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Biochemical changes in calcium chloride treated Hisar Arun (*Local*) and *Kashi Vishesh* (Hybrid) cultivars of Tomato fruit

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Abstract

Tomato (*Lycopersicum esculentum*) although, second largest crop produced across the world, is a highly perishable commodity. Consequently, pre and postharvest treatments are critical in order to enhance the shelf-life and nutrition. Therefore, two popular Indian tomato cultivars namely Hisar Arun and Kashi Vishesh in their Mature Green, Breaker and Mature Red stages were treated with 1%, 2% and 5% Calcium chloride in order to increase their shelf life and stored at 5°C, 10°C and 15°C temperatures. The changes in non-enzymatic antioxidants namely ascorbic acid, Lycopene, Carotenoid, and Total Phenol were recorded up to 21 days of storage. Our investigation revealed a linear increase in the ascorbic acid at 5°C when treated with 5% CaCl2 (~40%) while Lycopene (45%) and carotenoids (25%) have shown comparative moderate increment at 10°C storage when treated with 1% CaCl2. Our analysis suggests that low (1%) concentration of CaCl2 is the most effective in controlling the increase of investigated non-enzymatic antioxidants in both cultivars. Moreover, *hybrid* cultivar tomatoes exhibited delayed deterioration compared with local type and can be recommended for long-time storage.

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Introduction

Tomato (*Lycopersicum esculentum*) belongs to Solanaceous family, the tomato is utmost extensively used as a food crop. As tomato is a climacteric and perishable vegetable, it is prone to degradation in a small amount of time accounting to be 14 to 21 days[1]. In fact, tomatoes are known to possess a high quantity of sugar and acid contents which are the main factors contributing to its flavor[2]. Keeping the fresh appearance of tomatoes along with its nutrition

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even after harvesting is an undying task imposed by the consumers. External appearance, quality and nutritional content of tomato fruits can be maintained by controlled storage temperature[3]. Moreover, the temperature controlled environment has been known to have a direct control of the metabolic activities in the tomatoes and thus proven to enhance the shelf life of the tomatoes with preserved nutritional qualities. Further, it is undeniably one of the most significant measures invoked in maintaining and even augmenting the shelf-life of tomatoes[4].

Numerous biochemical and physiological processes of fruits and vegetables are controlled by the association of calcium ion which is believed to play a key role in retaining quality. The rate of ethylene production and respiration in tomato fruits reportedly decreased by increasing the level of calcium ions[5]. Thus, treatment of fruits with $CaCl_2$ resulted in improving the firmness of the fruits and decreased deteriorations physiologically thereafter leading to reduced respiration, decrement in ethylene production, and delay in fruit ripening[6]. Various investigations revealed that calcium chloride treatment on tomato reduced post-harvest decay quantitatively in terms of development in the lycopene and ascorbic acid content, controlled development of physiological disorders, strengthening of the skin constituting cell walls, and providing the tissues with improved resistance, reduced production of enzymes which thus limit infections, control ripening, and rotting thus inevitably improving the quality and shelf-life of tomato[7]. In parallel, storage temperature is an important factor in maintaining quality and increasing the shelf life of tomatoes by reducing moisture loss and decelerating biochemical changes during ripening. In the context of two varieties of the tomatoes abundantly consumed in India, we conducted the $CaCl_2$ treatment on them to find the optimum storage temperature and biochemical changes for ensuring better quality for consumption with optimized shelf life.

Materials and Methods

The experiments were conducted in the research laboratory of the SHUATS, Allahabad. Two cultivars of tomato fruit(*L. esculentum*) namely *properly. Ascorbic*, (a Local variety) and *Kashi Vishesh*, (a

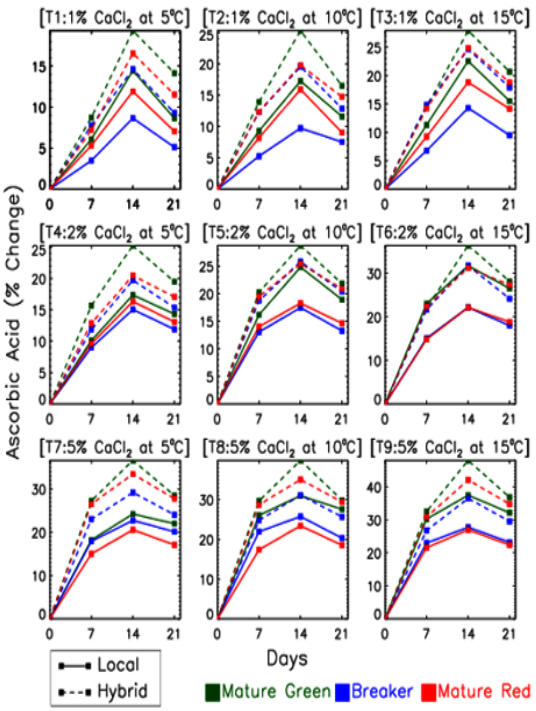

Fig. 1: Percent Change in the Ascorbic Acid content during storage in tomato fruits treated with different concentrations of $CaCl_2$ at different storage temperatures.

