Study on extension of shelf life of tomato using controlled atmosphere storage

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Abstract

Investigations were carried out to study the effect of different gas compositions of Controlled Atmosphere (CA) on the shelf life and quality of tomato variety Red Cloud. Mature green tomatoes were handpicked, sorted, washed, treated with permitted fungicide solution of known concentration and stored in CA conditions at $9\pm1C$, $90\pm5\%$ RH using 5% O_2 , 1% CO_2 and 5% O_2 , 2% CO_2 with N_2 as balance gas. Physicochemical and visual appearance of the tomatoes such as colour, texture, TSS, pH, acidity, sugars, pigments, ripening index and cumulative spoilage were periodically analysed. Results indicates that, tomato variety Red Cloud harvested at mature green stage could be stored in fresh and firm condition upto 8 weeks under CA conditions (5% O_2 and 2% CO_2 at $9\pm1C$) as compared to 4 weeks in control in cold room at $9\pm1C$. Physiological loss in weight (PLW) as well as cumulative spoilage was reduced considerably in CA stored fruits. At the end of 8 weeks of storage under CA conditions, tomatoes could ripe normally and developed full red color in 5 days at room temperature. Physicochemical changes after ripening were also studied and organoleptic quality characteristics of ripe fruits after CA storage were found very good. The above method of CA storage can be used for export of tomato variety Red Cloud in CA conditions by ship to distant countries.

Key words: Tomato, Storage, Controlled, Atmosphere, Shelflife

INTRODUCTION

A range of techniques are used to preserve post-harvest quality of fruits, vegetables, and other perishable produce. Refrigeration is the principal technique used but, in some instances, low temperature alone may be insufficient to retard ripening of fruit and prevent detrimental quality changes. Moreover, low temperature for prolonged periods may lead to physiological damage, e.g., low temperature breakdown in apples or chilling injuries in some other fruits [1]. Pioneering work of [2] led to the development of controlled atmosphere (CA) storage, a sophisticated technique that extends the life of some produce more than refrigeration alone. Where CA techniques are applied, the concentrations of CO_2 and O_2 are controlled at optima specific for each cultivator, facilitated by the recent development of automatic control systems.

Successful CA application requires that atmosphere surrounding fruit contains elevated CO_2 and reduced O_2 concentration. The most marked effects of reduced O_2 concentration at storage temperatures are seen at 5 or below 5% but below 2% problems of physiological disorders and alcohol formation may occur. The sensitivity of fruit to CO_2 concentration is cultivar-dependent, but at storage temperatures a number of physiological disorders can be exacerbated by high concentrations. To continue the beneficial effects of CA successfully into the marketing chain, any method used must therefore reduce O_2 and increase CO_2 concentrations sufficiently to get beneficial effects while not exceeding the limits set by the risk of physiological disorders.

In order to study the effect of CA on extension of shelf life and quality of mature green tomato of major commercial varieties (variety Red Cloud) and also to optimize the storage condition of tomatoes under controlled atmosphere storage, investigation was planned and carried out as follows. Mature green tomatoes were hand-picked, sorted, washed treated with permitted fungicide

solution (Benomyl, 500 ppm) surface dried and stored in CA condition at 10 1C, 90 5 % RH using different gas storage conditions of (CA1=1 % CO₂& 5% O₂), (CA2=2.5 % CO₂& 5% O₂) and (CA3=5 % CO₂& 5% O₂) with N₂ as balance gas. Changes in physicochemical and visual appearance of the tomatoes such as colour, texture, TSS, pH, acidity, chlorophyll, lycopene, ripening index, cumulative spoilage, marketability and sensory quality of fruits were periodically analyzed during storage period. The CA equipment and chamber used for storage study of tomato are shown in (Figures 1 and 2) respectively.

MATERIALAND METHODS

Chemicals

All reagents used were of analytical grade. Solvents used in HPLC analysis were from Ranbaxy, India and Merck, Germany. Standard for lycopene analysis was obtained from Sigma, USA. Other chemicals and reagents were obtained from SD Fine Chemicals, India and Ranbaxy, India.

Tomato Fruits

Tomato fruits (variety Red Cloud and variety Western Red) grown under uniform conditions in experimental plots (KhorasanRazavi Agricultural and Natural Resources ResearchCenter) were hand-harvested at the mature-green stage and used for the studies.

