax) by using ether as blank.

Lycopene

Lycopene was determined through method adopted in Sadasivam and Manickam (1992)[17]. 1.0g of tomato sample, as weighed into a conical flask, was transferred into a volumetric flask and filled with distilled water to reach 100 ml mark. Next, it undergone proper mixing and then transferred into a separating funnel in which 25 ml of petroleum ether was also added. It was shaking vigorously for about 15 minutes. The aqueous layer was run off and the absorbance of petroleum ether layer was recorded at 505 nm.

Total Phenol

Quantitative estimation of total phenol was done by the method described in Ragazzi and Veronese (1973)[18]. The 10 mg plant extract was dissolved in 10 ml of 50% MeOH: H_2O (1:1), overnight at the room temperature. Subsequently in a volume of 1.0 ml of the aforementioned solution, 1.0 ml of Folin's Reagent (1N) and 2.0 ml of Na_2CO_3 (20%) were added. The test mixture was mixed properly on cyclomixer, and then and maintained to 25 ml with water which is then kept at room temperature for 30 minutes. The absorbance of test mixture was measured at wavelength 725 nm on Varian Cary 50 Spectrophotometer. Graphs prepared using the standardized gallic acid solution of different concentrations and total phenol content have been expressed in mg/100 g material.

Statistical analysis

Each treatment had three replicates and all experiments were run at least twice, revealing similar results. All the data were collected and analyzed by multifactor ANOVA with SPSS 11.0 for windows. Significant effect was assessed at 5% ($p \leq 0.05$) level of significance and the mean was separated using least significant difference (LSD) procedure.

RESULTS AND DISCUSSION

Total Phenol

Total phenol content was affected by H_2O_2 concentration, temperature, maturity stages and cultivars (Fig 1. and Table 1). Similar to antioxidant activity total phenol content increases upto 14 days of storage and then it starts decline upto 21 days. Hybrid variety & lower concentration (1%) of H_2O_2 shows better treatments in terms of shelf life as the rate of change were very slow as compared to higher concentration (4%) & local varieties in all maturity stages at all the three varying temperature. The synthesis of phenylalanine ammonialyase (PAL) and hydroxycinnamoyl quinate transferase (HQT) enzymes is greatly assisted by the reduced storage temperature leading to increased total phenolics (Macheix *et al.,* 1990; Toor *et al.,* 2006)[19,20]. Additionally, the possibility that during storage of fruits, some compounds could be formed and react with the Folin–Ciocalteu's reagent and significantly enhance the phenolic content (Kallithraka *et al.,* 2009)[21] can also be accounted for increase in the total phenolics. Change in total phenol content during storage is a temperature dependent i.e. at 5°C temperature content were increase slowly followed by 10°C and 15°C during entire analysis. Initially content was high in mature green fruits but the rate of change was high in mature red stages followed by breaker and mature green.

Antioxidant activity

In this present study, the antioxidant activity of tomato fruits during storage treated with H_2O_2, was analyzed using DPPH radical scavenging assay, which is based on electron transfer reactions providing a scale of antioxidant reducing capacity. The percentage difference of the antioxidant activity, plotted in Fig. 2 (absolute values listed in Table 2) of tomato fruits of both the cultivars were found increased till 14 days of storage and then it starts

