

Post-harvest Shelf Life Extension and Nutritional Profile of Thompson Seedless Table Grapes Under Calcium Chloride and Modified Atmospheric Storage

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Abstract

Grapes are the fruits with highest rate of phenolic substances among all other fruits and vegetables but due to its enhanced susceptibility to *Botrytis cinerea*, its postharvest losses are also very high. In a country like Pakistan that has lesser advancement in post-harvest technological skills, a significant segment of this delicate fruit is lost. This study was conducted with an aim to minimize these losses by retaining healthy and attractive nutritional profile. Freshly harvested grapes after being sorted and graded were divided in three lots, two lots were immersed in aqueous solutions of 1% and 2% calcium chloride followed by modified atmosphere storage at 5% CO₂ level, and third lot was control sample that was simply dipped in tap water at ambient conditions, 80% relative humidity and 10 ± 1°C temperature was kept same for all three lots. After being analyzed at harvest, stored grapes were then analyzed for total polyphenols, firmness, acidity, total sugars, total soluble solids and total viable count at 4th, 8th and 12th days of storage respectively. Overall results designated that grapes pretreated with 2% CaCl₂ stored at 5% CO₂ level retained maximum firmness, acidity and phenolic substances with minimum increase in soluble solid contents and significantly reduced incidence of browning caused by gray mold as compared to water washed control sample that was spoiled at the 8th day of storage. Grapes were stored for 12 days and the effect of storage days on differently pretreated samples was analyzed.

Keywords: CaCl₂; Modified atmosphere; Total phenolic compounds (TPC); Total viable count (TVC); Polypropylene perforated storage bags

Introduction

Grapes (*Vitis vinifera*) belong to the *Vitaceae* family and are amongst the largest cultivated global fruit crop due to their excessive use in winemaking and as table fruit [1] and are considered as highly perishable and delightful fruit with strong nutritional profile. Healthy berries contain fiber and folic acid that are helpful in lowering the bodyweight, blood cholesterol and chances of severe hypertension [2]. Berries contain polyphenols that are primarily confined to skin and involve anthocyanin and resveratrol that have documented biological activities as antiatherogenic substances and minimizing the risks of atherosclerosis [3]. Food and Agriculture Organization of the United Nations reported that 75,866 km² of the world directly sanctified for grapes and practically one third of the global foodstuff manufactured for consumption is lost after being reaped [4]. In Pakistan, total grape production is 122,000 tons on an area of about 14,000 ha in 2008-09 that was recorded as the highest production of table grapes in the last nine successive years [5]. About 70% of the production of grapes in Pakistan is from Baluchistan Province with possible yield of almost 19 tons/ha only against the productive capacity of nearly 25 tons/ha, indicating poor infrastructure and postharvest technological skills [6].

Grapes experience many ripening changes during storage including biochemical and physical modifications in sugars, pH, acidity, total soluble solids, total polyphenols, and contents of vitamin C including minerals and different sensorial attributes. The ratio between sugars/acids is regarded to be the prime factor affecting taste and end quality of grapes and this ratio is highly exaggerated during prolonged storage [7]. Postharvest storage of fruits is highly susceptible to fungal infections such as *Botrytis*, *Aspergillus*, *Alternaria*, *Rhizopus* etc. that adversely affect the quality attributes of fruits [8]. Grey mold and stem browning caused by *Botrytis cinerea*, is a major biological hazard to horticultural produce as it can grow even below at -0.5°C and has the tendency to reproduce rapidly on berries skin [9]. To minimize the postharvest losses of grapes, several technologies have been introduced. The typical exercise is fumigation of the berries after being harvested with

conventional sulfur dioxide gas (SO₂) but severe health issues of its residues specifically to the people that are allergic to sulfites have been reported [9]. Controlled and modified atmosphere strategies [10], use of various bio-control agents [11], different inorganic salt patterns [12], several nonchemical compounds [13] and natural antimicrobials [14] have been used as substitutes of SO₂.

Applications of calcium salts are a well experienced example to replace conservative fungicide sprays in controlling the postharvest antimicrobial decay of fruiting berries [15]. Calcium salt has been shown to play an eccentric part in upholding the cell wall of the fruit cells for its role as preservative and firming agent. Postharvest application of different salts pretreatments including calcium chloride [16], sodium carbonate and bicarbonates [17], calcium lactate [18] and various others are the promising salt treatments for maintaining the quality characteristics of fruits.