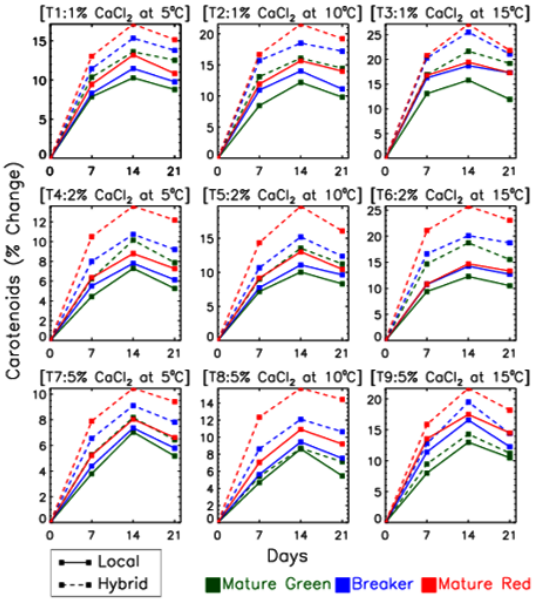

Fig. 2: Percent change in the Carotenoid content during storage in tomato fruits treated with different concentrations of $CaCl_2$ at different storage temperatures.

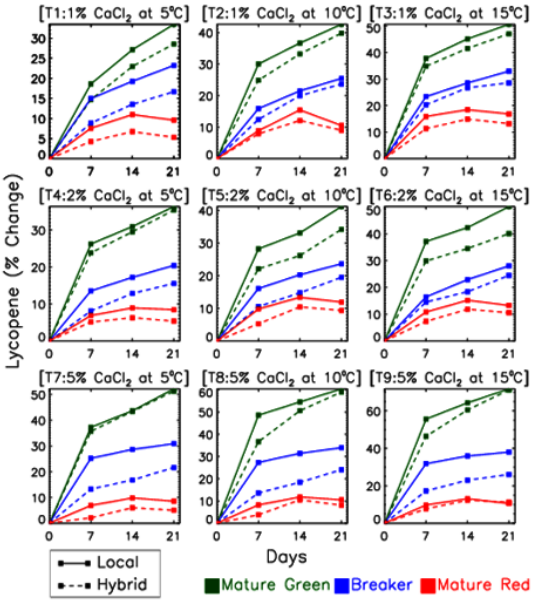

Fig. 3: Percent change in the Lycopene content during storage in tomato fruits treated with different concentrations of $CaCl_2$ at different storage temperatures

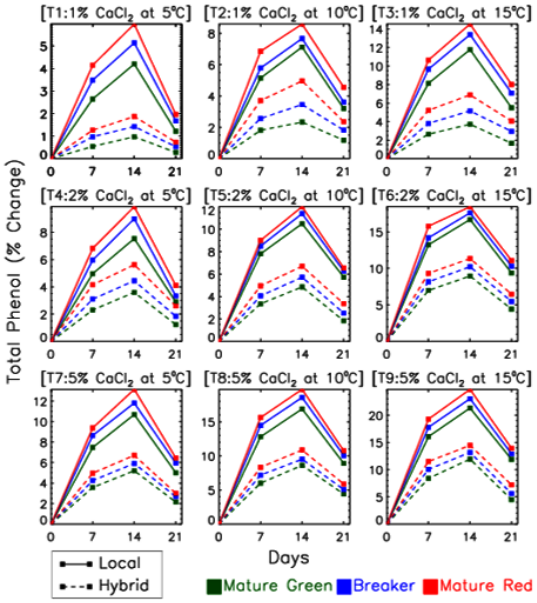

Fig. 4: Percent change in the total phenol content during storage in tomato fruits treated with different concentrations of $CaCl_2$ at different storage temperatures

hybrid variety) were harvested from the experimental field of Department of Forestry in SHUATS (25°24'50.4"N, 81°50'58.5"E). Following the color based maturity stages, as prescribed in the USDA color chart8, we picked the tomatoes corresponding to three particular maturity stages visually namely Green (Mature Green), Breaker and Red (Mature red) and further graded according to shape size, color, and appearance. After grading, tomatoes were washed with water and air dried. Next, the tomatoes were treated with 1%, 2% and5% $CaCl_2$ solution for 15 minutes and stored at 5°C, 10°C, and 15°C temperatures for 21 days, the analysis was done after every 7 days. The storage duration is chosen to be 21 days in order to perform a comprehensive comparison for all the maturity stages of the tomatoes as, after this time duration, the sample of the mature red stage of tomatoes would succumb to degradation and fungal activities. Over-ripened tomatoes of different treatments with the passage of time during storage were excluded from the trial.

Changes in quality of tomato during storage

Ascorbic Acid

The ascorbic acid content was estimated by using spectrophotometric method[9]. The 1.0 g fresh sample was extracted with 10ml of 0.4% freshly prepared oxalic acid and were then centrifuged with 8000 rpm for 20 min. The 3ml solution was maintained by the addition of 1 ml aliquots of supernatant and 2 ml of 0.4% oxalic acid afterward 7 ml of 2, 6-dichlorophenol indophenol dye solution was added. The absorbance of the test mixture was recorded instantaneously at 518nm after mixing the test solution properly.Ascorbic acid was estimated using a calibration curve prepared against a highly pure ascorbic acid.