decreasing. This trend is unanimously seen for all the storage temperatures, maturity stages and concentration level of H_2O_2. However, difference in increment was seen to be higher when fruits were stored upto 7 days during entire analysis. 15°C storage temperature resulted comparatively higher activities of antioxidant than 5°C and 10°C temperatures and displayed much more activities when treated with 4 % H_2O_2. The increment in the antioxidant activity during progressive stages of ripening, and storage, may be caused due to the deposition of total phenolics and carotenoids (Toor *et al.,* 2006; Bhandari and Lee, 2016)[22,23] However, in contrast to our results, the investigation of the antioxidant and total phenolic content in the H_2O_2 treated fresh-cut tomatoes, made by Kim *et al.,* (2007)[24], revealed a declining trend. This may be attributed to the fact that their study is subject to oxidation, and its use in lignin formation, led by post-harvest damage made during cutting the tomatoes which is not the case in our investigation. Pinelo *et al.,* (2005)[25] suggested the promotional tendency of poly phenols in the synthesis of polymerized compounds to be the cause of increase in antioxidant activity. Further, the decrease in antioxidant activity is the consequence of polymerization exceeding a critical value, which leads to enhanced molecular complexity and steric hindrance disrupting hydroxyl groups' reaction with the DPPH radicals (Piljac- Zegarac *et al.,* 2009)[26]. The local variety fruits (*Kashi Vishesh*) having a good source of antioxidants compared to hybrid variety (*Hisar Arun*), also responded remarkably to all the studied treatment supplements. The percent increment of antioxidant activity was found to be maximum in mature green stage in both local (*Kashi Vishesh*) as well as hybrid variety (*Hisar Arun*) fruits during storage. In particular, antioxidant activity of tomatoes harvested at mature green stage is found to be higher followed by breaker and mature red fruits for the entire analysis in both varieties. Further, increased concentration of H_2O_2 led to increase in the antioxidant activity during storage along with increasing temperatures. The activity in tomato fruits was achieved highest when treated with 4% H_2O_2, stored for 14 days at temperature 15°C but slow rate of increment was perceived in matured red fruits under these storage condition.

Table 1: Total Phenol Content (mg/100g FW) during storage in tomato fruits treated with hydrogen peroxide

Concentration	Temperature	Days	*Kashi Vishesh* (Hybrid)			*Hisar Arun* (Local)		
			Mature Green	Breaker	Mature Red	Mature Green	Breaker	Mature Red
		0 Days	12.013	10.273	9.841	16.226	14.792	10.418
1% H_2O_2	5°C	7 Days	12.119	10.4	9.991	16.639	15.234	10.788
		14 Days	12.246	10.51	10.098	17.016	15.586	11.041
		21 Days	12.112	10.391	9.991	16.749	15.363	10.891
	10°C	7 Days	12.335	10.593	10.185	17.13	15.682	11.074
		14 Days	12.549	10.77	10.365	17.459	15.975	11.286
		21 Days	12.371	10.621	10.219	17.192	15.751	11.124
	15°C	7 Days	12.571	10.821	10.419	17.46	16.048	11.403
		14 Days	12.867	11.051	10.643	17.954	16.573	11.756
		21 Days	12.668	10.912	10.506	17.654	16.248	11.508
4% H_2O_2	5°C	7 Days	12.562	10.775	10.354	17.171	15.727	11.114
		14 Days	12.807	10.978	10.548	17.566	16.072	11.368
		21 Days	12.661	10.875	10.442	17.293	15.86	11.226
	10°C	7 Days	12.811	11.026	10.615	17.771	16.294	11.557
		14 Days	13.11	11.261	10.859	18.347	16.841	11.922
		21 Days	12.876	11.081	10.692	17.834	16.366	11.608
	15°C	7 Days	13.181	11.356	10.959	18.536	16.98	12.026
		14 Days	13.56	11.686	11.275	19.08	17.499	12.379
		21 Days	13.301	11.458	11.097	18.705	17.114	12.108