The positive impacts of calcium chloride have formerly been testified on wide ranges of horticultural produce including various fruits such as strawberries, grapes, mangoes, apples and different vegetables [19]. Akhtar et al. [20] illustrated that CaCl₂ treated loquats exhibited higher degree of firmness as compared to water washed control loquats. Yousefi et al. [16] reported that nutritional profile of CaCl₂ treated apricots such as Ca, Mg, N, and vitamin C were significantly higher than non-treated apricot fruits at termination of storage duration. Extended duration of fruits storage in modified atmosphere where

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increased CO₂ level was used proved useful in postponing the softening of skin of many fruits [21]. Al-Quarshi and Awad [22] reported that table grape vines sprayed with 1% CaCl₂ and 10% ethanol solution effectively reduced the incidences of decay. Furthermore, -Ca salt didn't show any negative influence on the overall worth of the fruit. Microbial infestation caused by Botrytis is a major cause of spoilage in fruits specifically grapes due to its soft texture and high perishability. Chervin et al. [23] reported reduction in grey mold incidence when berries were treated with ethanol and 1% calcium chloride (CC) during modified atmosphere storage. This study was conducted with the aim to prolong the shelf life of white seedless table grapes to cause an increase in export and processing quality of the fruit.

Materials and Methods

Procurement of raw material

Freshly harvested Thompson seedless table grapes (*Vitis vinifera*) were procured from botanical garden, University of Agriculture, Faisalabad, Pakistan. The berries were then brought to Food Safety Laboratory of National Institute of Food Science and Technology, UAF for further processing and storage.

Post-harvest treatments

After sorting and grading, healthy clusters were divided in three equal lots. 1st lot was simply immersed in tap water and named as control sample (T₀), 2nd lot was dipped in 1% CaCl₂ (T₁) and the 3rd lot in 2% CaCl₂ solution (T₂).

This dipping pretreatment to all samples at ambient conditions was given for 5 minutes followed by packing in polypropylene perforated storage bags and stored at modified atmospheric chambers (ICH260 Memmert, Germany). In all three storage chambers, temperature and relative humidity were attuned at 10 ± 1°C and 80%, however the level of CO₂ for T₁ and T₂ inside the chambers was maintained at 5% and 0% for T₀ as shown in treatment plan (Table 1).

Total phenolic contents

Preparation of sample: Weighed amount (200 g) of samples were taken in glass bottles and the bottles were filled with methanol until a layer was formed above the sample. These samples were continuously shaken for 48 h with 3 h intervals at ambient temperature. After this, samples were filtered with filter paper and the extract obtained was concentrated to rotary evaporation for the removal of solvent from samples under vacuum. The distillation was stopped when the volume of the extract remains 1 mL. The solvent was further removed under purified gentle stream of N₂ gas. The sample was stored in freezer at -4°C till further analysis.

Determination of total polyphenols: The total phenolic compounds were estimated by Folin-Ciocalteu method by using an ultraviolet visible spectrophotometer (CECIL CE7200, UK) [24]. Extract mixture of 0.1 mL and 0.75 mL of Folin-Ciocalteu reagent that was previously diluted with deionized water was prepared in a test tube that was then allowed to stand at ambient conditions for 5 min. 0.75 mL of sodium carbonate 6% w/v was later added in the mixture. The absorbance of the samples was recorded from UV-Vis spectrophotometer at 275 nm. Gallic acid

Treatments	CaCl ₂ conc. (%)	CO ₂ level (%)
T ₀	0	0
T ₁	1	5
T ₂	2	5

Table 1: Treatment plan.

was run as a standard along with the samples and its standard curve was used for the calculation of the total phenolic contents in the samples.

Total sugars and TSS

The total sugar contents (glucose, fructose and sucrose) were determined in terms of g/100 g by following the procedure (Method No. 967.21) as described in AOAC [25] and total soluble solids (°Brix) were measured using digital hand Refractometer (Carl Zeiss Jena-Germany) after being calibrated against sucrose.

Titrateable acidity (%)

TA of the fruiting berries was determined by titrating diluted sample against 0.1 N NaOH with phenolphthalein indicator according to method described by AOAC [25].