Carotenoids

1g of the sample was ground in mortar and pestle with 80% methanol and then centrifuged. The supernatants obtained was concentrated to dryness and dried. The dried residue of the supernatant was then dissolved in 15 ml of diethyl ether. After this, 15 ml 10% methanolic KOH was added for removal of alkali the obtained mixture was then washed with 5% ice-cold saline water. The collective saline washings were extracted with ether (3:15 v/v). Both the ether extract obtained were mixed together and washed till alkali-free with cold water. The alkali-free ether extract was dried over anhydrous Na_2SO_4 for two hours in the dark. Using Ether as a blank

absorbance of filtered alkali-free ether extract were taken at 450nm[10].

Lycopene

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. After, proper mixing it was transferred into a separating funnel in which 25ml 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 505nm.[11]

Total Phenol

1 gm of fresh sample was centrifuged with 10 ml of 50% MeOH: H_2O (1:1). 3 ml of test mixture was prepared by the addition of 1 ml of FCR (1 N), 2 ml of freshly prepared 20% Na_2CO_3 in 1 ml aliquots of the supernatant. The mixture prepared was then mixed properly using cyclomixer and then maintained to 25 ml with water which is then kept at room temperature for 30 minutes. After completing 30 min absorbance was taken at 725 nm. Graphs were prepared using the standardized gallic acid solution of different concentrations and total phenol content have been expressed in mg/100 gmaterial[12].

Statistical Analysis

Each treatment has been repeated thrice to reduce human error, which is evident by the similar result obtained with this exercise. All the datasets were next subjected to multifactor ANOVA with SPSS 11.0 for windows. A significant effect was assessed at 5% ($p \leq 0.05$) level of significance and the mean was separated using the least significant difference (LSD) procedure.

Results and Discussions

Effect of Calcium Chloride treatment on Ascorbic Acid content of tomatoes

Figure 1 provides a comprehensive evolution of the two varieties of tomato (named *Hisar Arun* and *Kashi Vishesh*) for a duration of 21 days when treated with a varying amount of $CaCl_2$ concentration [1%, 2%, and 5%] and by changing the three storage temperature in the range of 5-15 degrees. For a better representation, while figure 1 shows the evolution derived in the form of relative difference (w.r.t. the value at day zero) in percentage, Table 1 provides the actual values recorded from the experiments. The evolution corresponding to Hisar Arun tomato is plotted with full lines while that corresponding to *Kashi Vishesh* tomato is shown in dotted. Further, as mentioned earlier, the three stages of tomato are put under investigation namely Mature Green, breaker, and full-ripe as shown in red, blue and green colors, respectively. In general, while we find the percentage increase in the ascorbic acid content in all the investigations, as following, we brief specific characteristics conclusions from this investigation.

A significant increase in the ascorbic acid content up to 14 days in all stages (Mature Green, Breaker and mature red), temperatures (5°C,10°C and 15°C) and concentrations (1%, 2% and 5 % $CaCl_2$) in both cultivars (*Kashi Vishesh* and *Hisar Arun*). On the other hand, storage after 14 days leads to a saturation in the acid content followed by a decreasing trend. From the data, it can be concluded that ascorbic acid content increased on increasing concentration of calcium chloride in all the observed condition. Breaker tomato fruit had the maximum ascorbic acid content followed by mature red and Mature Green. Maximum content (25.014 mg/100g) were found at 5% $CaCl_2$ concentration after 14 days of analysis stored at 15 °C in Hisar Arun variety while minimum (9.327 mg/100g) were found at 1% $CaCl_2$ concentration after 7 days of analysis stored at 5°C in *Kashi Vishesh* variety. From the figure, treatment with 5% concentration of $CaCl_2$ led to a significantly higher rate (up to 40 %) of increment of the acid in all stages, temperature and both cultivars. The rate of increment was observed higher in the Mature Green stage trailed by mature red and Breaker stage in both cultivars. The rate of increase in the ascorbic acid content was found to be maximum at Mature Green stage T9 (35-45%) in both cultivars however that recorded minimum corresponding to mature red stage of Hisar Arun variety (8%).

As we can see from plot T1, the ascorbic acid content in Mature Green stage increased up to 18 % in *Kashi Vishesh* variety (10.236 Mg/100g) after 14 days of storage under treatment, while in the case of Hisar Arun variety, it increases up to only 15 % (13.879 mg/100g) in same time interval and then both started decreasing. Mean value of ascorbic acid content to be ranging between 15 to 23mg/100g in tomato

Table 1: Changes in Ascorbic Acid content (mg/100gm FW) during storage under a different condition in CaCl2 treated tomato fruits