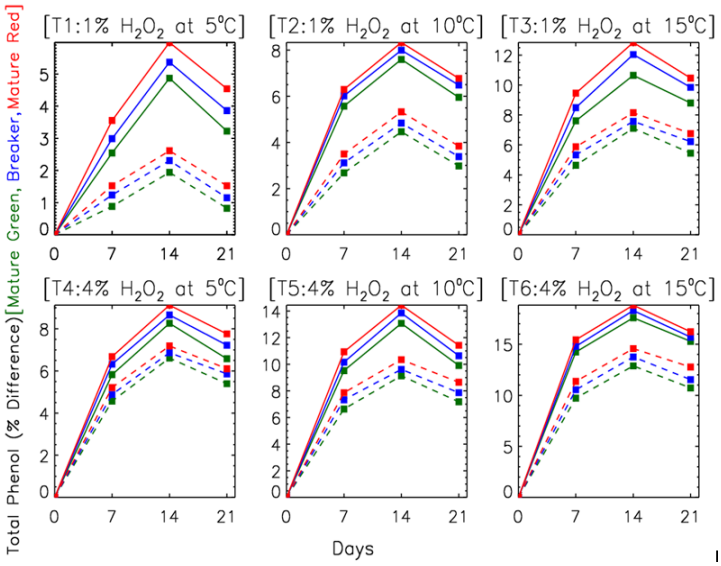

Fig. 1. Percentage difference in Total Phenol Content corresponding to different tomato-cultivars (*Hisar Arun* (full line); *Kashi Vishesh* (dotted line)), stored at various temperatures (5°C (left panel); 10°C (middle panel), and 15°C (right panel)), while being treated with different concentration of H_20_2 (1% (top row) and 4% (bottom row)) and investigated at different stages of ripening (Mature green (green color); Breaker (blue); and Mature red (red)

Ascorbic Acid

The evolution in ascorbic acid (AA) content were recorded during the storage period upto 21 days. It was observed that AA content (percentage difference in Fig. 3 and absolute values in Table 3) were strongly dependent on the maturity stages, temperature and H_2O_2 concentration. The present studies revealed that ascorbic acid rose upto 14 days of storage at all maturity stages; at each storage temperatures and H_2O_2 concentrations after that it

Table 2: Antioxidant activity during storage in tomato fruits treated with hydrogen peroxide

Concentration	Temperature	Days	*Kashi Vishesh* (Hybrid)			*Hisar Arun* (Local)		
			Mature Green	Breaker	Mature Red	Mature Green	Breaker	Mature Red
		0 Days	165.371	357.146	505.293	255.474	486.967	655.589
1% H_2O_2	5°C	7 Days	171.558	368.467	517.804	265.858	503.794	674.259
		14 Days	174.328	373.499	524.212	270.625	511.211	683.002
		21 Days	173.321	371.867	521.837	268.631	508.337	679.777
	10°C	7 Days	173.86	372.416	522.831	269.889	509.614	680.104
		14 Days	178.337	380.744	532.945	277.49	522.537	695.041
		21 Days	177.051	377.872	529.253	274.888	518.23	689.769
	15°C	7 Days	178.88	381.063	532.055	278.729	522.551	695.019
		14 Days	183.868	390.938	544.937	287.627	537.763	712.91
		21 Days	182.027	387.86	540.946	283.407	532.517	706.79
4% H_2O_2	5°C	7 Days	179.33	385.493	540.306	278.255	527.256	704.871
		14 Days	183.497	393.092	553.179	284.763	538.843	720.288
		21 Days	182.201	390.692	549.501	282.689	535.162	715.333
	10°C	7 Days	182.727	390.5	547.737	284.04	534.909	713.973
		14 Days	189.538	403.791	565.809	295.67	554.261	737.422
		21 Days	187.387	400.178	560.15	291.24	548.631	730.316
	15°C	7 Days	187.901	401.696	562.512	292.369	549.681	733.761
		14 Days	195.59	417.409	583.986	304.894	571.653	762.277
		21 Days	193.275	413.302	577.67	300.81	565.771	754.083

starts declining at all storage conditions. Arthur *et al.,* (2015)[27] suggested the respiration and transpiration physiological process to be inducing the reduction in ascorbic acid content. Rate of increment was found directly proportional to the concentration of H_2O_2 but major change was recorded in mature green fruits. However, temperature also played important role in the synthesis of Ascorbic acid i.e. at low temperature rate of change were slow and at higher temperature, the change was higher during storage periods in all stages. Positive effect of increase in the storage temperature on ascorbic acid synthesis may be an indication of active ripening process (Sammi and Masud, 2007)[28] while its decrement is indicative of senescent fruit. The ascorbic acid change in fruits of local variety treated with 1 % H_2O_2 kept at 15°C after 14 days of storage were found almost similar at 4 % H_2O_2 stored at temperature 5°C. Chemical H_2O_2 @4% accelerated upto 30% increase in AA while at low concentration (@1%) displayed increment only upto 15% in mature green fruits at 14 days stored at the temperature 15°C. Whereas, only 6% and 4% increment were found respectively in mature red and breaker at same condition in local variety. In particular, it is noted that the rate of change of ascorbic acid is slower in hybrid variety when compared to local variety in all the applied treatments.