Firmness (N/mm)

Firmness of the berries was measured by using texture measuring system fitted with needle probe. Berries were randomly selected from each treatment and placed at the base of the texture analyzer (Mod. TA-XT2, Surrey, UK). The force (N) required to penetrate the fruit surface up to a specific depth (mm) was recorded and expressed in terms of N/mm [26].

Total viable count (log CFU/g)

Fruits of known weight from treatments were washed in sterile distilled water using a shaker, with fruit to distilled water ratio as 1:9. The wash water was then further diluted using peptone water, up to 105 and plated in triplicate on potato dextrose agar prepared as per manufacturer's instructions and incubated at 25°C for 24 hours to count the microbial colonies [27].

Statistical analysis

The data was scrutinized statistically to determine the level of significance [28]. Experiment was a completely randomized design with factorial arrangement. Difference and comparison between the means were evaluated by LSD multiple range test at 5% level of significance.

Results and Discussion

The effects of calcium chloride and modified atmosphere storage showed a significant effect in maintaining the nutritional profile and physicochemical attributes of the berries as depicted (Table 2).

Total phenolic compounds (TPC)

Results: Present study investigations revealed that polyphenols of the berries were continuously decreasing throughout the storage irrespective of the given treatments and storage conditions; however the rate of this fall was highly dependent upon the given treatments (Figure 1). Maximum loss in TPC was observed in control sample. At harvest, TPC were recorded as 159.14 ± 6 mg GAE/100 g that reached to 116.2 and 94.6 at 4th and 8th day of storage, moreover significant lost was recorded at 12th day when the recorded value for trait reached 58.1 in T₀. Likewise, in T₁, a gradual decrease in phenolic contents was recorded that reached 99.2 at storage termination. Minimum fall in polyphenol count was recorded in T₂ under which grapes were treated with 2% CaCl₂ and stored at 5% CO₂ where value from harvest of 159.14 reached 124.2 at 12th day of storage indicating best and statistically significant results as compared to T₀ and T₁. This concludes that treated grapes with 2% CaCl₂ could adopt a sturdier defense mechanism which T₀ failed to adopt.

Storage Days	Treatments	TSS (° Brix)	Total Sugars (g/100 g)	Titrateable Acidity TA (%)	Firmness (N/mm)	Polyphenols (mg of GAE 100g ⁻¹)	TVC (log CFU g ⁻¹)
0	--	17.8 ± 0.9	14.12 ± 0.2	0.89 ± 0.03	2.74 ± 0.2	159.14 ± 6.0	2.73 ± 0.06
4	T ₀	22.5 ± 1.3	16.56 ± 0.9	0.61 ± 0.08	2.12 ± 0.3	116.2 ± 5.1	3.2 ± 0.1
	T ₁	19.0 ± 0.8	14.40 ± 0.2	0.83 ± 0.04	2.69 ± 0.2	138.3 ± 5.6	2.4 ± 0.1
	T ₂	17.9 ± 1.0	14.21 ± 0.2	0.88 ± 0.07	2.73 ± 0.1	154.3 ± 9.1	2.3 ± 0.08
8	T ₀	26.9 ± 2.0	19.10 ± 0.8	0.52 ± 0.04	1.5 ± 0.13	94.6 ± 4.8	3.9 ± 0.09
	T ₁	19.9 ± 1.0	15.41 ± 0.3	0.80 ± 0.03	2.45 ± 0.1	119.7 ± 5.9	2.5 ± 0.07
	T ₂	18.1 ± 1.0	14.50 ± 0.3	0.86 ± 0.05	2.7 ± 0.2	142.1 ± 6	2.4 ± 0.05
12	T ₀	29.5 ± 2.9	20.40 ± 1.6	0.40 ± 0.04	0.7 ± 0.08	58.1 ± 4.9	4.95 ± 0.1
	T ₁	23.6 ± 2.1	17.44 ± 0.2	0.68 ± 0.03	2.0 ± 0.09	99.2 ± 6.5	2.79 ± 0.2
	T ₂	18.7 ± 0.5	14.59 ± 0.4	0.82 ± 0.08	2.66 ± 0.1	124.2 ± 5.5	2.6 ± 0.06

T₀ = Water Washed (0% CO₂), T₁ = 1% CaCl₂ (5% CO₂), T₂ = 2% CaCl₂ (5% CO₂), Temperature (10 ± 1°C) and Relative Humidity (85%) was kept same in all. Given data are the mean values with 3 replications ± SD.