			Hybrid (Kashi Vishesh)			Local (Hisar Arun)		
Conc.	**Temp.**	**Days**	**Mature green**	**Breaker**	**Mature red**	**Mature green**	**Breaker**	**Mature red**
		0 Day	8.578± 0.274	14.965± 0.479	12.872± 0.412	12.127± 0.388	19.584± 0.627	15.989± 0.512
	5°C	7 Days	9.327± 0.298	16.128± 0.516	13.804± 0.442	12.865± 0.412	20.266± 0.649	16.838± 0.539
		14 Days	10.236± 0.328	17.151± 0.549	15.004± 0.480	13.879± 0.444	21.278± 0.681	17.894± 0.573
		21 Days	9.791± 0.313	16.357± 0.523	14.356± 0.459	13.174± 0.422	20.584± 0.659	17.116± 0.548
	10°C	7 Days	9.772± 0.313	16.808± 0.538	14.460± 0.463	13.251± 0.424	20.613± 0.660	17.298± 0.554
1% $CaCl_2$		14 Days	10.747± 0.344	17.892± 0.573	15.415± 0.493	14.218± 0.455	21.488± 0.688	18.533± 0.593
		21 Days	9.991± 0.320	16.888± 0.540	14.767± 0.473	13.531± 0.433	21.057± 0.674	17.432± 0.558
	15°C	7 Days	9.821± 0.314	17.187± 0.550	14.705± 0.471	13.498± 0.432	20.911± 0.669	17.468± 0.559
		14 Days	10.972± 0.351	18.657± 0.597	16.068± 0.514	14.862± 0.476	22.383± 0.716	18.998± 0.608
		21 Days	10.351± 0.331	17.646± 0.565	15.293± 0.489	14.007± 0.448	21.440± 0.686	18.248± 0.584
	5°C	7 Days	9.921± 0.317	16.742± 0.536	14.525± 0.465	13.354± 0.427	21.344± 0.683	17.525± 0.561
		14 Days	10.743± 0.344	17.914± 0.573	15.504± 0.496	14.231± 0.455	22.529± 0.721	18.598± 0.595
		21 Days	10.248± 0.328	17.264± 0.552	15.067± 0.482	13.864± 0.444	21.913± 0.701	18.078± 0.578
	10°C	7 Days	10.315± 0.330	17.767± 0.569	15.381± 0.492	14.087± 0.451	22.139± 0.708	18.231± 0.583
2% $CaCl_2$		14 Days	11.042± 0.353	18.824± 0.602	16.147± 0.517	15.135± 0.484	23.003± 0.736	18.909± 0.605
		21 Days	10.448± 0.334	18.013± 0.576	15.561± 0.498	14.420± 0.461	22.181± 0.710	18.326± 0.586
	15°C	7 Days	10.467± 0.335	18.207± 0.583	15.745± 0.504	14.917± 0.477	22.538± 0.721	18.364± 0.588
		14 Days	11.697± 0.374	19.721± 0.631	16.891± 0.541	15.973± 0.511	23.924± 0.766	19.527± 0.625
		21 Days	10.991± 0.352	18.578± 0.594	16.384± 0.524	15.343± 0.491	23.104± 0.739	18.995± 0.608
	5°C	7 Days	10.916± 0.349	18.408± 0.589	16.285± 0.521	14.335± 0.459	23.093± 0.739	18.387± 0.588
		14 Days	11.717± 0.375	19.331± 0.619	17.175± 0.550	15.062± 0.482	24.033± 0.769	19.273± 0.617
		21 Days	11.023± 0.353	18.561± 0.594	16.445± 0.526	14.792± 0.473	23.524± 0.753	18.719± 0.599
	10°C	7 Days	11.121± 0.356	18.674± 0.598	16.567± 0.530	15.285± 0.489	23.881± 0.764	18.767± 0.601
5% $CaCl_2$		14 Days	11.998± 0.384	19.625± 0.628	17.380± 0.556	15.883± 0.508	24.624± 0.788	19.732± 0.631
		21 Days	11.138± 0.356	18.804± 0.602	16.657± 0.533	15.468± 0.495	23.560± 0.754	18.949± 0.606
	15°C	7 Days	11.369± 0.364	18.974± 0.607	16.845± 0.539	15.793± 0.505	24.082± 0.771	19.436± 0.622
		14 Days	12.695± 0.406	20.424± 0.654	18.286± 0.585	16.668± 0.533	25.014± 0.800	20.299± 0.650
		21 Days	11.732± 0.375	19.382± 0.620	17.346± 0.555	16.022± 0.513	24.122± 0.772	19.566± 0.626

All values are mean ± standard deviation (n = 3) and are significant at P=<0.05

Table 2: Changes in carotenoids content (mg/100gm FW) during storage under a different condition in CaCl2 treated tomato fruits