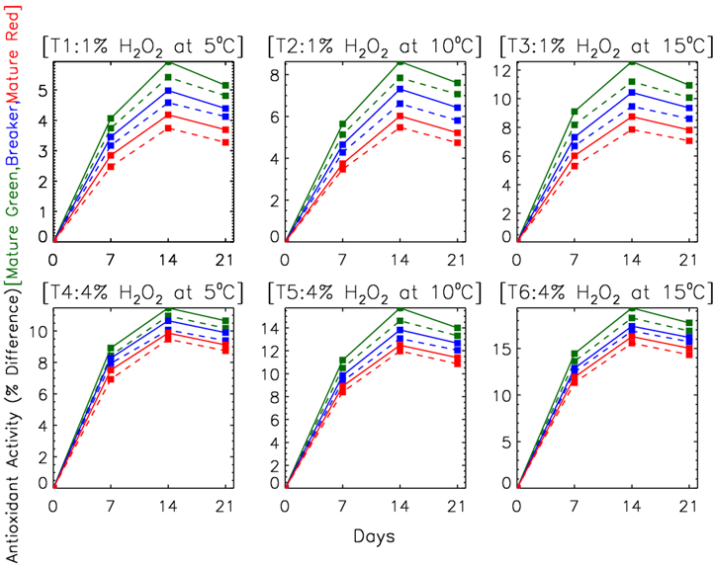

Fig. 2. Percentage difference in Antioxidant Activity for 21 days of investigation of tomato fruits stored at different temperature and H_2O_2 concentration. The color code and line style were kept similar to that used in Figure 1

Carotenoids

Figure 4 shows the percentage difference in the carotenoid content (Table 4 contains absolute values), recorded every 7 days within the total storage duration of 21 days. We witnessed changes in carotenoid content to increase until 14 days after which the same started declining. The rate of change was maximum (21%; T2) in mature red fruits of hybrid variety after 14 days of storage treated with 1 % H_2O_2 which was kept at 15°C while minimum (T3; 3%) were in mature green fruits of local variety after 14 days of storage when treated with 4% H_2O_2 and kept at 5°C. The changes were higher in hybrid variety and at lower concentration of H_2O_2. In fruits treated with 1% H_2O_2, changes were measured up to 22 % which is the maximum value for mature red fruits of hybrid variety. From T3 and T4, we determined that the change in carotenoid content got doubled in for increased H_2O_2 concentration but the rate of change was slower at low temperature (5°C). The investigation of Yumbya *et al.,* (2014)[29] revealed similar trend in the carotenoids, however, in mangoes and passions fruits. During storage, in the sequence of ripening process of tomatoes, decomposition of chlorophyll occurs which results in subsequent release of carotenoids (Joyce *et al.,* 2016)[30] leading to increased carotenoids as revealed in our investigation.

Lycopene

Change in lycopene content during storage in two cultivars of tomato fruits treated with H_2O_2,

stored at different temperatures were evaluated as shown in Fig. 5 and enlisted in Table 5. Contrary to the aforementioned non-enzymatic characteristics which increased only until 14 days, Lycopene content is recorded to keep increasing throughout the storage duration of 21 days however the rate of change depends on concentration, maturity stage, temperature, and storage time. Similar variation in lycopene content have been also reported previously, however in other cultivars (Ilahy

Table 3: Ascorbic acid content (mg/100g FW) in tomato fruits treated with hydrogen peroxide