Total Acidity

Titrable acidity (TA) was determined according to [4] Ten-gram sample was macerated with distilled water and extract made up to 100 ml. Aliquot of the filtrate was titrated against 0.1 N NaOH using phenolphthalein as indicator. Titrable acidity was expressed as percent citric acid (anhydrous).

Pigments

Colorimetric method, Tomato pigments were extracted and

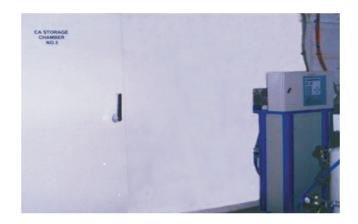








Figure 1. Controlled Atmosphere equipment with control system

quantified using the colorimetric method described by ^[4]. Chlorophyll was extracted with acetone, partitioned in diethyl ether and optical density of the extract measured at 660 nm and 642.4 nm after appropriate dilution, using Beckman spectrophotometer.

Lycopene was extracted in petroleum ether and optical density measured at 503 nm and at 452 nm for total carotenoeids.

Estimation of Lycopene content by HPLC method

Lycopene extraction procedure was similar to the published procedure for carotenoids extraction from vegetables and fruit^[5]. For every storage condition the 2 replicate samples of tomato (10-20 g, in different stage of ripening), vortexed for 1 min, and transferred into a glass fibre filter (10 to 20 µm) Buchner funnel. Forty ml of tetrahydrofuran and methanol (1:1 v/v THF: MeOH) were added and the suspension filtered under vacuum. When needed for an additional removal of colour, a second extraction was done with 20 ml THF/ MeOH as described to produce a grey/white precipitate. The combined filtrate was transferred to a reparatory funnel. Twenty ml of petroleum ether (40 to 60°C fraction) and 20 ml 10% sodium chloride solution were added and mixed by careful shaking. The lower THF/MEOH/aqueous phase was drawn off. The upper soluble materials, transferred into a 50 ml flask, and evaporated to dryness under nitrogen. The residue was dissolved, to a final volume of 4 ml of hexane, filtered (0.45μm) and analyzed by high performance liquid chromatography (HPLC). All procedures were performed under reduced light.



Figure 2. Controlled Atmosphere storage chamber

Chromatography

Reverse phase HPLC was performed on a C18 (201 TP540) analytical column (5 µm,25 cm X 4.6 mm; VYDAC, Hesperial, Calif., U.S.A). A 20 µl loop was used for solvent injection. Solvent delivery was achieved with spectra physics Sp8800 system at a flow rate of 1 ml/min. An isocratic mobile phase system of acetonitrile: methanol: 2-propanol (44:54:2 by vol) was used. Detection was monitored with a diode array 1040 A Hewlett Packard absorbance detector that also stored spectral data over the range of 190 to 600 nm for spectrophotometric peak identification. The chromatograms were simultaneously monitored at 350, 470 and 503 nm. Lycopene standard was obtained from Sigma Chemical Co. (St. Louis, MO., U.S.A). Peak identification was based on retention time and published absorbance spectral data. Lycopene in extracts of tomato powder was quantified spectrophotometrically using photo diode array detector (max=470 nm) using UV-Visible spectrophotometer, Beckam Instruments Inc., U.S.A

Color Value (Lab)

Hunter lab system, Minolta spectrophotometer CM-3500 d, Japan, with observation angle of 2, was used to monitor the whole fruit colour by measuring L, a, b values. These values were computed as the average of 4 measurements taken by rotating a tomato a quarter of turn after each measurement.

L, a, b are Hunter colour values and 'L' gives a measure of lightness on a scale ranging from zero (black) to 100 (white), 'a' denotes greenness when negative and redness when positive, 'b' denotes blueness when negative and yellowness when positive.

Changes in tomato colour was expressed as a/b ratio, known as red colour index, during storage period^{[6-7].}

Ripening Index

The method described by [8] given below was used to calculate the ripening index. Fruits were classified according to their degree of ripeness as mature-green, beaker with a tinge of yellow on an otherwise completely green surface, turning with about 50-70 % yellowing and a persistent green colour, light pink with complete

disappearance of green but with the appearance of slight pink or red colour, medium red, the fruits being wholly red but not intensive, and deep-red with full and intensive red colour.

The ripening rate of the fruit in terms of colour development was expressed as ripening index. This was monitored periodically by sorting out the fruit at each observation period (replicate-wise under each treatment) into different stages of ripening and assigning scores to each stage as follows:

MATURE green=0

Beaker=1

Turning = 2

Light pink=3

Medium red = 4

Deep red = 5

The ripening index (RI) was calculated using the relationship given below:

$$RI = [(A+B+C+D+E+F)/(YxZ)x100]$$

Where, A to F: product of the scores assigned and the number of fruits used at each stage of ripening.