			Hybrid (Kashi Vishesh)			Local (Hisar Arun)		
Conc.	**Temp.**	**Days**	**Mature green**	**Breaker**	**Mature red**	**Mature green**	**Breaker**	**Mature red**
		0 Day	48.785 ± 1.561	45.026 ± 1.441	42.573 ± 1.362	58.254 ± 1.864	56.473 ± 1.807	54.797 ± 1.754
	5°C	7 Days	53.841 ± 1.723	50.179 ± 1.606	48.126 ± 1.540	62.841 ± 2.011	61.165 ± 1.957	59.973 ± 1.919
		14 Days	55.425 ± 1.774	51.939 ± 1.662	49.872 ± 1.596	64.244 ± 2.056	62.957 ± 2.015	62.032 ± 1.985
		21 Days	54.903 ± 1.757	51.233 ± 1.639	49.029 ± 1.569	63.377 ± 2.028	61.996 ± 1.984	60.739 ± 1.944
	10°C	7 Days	55.186 ± 1.766	52.103 ± 1.667	49.677 ± 1.590	63.185 ± 2.022	62.672 ± 2.006	61.337 ± 1.963
1% $CaCl_2$		14 Days	56.643 ± 1.813	53.371 ± 1.708	51.762 ± 1.656	65.372 ± 2.092	64.402 ± 2.061	63.394 ± 2.029
		21 Days	55.854 ± 1.787	52.783 ± 1.689	50.768 ± 1.625	63.987 ± 2.048	62.772 ± 2.009	62.487 ± 2.000
	15°C	7 Days	57.027 ± 1.825	54.153 ± 1.733	51.404 ± 1.645	65.873 ± 2.108	65.657 ± 2.101	63.984 ± 2.047
		14 Days	59.340 ± 1.899	56.503 ± 1.808	54.116 ± 1.732	67.459 ± 2.159	67.031 ± 2.145	65.436 ± 2.094
		21 Days	58.142 ± 1.861	54.503 ± 1.744	51.852 ± 1.659	65.191 ± 2.086	66.257 ± 2.120	64.271 ± 2.057
	5°C	7 Days	51.826 ± 1.658	48.626 ± 1.556	47.043 ± 1.505	60.842 ± 1.947	59.583 ± 1.907	58.273 ± 1.865
		14 Days	53.735 ± 1.720	49.864 ± 1.596	48.358 ± 1.547	62.503 ± 2.000	60.879 ± 1.948	59.616 ± 1.908
		21 Days	52.627 ± 1.684	49.172 ± 1.574	47.762 ± 1.528	61.317 ± 1.962	59.942 ± 1.918	58.779 ± 1.881
	10°C	7 Days	53.176 ± 1.702	49.827 ± 1.594	48.687 ± 1.558	62.427 ± 1.998	60.847 ± 1.947	59.815 ± 1.914
2% $CaCl_2$		14 Days	55.391 ± 1.773	51.871 ± 1.660	50.968 ± 1.631	64.103 ± 2.051	62.736 ± 2.008	61.937 ± 1.982
		21 Days	54.269 ± 1.737	50.593 ± 1.619	49.415 ± 1.581	63.097 ± 2.019	61.916 ± 1.981	60.525 ± 1.937
	15°C	7 Days	55.943 ± 1.790	52.513 ± 1.680	51.557 ± 1.650	63.715 ± 2.039	62.548 ± 2.002	60.716 ± 1.943
		14 Days	57.917 ± 1.853	54.076 ± 1.730	53.539 ± 1.713	65.402 ± 2.093	64.503 ± 2.064	62.843 ± 2.011
		21 Days	56.356 ± 1.803	53.456 ± 1.711	52.392 ± 1.677	64.371 ± 2.060	63.643 ± 2.037	62.097 ± 1.987
	5°C	7 Days	51.359 ± 1.643	47.975 ± 1.535	45.932 ± 1.470	60.467 ± 1.935	58.952 ± 1.886	57.667 ± 1.845
		14 Days	52.772 ± 1.689	49.125 ± 1.572	47.022 ± 1.505	62.356 ± 1.995	60.626 ± 1.940	59.203 ± 1.894
		21 Days	51.923 ± 1.662	48.543 ± 1.553	46.577 ± 1.490	61.268 ± 1.961	59.742 ± 1.912	58.419 ± 1.869
	10°C	7 Days	51.416 ± 1.645	48.904 ± 1.565	47.831 ± 1.531	60.974 ± 1.951	59.649 ± 1.909	58.654 ± 1.877
5% $CaCl_2$		14 Days	53.027 ± 1.697	50.473 ± 1.615	49.274 ± 1.577	63.279 ± 2.025	61.803 ± 1.978	60.775 ± 1.945
		21 Days	52.256 ± 1.672	49.819 ± 1.594	48.721 ± 1.559	61.441 ± 1.966	60.723 ± 1.943	59.842 ± 1.915
	15°C	7 Days	53.404 ± 1.709	50.772 ± 1.625	49.331 ± 1.579	62.917 ± 2.013	62.916 ± 2.013	62.218 ± 1.991
		14 Days	55.768 ± 1.785	53.816 ± 1.722	51.831 ± 1.659	65.826 ± 2.106	65.843 ± 2.107	64.389 ± 2.060
		21 Days	54.265 ± 1.736	51.513 ± 1.648	50.319 ± 1.610	64.362 ± 2.060	63.402 ± 2.029	62.747 ± 2.008

fruits[13]. Further, the range may vary within 8 and 59 mg/100 based on the cultivar variety, which agrees to the results obtained in our investigation. This reduction of nutrients and deterioration are reported to be instigated by transpiration and respiration, the key physiological processes[14]. The non-linear rate of change in the AA content over the storage period of 21 days may be attributed to the oxidation process, which is understood to be caused by the catalyst and oxidizing enzymes[15]. Moreover, according to some researchers[7], the reason for high ascorbic acid content in calcium-treated fruits might be that metabolic activities were fast as compared to that in untreated fruits. In this context, the increase in the content of ascorbic acid as the storage temperature is subject to an increment that is indicative of the fact that fruit is still undergoing the process of ripening[16], while a decrease in the aforementioned content indicates a senescent fruit. The ascorbic acid content in tomato deteriorated from 21.63 mg/100g at the start of the trial, attaining the minimum 13.53 mg/100g in 1.5 % $CaCl_2$ after 9 days of storage[17]. In agreement with our findings, ascorbic acid content to be increasing with the ripening of the tomatoes until a stage where the same has shown a decreasing trend[18]. This suggests that harvesting the tomatoes at a suitable maturity stage may retain the nutrition level over the long storage period.