Concentration	Temperature	Days	*Kashi Vishesh* (Hybrid)			*Hisar Arun* (Local)		
			Mature Green	Breaker	Mature Red	Mature Green	Breaker	Mature Red
		0 Days	8.433	15.783	12.947	13.876	21.578	17.241
1% H_2O_2	5°C	7 Days	8.719	16.144	13.174	14.645	22.576	17.937
		14 Days	8.987	16.579	13.45	15.132	23.337	18.52
		21 Days	8.832	16.318	13.308	14.91	22.964	18.219
	10°C	7 Days	8.992	16.647	13.541	15.308	23.556	18.593
		14 Days	9.412	17.465	14.169	16.008	24.616	19.399
		21 Days	9.212	17.042	13.857	15.641	24.038	19.061
	15°C	7 Days	9.22	17.127	13.998	15.629	24.148	19.199
		14 Days	9.586	17.818	14.515	16.427	25.241	20.044
		21 Days	9.435	17.55	14.311	16.241	24.839	19.74
4% H_2O_2	5°C	7 Days	9.125	17.018	13.895	15.416	23.892	18.981
		14 Days	9.407	17.547	14.329	15.829	24.523	19.502
		21 Days	9.273	17.288	14.12	15.584	24.131	19.207
	10°C	7 Days	9.528	17.741	14.472	16.413	25.277	19.99
		14 Days	9.874	18.381	14.96	17.213	26.496	20.974
		21 Days	9.653	17.922	14.627	16.809	25.917	20.58
	15°C	7 Days	9.911	18.415	14.908	17.095	26.302	20.804
		14 Days	10.311	19.129	15.553	18.159	27.94	22.02
		21 Days	10.105	18.694	15.215	17.616	27.146	21.505

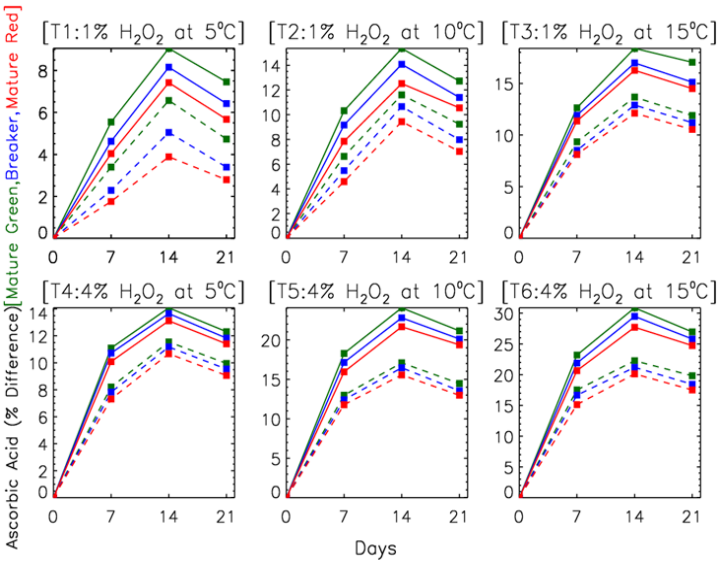

Fig. 3. Percentage difference in Ascorbic Acid content for 21 days of investigation of tomato fruits stored at different temperature and H_2O_2 concentration. The color code and line style were kept similar to that used in Figure 1

et al., 2011; Jarqu´ın-Enr´ıquez *et al.,* 2013)[31,32]. The transformation of chloroplast in chromoplast during the ripening process of tomatoes (Bhandari and Lee, 2016; Dibbisa *et al.,* 2016)[23,33] can be understood to cause the increase in lycopene content. Furthermore, progression in the enzyme activity associated with phytoene synthase I is also attributed to contribute to the synthesis of lycopene

Table 4: Carotenoids content (mg/100g FW) during storage in tomato fruits treated with hydrogen peroxide