Y: Total number of fruits in the determination A, B, C, D, E & F

Z: The number of stages of ripening in the replicate.

Firmness (Texture)

The puncture test, which is a force measuring method that has the dimension mass, length, and time, is probably the most frequently used method for textural evaluation. It consists of measuring the force and/or deformation required to push a probe or punch into a food to a depth that causes irreversible damage or failure^[9]. The photograph illustrates use of a 8 mm flat head stainless steel cylindrical probe to measure maximum force required to penetrate a tomato pricarp. Puncture force depends on two different properties of the sample (e.g., compressive and

Table 1: Physicochemical characteristics of tomato samples after storage at CA condition and ripening at room temperature

Treatment	Physicochemical characteristics								
	a/b	Ripening index (%)	Firmness (N)	Lycopene (mg/100g)	Chlorophyll (mg/100g)	T.S.S. (? Brix)			
CA 1	1.65 ±0.02	100	9.5 ±0.1	4.90 ±0.03	0.0	5.70 ±0.02			
CA 2	1.60 ±0.03	100	9.8 ±0.2	4.78 ±0.02	0.0	5.70 ±0.03			
CA 3	0.67 ± 0.01	73 ±2	7.6 ± 0.2	3.20 ±0.01	0.3±0.03	5.52 ±0.03			
Control	1.76 ±0.03	100	8.9 ±0.3	4.90 ±0.02	0.0	5.68 ±0.02			

shear strengths) and on both the probe area and perimeter, [10].

Firmness of each tomato was measured with a puncture probe (8 mm) penetrating to a dept of 7.8 mm using a Universal Texture Measuring System (Model LR5K, LLOYD, Japan). operated at cross head speed of 10 kg full scale load. Average of two maximum force recording (N) was taken at two diametrically opposite positions on the circumference of each tomato.

Cumulative spoilage

Observations were made at regular intervals for microbial spoilage in both the treated and control fruit samples. The number of spoilt fruit were expressed as percentage of the original number of fruits and recorded as percent spoilage.

Sensory evaluation

Sensory evaluation was carried out at the end of storage period to ascertain consumer acceptability of the ripened fruits. Tomato samples from each treatment were evaluated by 25 panellists from the research staff of Fruits and Vegetable Technology Department

and other staff members of CFTRI.

This was done by sensory category scaling for physical appearance (shrivelling), colour, firmness (Finger-feel) texture (bite firmness), taste, and overall quality using intensity scale ranging from 1 (poor) to 10 (very good). Final scores were calculated as means of the number of observations.

RESULTS

Colour Index

Colour values expressed as a/b are presented in (Figures 3). Progress of a/b value was delayed by storage of tomato samples in Controlled Atmosphere Condition for the both the varieties of the tomatoes. The rate of inhibition inside the chambers was different based on the gas concentration and tomato variety. Colour values of control fruits, openly kept in same temperature, increased from initial value of -0.53 to 1.32 in CA2 during 8 weeks of storage period.

Although colour development was retarded in samples stored

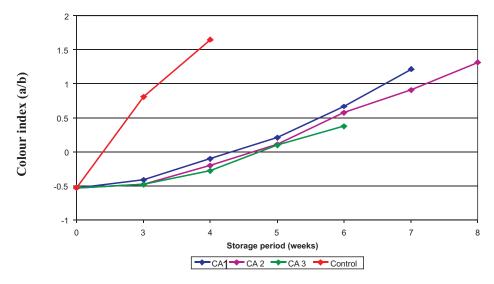


Fig 3: Changes in color index (a/b) of tomatoes during storage under different Controlled Atmosphere (CA) conditions (varietyRed Cloud)

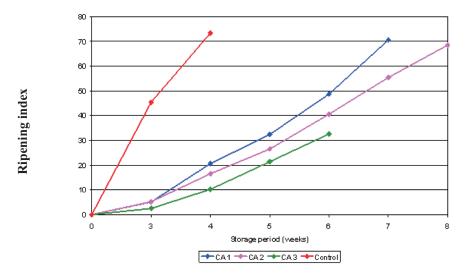


Fig 4: Changes in ripening index (%) of tomatoes during storage under different Controlled Atmosphere (CA) conditions (varietyRedCloud)