Effect of $CaCl_2$ treatment on Carotenoid content in tomatoes during storage

The lycopene is considered accountable for the bright red color of most fruits e.g. Pineapple, orange, lemon, grapefruit, strawberry and tomato etc.19. As tomatoes reach to the maturity stage, the carotenoid content decreases. Investigation of carotenoid content for the *Hisar Arun* and *Kashi Vishesh* cultivars revealed a generally increasing trend up to 14 days after which it starts decreasing (actual values reported in Table 2).

Rates of increment were found to be higher in the mature red stage of Hisar Arun variety in all set of observations. From the figure 2, it can be concluded that on increasing concentration of calcium chloride, the rate of increment slows down as noted in T3 where the percentage increase is up to 27% while in T9 the same reached only up to 21%. Further, the temperature is found to play an important role in affecting carotenoid's content during storage. At temperature 5°C the content increases slowly (8-16%) followed by that resulted for 10°C temperature (15-22%) while that corresponding to 15°C revealed maximum increment (20-27%). After 14 days of storage, carotenoids content start decreasing in all the samples under study. The increase in carotenoids can be attributed to the unmasking of the carotenoids following chlorophyll degradation during ripening of tomatoes[17]. A similar trend in the carotenoids has been reported in mangoes and passions fruits[20].

The investigation of Lycopene content is crucial as the same is found to increases in tomato fruits during ripening. The evolution of the Lycopene content, recorded over a period of 21 days for which the tomatoes were stored, is shown in figure 3 (actual experimentally recorded values are given in Table 3).The overall evolution enables us to note that during primary stages of ripening, lycopene content progressively increased while after a certain level, the same started decreasing. Similar trends were also found in the investigation of ascorbic acid content. Lycopene content increased relatively slowly in the *Kashi Vishesh* variety compared with that of the Hisar Arun variety at each level of concentration, temperature, and days of storage. Further, the rate of increment in the lycopene content after 21 days of storage was found to be maximum (~ 65%) in T9 while minimum (~33%) in T1 corresponding to the Mature Green stage of Hisar Arun variety. Moreover, lycopene content is found to be maximum in all the stages treated with 5% $CaCl_2$ followed by that for 2% $CaCl_2$ and then for 1% concentration of $CaCl_2$.Minimum lycopene content with the value of 0.03mg/100g in fruit when treated with 1% $CaCl_2$ followed by 3% $CaCl_2$ with a value of 0.06 mg/ 100g,[21] in agreement to the rate of increment of the lycopene content recorded in our analysis. Further, thermal processing of tomatoes, the increase in the extractable lycopene content in processed products when compared with the fresh tomatoes[21]. The change in lycopene content in the fruits and vegetables may be attributed to its reaction with free radicals. This results in disruption of polyene chain[22,23].

Effect of $CaCl_2$ treatment on the Total Phenol content in tomatoes during storage

It is crucial to quantify the post-harvest change in the total phenol content of tomatoes as such extensively

Table 3: Changes in Lycopene content (mg/1000gm FW) during storage under a different condition in $CaCl_2$ treated tomato fruits