Table 2: Post-harvest quality attributes of Thompson seedless table grapes under different concentrations of CaCl₂ and modified atmosphere storage.

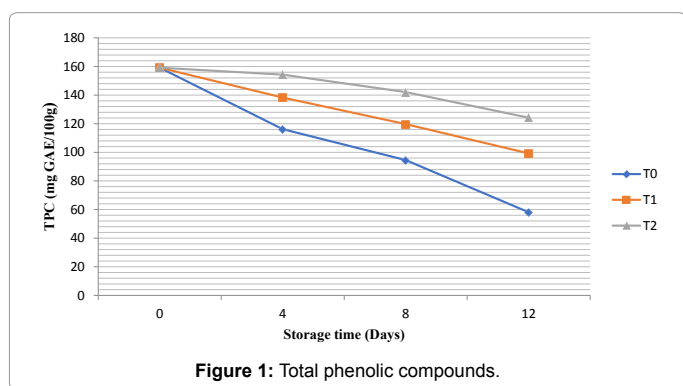


Figure 1: Total phenolic compounds.

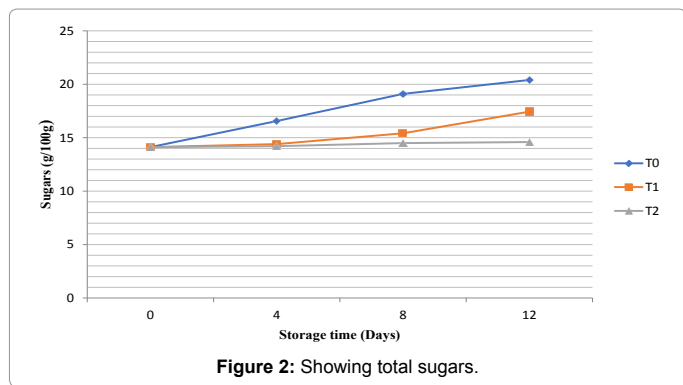


Figure 2: Showing total sugars.

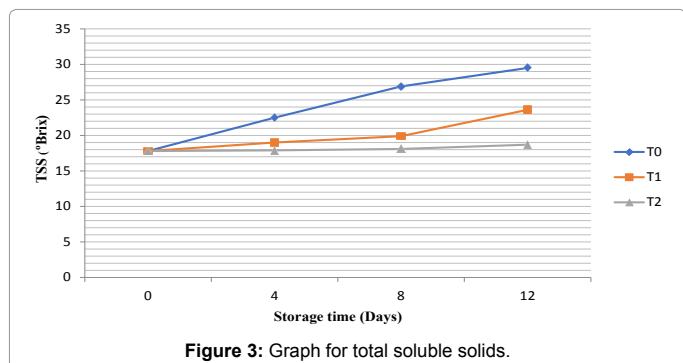


Figure 3: Graph for total soluble solids.

Discussion: Polyphenols are phytochemicals by nature and are present in highest amount in grapes than any other fruit; however, wine grapes have increased level of flavonoids, anthocyanin and phenolic

contents as compared to table grapes [29]. This polyphenol composition also varies with berries skin color, cultivar, species, environmental and postharvest management skills [2]. Results of study in hand closely resemble with the investigations of Al-Quarshi and Awad [22] who reported that total polyphenols were significantly higher in CaCl₂ treated grapes as compared to control grapes and in promise with the fallouts of Pinheiro et al. [30] who explored total phenolic contents and anthocyanin in grape cultivars and illustrated that TPC of grapes were significantly decreasing with storage days irrespective of the given treatments.

Total sugars and total soluble solids (TSS)

Results: A gradual increase in total sugars composition and TSS was observed with progression in storage days. Amongst treatments, a similar behavior was shown by all treatments indicating a significant effect ($P < 0.05$) of storage days on total sugars (Figure 2) and TSS (Figure 3), however this escalation was magnificently higher in T₀ as compared to CaCl₂ treated berries (T₁ and T₂).