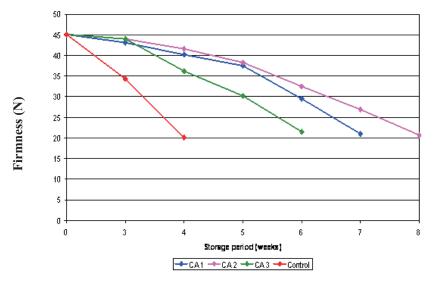


Fig 5 : Changes in firmness (N) of tomatoes during storage under different Controlled Atmosphere (CA) conditions

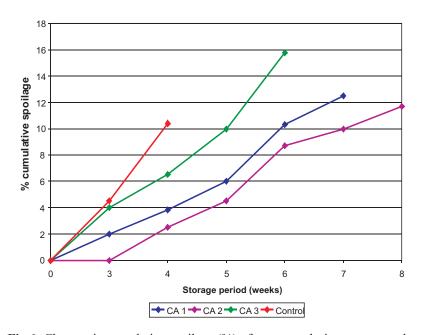


Fig 6: Changes in cumulative spoilage (%) of tomatoes during storage under different Controlled Atmosphere (CA) conditions

in Controlled Atmosphere during storage, control fruits reached a high value of 1.65 corresponding to the medium red by 4 weeks of storage period for variety RedCloud. Results showed a greater effect of reduced $\rm O_2$ level and elevated $\rm CO_2$ concentration on reduction of respiration and delay of ripening reactions.

The development of red colour in tomatoes is due to chlorophyll destruction, synthesis of carotenoids and lycopene $^{[11]}$ Synthesis of lycopene and carotene are dependent on the availability of O_2 . This would account for the delayed development of the red colour in fruits in CA. Although O_2 concentration was same in storage chambers, colour development was more inhibited at CA3where concentration of CO_2 was higher i.e. 5%. $^{[12]}$ showed that there was a clear correlation between the CO_2 levels and red colour development of tomatoes and the same correlation existed between red colour and lycopene content of tomato fruits.

Elevated CO_2 can result in detrimental effects on colour of some commodities during CA storage and following transfer to air. Examples include uneven red colour development in tomato, browning and scald-like blemish usually at the blossom end of the tomato. Injury was partially observed in tomatoes held continuously under 5% CO_2 . About 7% of all the fruit stored in this atmosphere were injured 6 weeks.

Ripening Index

Tomato openly kept in normal atmosphere as control in same temperature, ripened to a medium to full red colour after 4 weeks. Adding 1, 2.5, and 5% CO₂ to a low O₂ atmosphere inhibited ripening development, and tomatoes in these atmosphere were at turning or light pink stage after 6 weeks where ripening was slow as compared to the control fruits in same storage temperature. Control samples attained a ripening index of 67.2 after 4 weeks of storage, while it was always below 20%

considered as breaker stage for all the CA samples in same period (Figures 4). Tomatoes stored in CA2 chamber demonstrated regular ripening changes and storage life could be extended by 8 weeks, Similar results were reported by [13]indicating direct regulatory effect of low O₂ and high CO₂ levels on mechanism of fruit ripening in general and cell-wall enzyme activities in particular, in addition to their effect on ethylene biosynthesis. There were differences in ripening of tomatoes between modified atmosphere packaging and controlled atmosphere storage where mature green fruits in modified atmosphere packaging ripened earlier than the same fruit in controlled atmosphere storage. The difference was probably due to ethylene accumulation in modified atmosphere packaging which would not occur in controlled atmosphere storage because of the continuous gas flushing and ethylene scrubbing.

Firmness (Texture)

Controlled Atmosphere storage showed a considerable influence on the textural quality of stored tomato fruits. The

Firmness of tomato fruits stored in CA condition was greater than control samples stored in air at the same temperature. The firmness of control fruits fell rapidly from 45.1 to 20.1 (Figure 5). In spite of expected effect of more CO₂ concentration on maintaining the firmness value in CA₃ samples, more decline was observed after 4th week of storage and fruits were softer after 6 weeks which could be due to slight CO₂ injury leading to more internal softening in this concentration. Reduction of O₂ level and more important increases in the CO₂ level showed a significant effect on delay of softening due to its effect on cell-wall enzyme activities which are associated with softening.

The greater firmness of CA tomatoes is an advantage in handling and acceptance. There would be less mechanical damage during shipping and handling of the CA stored tomatoes, and they would retain firmness acceptable to consumers for a longer period of time.