			Hybrid (Kashi Vishesh)			Local (Hisar Arun)		
Conc.	**Temp.**	**Days**	**Mature green**	**Breaker**	**Mature red**	**Mature green**	**Breaker**	**Mature red**
		0 Day	15.651± 1.424	25.456± 2.316	34.144± 3.107	20.951± 1.907	36.482± 3.320	45.009± 4.096
	5°C	7 Days	17.953± 1.634	27.712± 2.522	35.610± 3.241	24.850± 2.261	41.951± 3.818	48.427± 4.407
		14 Days	19.246± 1.751	28.903± 2.630	36.451± 3.317	26.635± 2.424	43.512± 3.960	49.955± 4.546
		21 Days	20.122± 1.831	29.704± 2.703	35.961± 3.272	27.958± 2.544	44.953± 4.091	49.334± 4.489
	10°C	7 Days	19.545± 1.779	28.627± 2.605	36.843± 3.353	27.241± 2.479	42.273± 3.847	49.008± 4.460
1% $CaCl_2$		14 Days	20.849± 1.897	30.546± 2.780	38.285± 3.484	28.632± 2.606	44.331± 4.034	51.959± 4.728
		21 Days	21.879± 1.991	31.485± 2.865	37.221± 3.387	29.876± 2.719	45.762± 4.164	49.754± 4.528
	15°C	7 Days	21.111± 1.921	30.635± 2.788	38.025± 3.460	28.881± 2.628	45.031± 4.098	52.178± 4.748
		14 Days	22.162± 2.017	32.273± 2.937	39.241± 3.571	30.421± 2.768	46.911± 4.269	53.337± 4.854
		21 Days	23.024± 2.095	32.741± 2.979	38.661± 3.518	31.568± 2.873	48.522± 4.416	52.603± 4.787
	5°C	7 Days	19.382± 1.764	27.524± 2.505	35.901± 3.267	26.447± 2.407	41.422± 3.769	48.115± 4.378
		14 Days	20.272± 1.845	28.733± 2.615	36.291± 3.302	27.431± 2.496	42.756± 3.891	49.028± 4.462
		21 Days	21.191± 1.928	29.412± 2.676	35.983± 3.274	28.562± 2.599	43.932± 3.998	48.819± 4.443
	10°C	7 Days	19.114± 1.739	28.142± 2.561	35.952± 3.272	26.863± 2.445	42.345± 3.853	49.421± 4.497
2% $CaCl_2$		14 Days	19.747± 1.797	29.214± 2.658	37.714± 3.432	27.889± 2.538	43.886± 3.994	51.038± 4.644
		21 Days	21.003± 1.911	30.423± 2.768	37.333± 3.397	29.577± 2.692	45.112± 4.105	50.370± 4.584
	15°C	7 Days	20.324± 1.849	29.152± 2.653	36.643± 3.335	28.723± 2.614	42.475± 3.865	49.867± 4.538
		14 Days	21.049± 1.915	30.136± 2.742	38.186± 3.475	29.816± 2.713	44.833± 4.080	51.843± 4.718
		21 Days	21.924± 1.995	31.681± 2.883	37.752± 3.435	31.452± 2.862	46.699± 4.250	50.991± 4.640
	5°C	7 Days	21.236± 1.932	28.835± 2.624	34.872± 3.173	28.754± 2.617	45.676± 4.157	48.114± 4.378
		14 Days	22.445± 2.042	29.726± 2.705	36.203± 3.294	30.093± 2.738	46.933± 4.271	49.431± 4.498
		21 Days	23.675± 2.154	30.963± 2.818	35.873± 3.264	31.876± 2.901	47.751± 4.345	48.873± 4.447
	10°C	7 Days	21.387± 1.946	28.923± 2.632	35.443± 3.225	31.123± 2.832	46.454± 4.227	48.721± 4.434
5% $CaCl_2$		14 Days	23.564± 2.144	30.148± 2.743	37.745± 3.435	32.372± 2.946	47.939± 4.362	50.314± 4.579
		21 Days	24.881± 2.264	31.581± 2.874	36.903± 3.358	33.601± 3.058	48.862± 4.446	49.741± 4.526
	15°C	7 Days	22.920± 2.086	29.851± 2.716	36.773± 3.346	32.611± 2.968	48.099± 4.377	49.393± 4.495
		14 Days	25.125± 2.286	31.317± 2.850	38.354± 3.490	34.433± 3.133	49.585± 4.512	50.925± 4.634
		21 Days	26.802± 2.439	32.094± 2.921	38.002± 3.458	35.991± 3.275	50.363± 4.583	49.772± 4.529

Table 4: Changes in Total Phenol content (mg/100gm FW) during storage under a different condition in CaCl2 treated tomato fruits

Conc.	Temp.	Days	Hybrid (Kashi Vishesh)			Local (Hisar Arun)		
			Mature green	Breaker	Mature red	Mature green	Breaker	Mature red
		0 Day	12.591± 0.755	10.876± 0.653	9.231± 0.554	16.771± 1.006	14.663± 0.880	10.997± 0.660
	5°C	7 Days	12.660± 0.760	10.982± 0.659	9.348± 0.561	17.215± 1.033	15.174± 0.910	11.453± 0.687
		14 Days	12.714± 0.763	11.031± 0.662	9.404± 0.564	17.476± 1.049	15.416± 0.925	11.654± 0.699
		21 Days	12.626± 0.758	10.934± 0.656	9.299± 0.558	16.975± 1.018	14.909± 0.895	11.213± 0.673
	10°C	7 Days	12.819± 0.769	11.155± 0.669	9.573± 0.574	17.634± 1.058	15.511± 0.931	11.750± 0.705
1% $CaCl_2$		14 Days	12.885± 0.773	11.252± 0.675	9.689± 0.581	17.966± 1.078	15.787± 0.947	11.942± 0.717
		21 Days	12.740± 0.764	11.074± 0.664	9.449± 0.567	17.307± 1.038	15.193± 0.912	11.498± 0.690
	15°C	7 Days	12.925± 0.775	11.290± 0.677	9.713± 0.583	18.135± 1.088	16.080± 0.965	12.167± 0.730
		14 Days	13.060± 0.784	11.437± 0.686	9.867± 0.592	18.746± 1.125	16.628± 0.998	12.595± 0.756
		21 Days	12.801± 0.768	11.197± 0.672	9.606± 0.576	17.693± 1.062	15.703± 0.942	11.878± 0.713
	5°C	7 Days	12.881± 0.773	11.213± 0.673	9.614± 0.577	17.603± 1.056	15.538± 0.932	11.747± 0.705
		14 Days	13.044± 0.783	11.359± 0.682	9.750± 0.585	18.035± 1.082	15.981± 0.959	12.083± 0.725
		21 Days	12.746± 0.765	11.076± 0.665	9.471± 0.568	17.255± 1.035	15.151± 0.909	11.448± 0.687
	10°C	7 Days	13.012± 0.781	11.317± 0.679	9.688± 0.581	18.079± 1.085	15.908± 0.955	11.987± 0.719
2% $CaCl_2$		14 Days	13.202± 0.792	11.498± 0.690	9.849± 0.591	18.531± 1.112	16.333± 0.980	12.317± 0.739
		21 Days	12.822± 0.769	11.150± 0.669	9.541± 0.572	17.731± 1.064	15.582± 0.935	11.717± 0.703
	15°C	7 Days	13.470± 0.808	11.764± 0.706	10.091± 0.605	18.990± 1.139	16.743± 1.005	12.732± 0.764
		14 Days	13.717± 0.823	11.988± 0.719	10.278± 0.617	19.570± 1.174	17.244± 1.035	13.026± 0.782
		21 Days	13.147± 0.789	11.470± 0.688	9.828± 0.590	18.342± 1.101	16.173± 0.970	12.215± 0.733
	5°C	7 Days	13.041± 0.782	11.338± 0.680	9.689± 0.581	18.025± 1.081	15.930± 0.956	12.029± 0.722
		14 Days	13.246± 0.795	11.519± 0.691	9.850± 0.591	18.563± 1.114	16.395± 0.984	12.443± 0.747
		21 Days	12.862± 0.772	11.167± 0.670	9.508± 0.570	17.610± 1.057	15.534± 0.932	11.706± 0.702
	10°C	7 Days	13.346± 0.801	11.657± 0.699	10.000± 0.600	18.918± 1.135	16.786± 1.007	12.715± 0.763
5% $CaCl_2$		14 Days	13.677± 0.821	11.911± 0.715	10.234± 0.614	19.604± 1.176	17.387± 1.043	13.170± 0.790
		21 Days	13.145± 0.789	11.426± 0.686	9.773± 0.586	18.265± 1.096	16.137± 0.968	12.179± 0.731
	15°C	7 Days	13.648± 0.819	11.969± 0.718	10.292± 0.618	19.465± 1.168	17.270± 1.036	13.117± 0.787
		14 Days	14.093± 0.846	12.307± 0.738	10.565± 0.634	20.346± 1.221	18.040± 1.082	13.719± 0.823
		21 Days	13.155± 0.789	11.485± 0.689	9.896± 0.594	18.763± 1.126	16.554± 0.993	12.527± 0.752