Concentration	Temperature	Days	*Kashi Vishesh* (Hybrid)			*Hisar Arun* (Local)		
			Mature Green	Breaker	Mature Red	Mature Green	Breaker	Mature Red
		0 Days	49.432	46.321	43.043	64.224	59.482	56.276
1% H_2O_2	5°C	7 Days	53.123	50.204	47.1	67.451	62.94	59.963
		14 Days	54.783	51.748	48.59	69.118	64.499	61.738
		21 Days	53.941	51.017	47.801	68.432	63.803	60.851
	10°C	7 Days	54.781	51.732	48.538	69.175	64.894	61.928
		14 Days	55.962	52.8	49.662	70.424	66.05	63.022
		21 Days	55.55	52.35	49.279	70.103	65.703	62.725
	15°C	7 Days	57.655	54.45	50.939	72.012	67.083	63.929
		14 Days	59.227	55.923	52.342	73.316	68.502	65.252
		21 Days	58.716	55.426	51.794	73.137	68.053	64.893
4% H_2O_2	5°C	7 Days	51.578	48.503	45.318	66.05	61.332	58.245
		14 Days	52.278	49.197	45.936	66.543	61.857	58.742
		21 Days	51.801	48.75	45.565	66.52	61.759	58.592
	10°C	7 Days	53.34	50.212	46.85	68.2	63.402	60.315
		14 Days	54.083	50.832	47.386	68.909	64.157	60.989
		21 Days	53.653	50.505	47.184	68.517	63.751	60.632
	15°C	7 Days	54.748	51.536	48.151	69.655	64.768	61.52
		14 Days	55.689	52.384	48.955	71.039	66.071	62.712
		21 Days	55.086	51.835	48.383	70.324	65.345	62.066

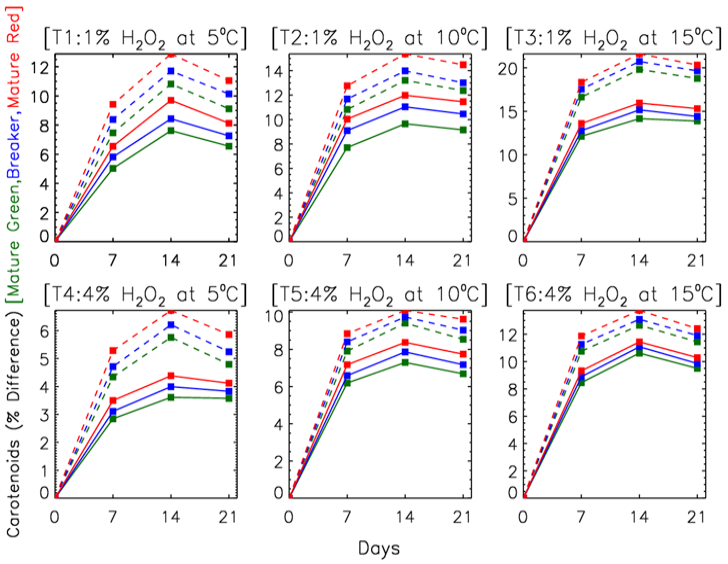

Fig. 4. Percentage difference in Carotenoids content for 21 days of investigation of tomato fruits stored at different temperature and H_2O_2 concentration. The color code and line style were kept similar to that used in Figure 1

content during the storage and ripening process (Ronen *et al.,* 1999; Paul and peter, 2004; Ilahy *et al.,* 2011)[34,35,31]. Concentration of H_2O_2 is directly proportional to the change in the lycopene content. From Fig. 5, it can be noted clearly that the rate got doubled in T4 when compared with that in T2 while in case of T3, the rate of increment was slow, probably due to low storage temperature. On the other hand, the storage temperature has shown a dominating effect in the form of significant reduction (by 50%) in the lycopene content for both the cases of H_2O_2 concentrations. Thus lower temperature can help in depressing the increment in the lycopene content. Further, the change was higher in local variety than hybrid variety. Also, the lycopene content increased faster in fruits harvested at mature green stage.

Table 5: Lycopene content (mg/1000g FW) during storage in tomato fruits treated with hydrogen peroxide