Cumulative Spoilage

Generally, the incidence of decay was higher with the control

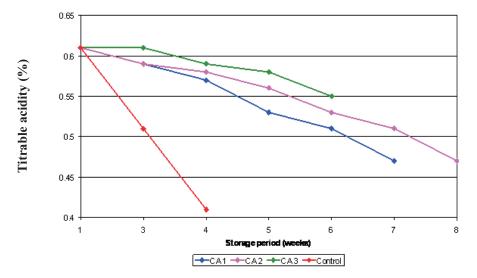


Fig 7: Changes in titrable acidity (%) of tomatoes during storage under different Controlled Atmosphere (CA) conditions

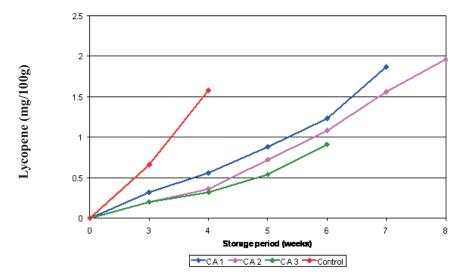


Fig 8 : Changes in lycopene (mg/100g) in tomatoes during storage under different Controlled Atmosphere (CA) conditions

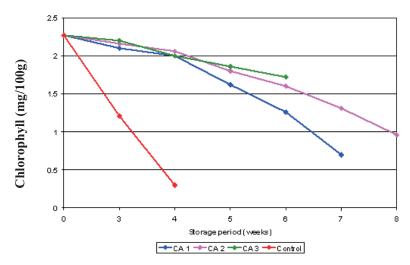


Fig 9: Changes in chlorophyll (mg/100g) of tomatoes during storage under different Controlled Atmosphere (CA) conditions

Table 2: Changes in overall sensory quality of tomatoes dur	ring storage under
different Controlled Atmosphere (CA) conditions and ripenir	ng at room temperature

Treatment	Storage+ Ripening period (days)							
	21+5	28+5	35+5	42+5	49+5	56+5		
CA 1	8.4±0.1	8.2±0.2	7.9±0.1	7.0±0.2	6.6±0.1			
CA 2	8.6±0.2	8.5±0.1	8.1±0.2	7.5±0.1	7.4±0.2	6.9±0.1		
CA 3	8.6±0.1	8.2±0.1	7.4±0.1	6.0±0.1	_			
Control	7.3±0.2	6.6±0.2		_	_			

fruits than those kept in CA storage (Figure 6). No or very less decay was found until 21 days storage in CA2 condition. Decay levels increased in all the treatments during storage. In comparison, there was a significant difference between storage condition of CA1 and CA2 samples where lower decay was observed in combination of 5 % O₂ and 2.5 % CO₂ in both the varieties. This is related to inhibitory effect of lowering O₂ level and high CO₂ concentration on decay of fruits. Shelf life limited where the spoilage was detected more than 10 %. General decay was observed by Alternaria rot and mould rot. By the time (after 4 weeks) some decay and local water accumulation occurred in the tomatoes, which were exposed, to the 5 % CO, indicating slight CO₂ injury. In tomatoes, a soft, water-soaked lesion may occur on any part of the fruit and enlarge rapidly. This disease is sometime called 'water rot [14].CO₂ injuries were reported by [15] in low O₂ (0-3 %) in combination with 3-5 % CO₂ conditions. Uneven ripening and a water-soaked appearance have been associated with CO₂ toxicity and this will be higher in cases where more concentration of this gas accumulates. Samples could be deep green in colour with sharply defined edges. In subsequent storage the damaged tissue turns brown.

Titrable Acidity

Titrable acidity (Figure 7) was higher in tomatoes stored at controlled atmosphere condition. There was a general trend to decrease in titrable acidity with time of storage both in control and CA stored tomatoes. This trend was more pronounced in control

fruits. The decrease in acidity in control samples is probably associated with faster ripening and therefore these fruits had earlier senescence compared with those stored under CA. These results are in agreement with [16].

There is little information on acidity changes during CA storage of tomatoes $^{[17]}$ found that titrable acidity increased with increasing CO₂ concentration from 0 to 5 % CO₂ during CA storage. $^{[18]}$ reported that the acidity level of tomatoes stored in either 12.2 % CO₂ or 15.6 % CO₂ with 5.5 % O₂ was lower than the acidity level of tomatoes stored in lower than 10 % CO₂ level. $^{[19]}$ reported that low temperature affected citric and malic acids, which are most closely associated with titrable acidity.