All values are mean ± standard deviation (n = 3) and are significant at P=<0.05

distributed naturally occurring substance in fruits and vegetables have the capability of scavenging superoxide and hydroxyl radical. In this regard, as shown in Figure 4, the percentage difference of total phenol content, as determined in the two varieties of the tomatoes, treated with 1%, 2% and 5% $CaCl_2$ and subsequently stored at various temperature for 21 days while the actual recorded values of TP content are provided in Table 4. In general, we found an increasing trend until 14 days of storage in the total phenol content in tomato in the course of ripening. In particular, the same have shown a significant increase in the fruits, which were treated with 1% calcium chloride and stored at 5° C during the entire analysis. According to many authors, during initial progression phase, a reduction was found in total phenolics content in fruits where the fruit is dignified to be physiologically mature and thus prone to initiation of ripening. The presence of higher concentration of phenolics during the early stage of development provides a protection mechanism to the phytohormones like auxins, gibberellin, and cytokines[24], which plays an important role in cell division and cell enlargement. In the present investigation, the rate of falling of total phenolic was maximum (25%-10%) in T9 where the elevation rate was also large in all maturity stages in both cultivars. From the figure, we can evaluate that on increasing concentration of calcium chloride from 1% to 5%, the rate of increase of total phenolic content enhanced during storage in all maturity stages.

In particular, tomato fruits stored at 5°C depict slower increase rates in total phenol content in all treated samples. Soluble phenolic contents are understood to be accumulated in the main compartment of a cell namely vacuole. The effect of storage temperature reflects in the form of building up of toxic intermediate in cells owing to change in the cell membrane permeability and activity of an enzyme associated with the membrane[25]. This explains the increment of total phenol content recorded in our investigation. Moreover, calcium prevents senescence-induced stress by main¬taining the strength of its membrane[6], and thus delays the release of phenolic compounds.

Conclusion

Tomato is used as an integral part of the human diet. Being a climacteric fruit, the tomato is prone to irreversible changes leading to a reduction in its shelf life. Therefore, this paper investigated the evolution of non-enzymatic antioxidants of the two most commonly consumed Indian tomato cultivars namely Hisar Arun (a Local variety), and *Kashi Vishesh* (a hybrid variety) which were harvested at different maturity stages namely Mature green, Breaker, and Mature red and treated with varied concentration (1%, 2% and 5 %) of calcium chloride ($CalCl_2$) at various storage temperatures 5°C,10°C and 15°C. Changes in Ascorbic acid, Lycopene, Carotenoid, and Total Phenol contents were recorded during storage and it was found that above mentioned non-enzymatic antioxidants except carotenoids increased till 14 days after which there was a decline in the quality of the parameters. On the other hand, carotenoid content in tomatoes increased up to 21 days for all maturity stages. It is to 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.

In conclusion, tomato fruits harvested at breaker stage retained the significantly higher amount of Ascorbic Acid after 14 days of storage compared to fruit harvested at the mature green and red stage. Additionally, our investigations are suggestive of 1% $CaCl_2$ 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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