Concentration	Temperature	Days	*Kashi Vishesh* (Hybrid)			*Hisar Arun* (Local)		
			Mature Green	Breaker	Mature Red	Mature Green	Breaker	Mature Red
		0 Days	16.003	24.792	32.138	20.893	34.793	43.108
1% H2O2	5°C	7 Days	17.072	26.311	33.982	22.896	37.942	46.633
		14 Days	17.311	26.687	34.469	23.284	38.487	47.366
		21 Days	17.513	26.995	34.868	23.499	38.899	47.829
	10°C	7 Days	17.609	27.137	34.99	23.682	39.26	48.309
		14 Days	17.915	27.602	35.526	24.037	39.831	49.093
		21 Days	18.125	27.934	35.958	24.261	40.205	49.594
	15°C	7 Days	17.786	27.442	35.404	24.253	40.165	49.504
		14 Days	18.076	27.854	35.901	24.726	40.913	50.447
		21 Days	18.353	28.274	36.446	25.159	41.608	51.227
4% H2O2	5°C	7 Days	17.569	27.103	34.993	24.09	39.992	49.39
		14 Days	17.891	27.65	35.692	24.44	40.597	50.169
		21 Days	18.196	28.115	36.37	24.77	41.133	50.819
	10°C	7 Days	18.865	29.078	37.47	25.883	42.895	52.869
		14 Days	19.285	29.68	38.235	26.297	43.603	53.703
		21 Days	19.619	30.184	38.985	26.67	44.311	54.665
	15°C	7 Days	19.868	30.553	39.263	28.097	46.422	57.061
		14 Days	20.33	31.288	40.168	28.837	47.654	58.621
		21 Days	20.805	32.005	41.195	29.514	48.808	60.05

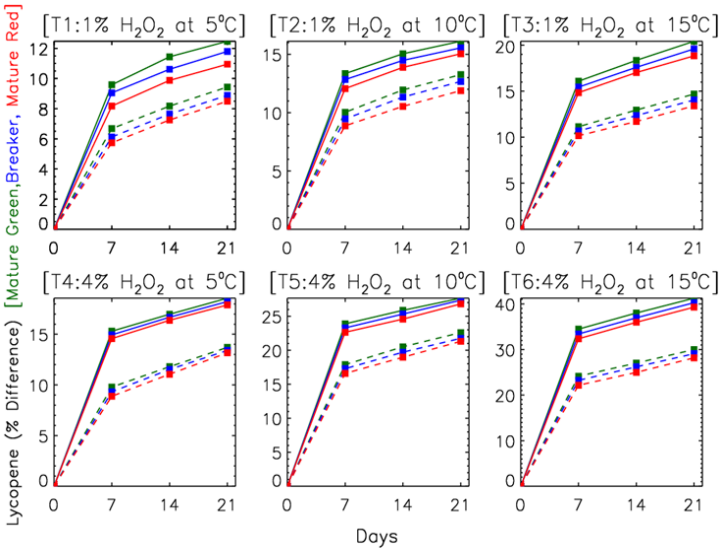

Fig. 5. Percentage difference in Lycopene content for 21 days of investigation of tomato fruits stored at different temperature and H_2O_2 concentration. The color code and line style were kept similar to that used in Figure 1

CONCLUSION

Tomato is used as an integral part of human diet. Being a climacteric fruit, tomato is prone to irreversible changes leading to reduction in its shelf life. Therefore, in this paper, we investigate the variation of non-enzymatic antioxidant content of the two most commonly consumed Indian tomato cultivars namely Hisar arun (a Local variety), and *Kashi Vishesh* (a *Kashi Vishesh* variety) which were harvested at different maturity stages namely Mature green, Breaker, and Mature red, when treated with varied concentration (1% and 4 %) of H_2O_2 and stored at various temperature 5°C, 10°C and 15°C. In particular, we record Antioxidant activity, Ascorbic acid, Lycopene, Carotenoid, and Total Phenol contents every 7 days and until 21 days of complete storage time. Our investigation revealed the aforementioned contents to be increasing till 14 days after which they show a decreasing trend. It is of note that the rate of increase in the carotenoid content is found to be high for the mature red stage in comparison to the mature green stage.

Summarily, we found tomato fruits, harvested at mature green stage, retained significantly higher amount of Ascorbic Acid after 14 days of storage compared to fruit harvested at the breaker and red stage. Additionally, our investigations are suggestive of 1% H_2O_2 treatment to be the most effective in terms of offering a definitive control in the rate of evolution of non-enzymatic antioxidants of the tomatoes such as lycopene and carotenoids. Further, although *Hisar arun* (Local) variety is found to retain more nutritional content than *Kashi Vishesh* (hybrid) variety, latter shows higher shelf life.

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