Lycopene

Lycopene content of the control tomatoes significantly over the period of storage while there was no considerable change in lycopene development in tomatoes kept in CA storage until 21 days storage and later increased slightly up to the end of storage. Although the lycopene content of tomatoes increased Steadily during the storage period in both, control and CA stored samples, the levels were low in the CA stored samples as compared with control (Figure8).

This would indicate the delayed development of red colour in fruits stored under CA. Although O_2 concentrations were similar in the storage containers, lycopene synthesis was more delayed under 5 % CO_2 (CA3) and 2.5 % CO_2 (CA2) as compared to 1 %

 $\mathrm{CO_2}$ (CA1). It could be concluded that the reduction in the rate of development of lycopene content in tomato during storage was affected by the level of $\mathrm{CO_2}$ in the atmosphere around them. This effect had previously been reported by [20].

DISCUSSIONS

Total Chlorophyll, Chlorophyll breakdown (Figures 9) was affected negatively by low O_2 and high CO_2 concentration provided in CA storage. Delay in the degradation of chlorophyll was observed in CA stored samples as compared to control during storage. Chlorophyll content in varietyRed Cloud stored under CA2 decreased from initial value of 2.27 mg/100g to 0.96 in the 8^{th} week of storage as against 2.27 mg/100g to 0.3 at the end of 4^{th} week of storage in control sample. [21-22] reported similar results.

Ripening behaviour of stored tomato samples, Results of analysis of tomato fruits after removal from CA condition at the end of storage period and ripening at room temperature for 5 days are presented in (Table 1). Tomato samples stored in CA1, and CA2 condition showed similar behaviour after ripening. Chlorophyll breakdown and lycopene synthesis increased uniformly as soon as fruits were subjected to ripening at room condition and fruits exhibited good firmness and maintained acceptable condition. Whereas in tomato samples stored in CA3 condition, partial CO₂ injuries delayed lycopene synthesis and also showed more softening and decay. Hence, tomato fruits in CA3 condition could not attain regular ripening and fruits remained in partial green colour even after 5 days of storage at room condition.

Overall sensory quality, The results of overall sensory quality for fruits stored in CA condition and ripened at room condition are presented in (Table 2). Ratings for overall sensory quality of CA stored tomatoes were higher than that of control fruits. As expected from quantitative physiological experiments, sensory panellists scored CA2tomato samples as the highest quality in comparison with the other treatments as well as control fruits. It indicates that CA2 samples maintained very good overall sensory quality characteristics after 56 days of storage and 5 days of further ripening with score of 6.9 (very good) for Variety Red Cloud.

When length of storage is considered, the overall quality attributes of tomato samples slightly decreased from early to later stages of storage period, but the changes took place in very good and good ranges as expressed by the sensory panellist.

Scale

Poor=1-2 Fair = 3-4 Good= 5-6 Very good = 7-8 Excellent = 9-10

CONCLUSION

Results confirm and demonstrate the effects of Controlled Atmosphere Storage (CAS) on the post-harvest physiology of green mature tomatoes by reducing the activity of tissue metabolism of fresh tomato. Chlorophyll break down was affected negatively by low O₂ and high CO₂ concentration. Red colour development as an indicator of ripening and maturity index was delayed in tomatoes stored under CA as compared to the control samples, openly kept at the same temperature.

Spoilage in CA tomatoes was reduced significantly and tomatoes reserved a high marketability without a significant impairment of overall sensory quality after storage and subsequent ripening at room temperature. Physiological loss in

weight (PLW) of tomatoes was reduced considerably in CA stored tomatoes. As differences of gas composition is concerned, storage of tomatoes under 5% $\rm O_2$ and 2.5% $\rm CO_2$ (CA2) showed the best composition, since a small percentage of fruits were injured at the higher (5%) $\rm CO_2$ level (CA3). This concentrationwas more effective for shelf life extension as compared to 1% $\rm CO_2$ and 5% $\rm O_2$ treatment alone.

Tomato varietyRed Cloud had a shelf life of 8 weeks at CA (5% O₂ and 2.5% CO₂) as compared to 4 weeks for control tomatoes. After removal from CA storage, fruits developed full red colour in 5 days at room temperature and were very good in overall sensory quality. The above method of CA storage can be used for export of tomato in CA condition by ship to distant countries.

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