Freshly procured grapes showed mean value of 14.1 g/100 g of total sugars at harvest that increased with storage period varying upon storage conditions and given treatments. Maximum increase in total sugars was observed in T₀ with mean values of 16.5, 19.1 and 20.4 at 4th, 8th and 12th day of storage respectively. Likewise, T₁ showed sugar contents of 14.1 at harvest to 17.44 at termination. Minimum nonconformity in total sugars was recorded in T₂ that had sugar contents of 14.59 at termination day indicating best positive results gained by 2% CaCl₂ given pretreatment followed by modified atmosphere storage at 5% CO₂ level.

Similarly, total soluble solids determined at harvest in fresh berries were 17.8 ± 0.9 °Brix with no noticeable changes in grapes stored at modified atmospheric conditions with 5% CO₂ level after being pretreated with CaCl₂ (1% and 2%). At the end of storage duration, TSS in CaCl₂ treated fruits were significantly lower and closer to the value at harvest. Contrary, a highly significant elevation in TSS level was found in T₀ where TSS were recorded as 22.5°, 26.9° and 29.5° at 4th, 8th and 12th days of storage respectively showing a greater degree of deviation in soluble solid contents. Likewise, T₁ also showed escalation in soluble solids reaching 19.0°, 19.9° and 23.6° during storage intervals. Minimum increase in total soluble solids was noticed in T₂ that stretched 17.8° from initiation to termination.

Discussion: A large percentage of the soluble solids in grapes are sugars, mainly glucose and fructose that are central sugars and are involved in cell respiration and synthesis and the third sugar is sucrose

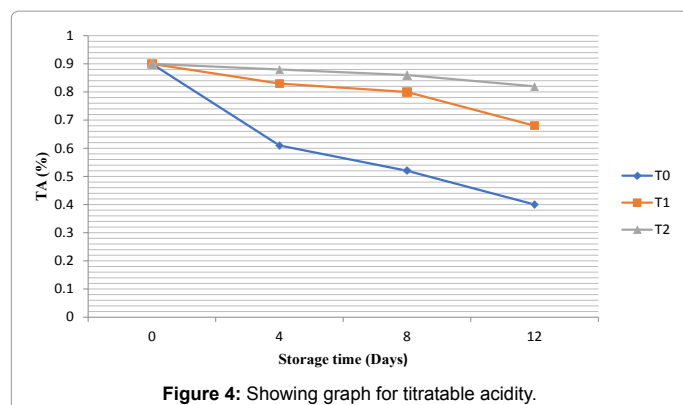


Figure 4: Showing graph for titratable acidity.

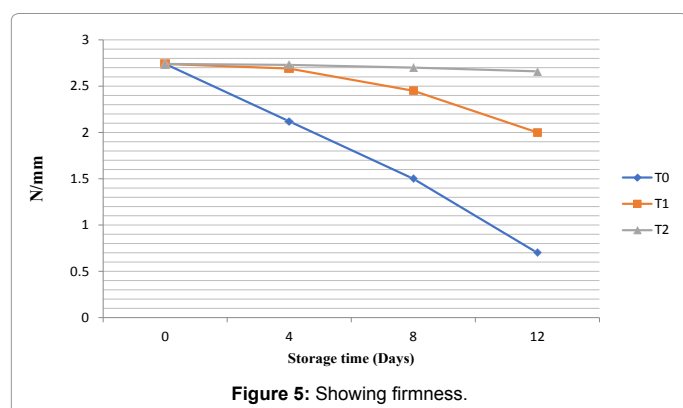


Figure 5: Showing firmness.

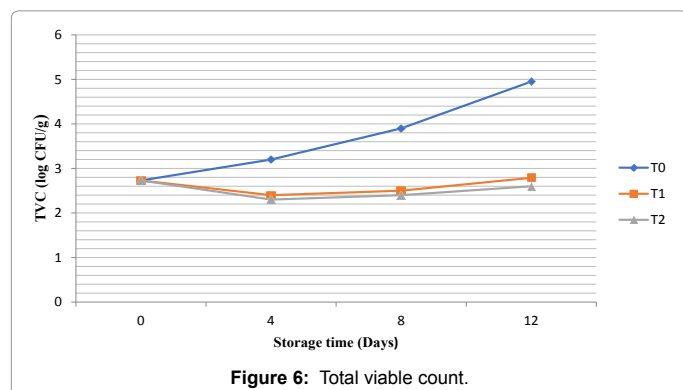


Figure 6: Total viable count.

that is non-reducing by nature and present relatively in smaller amounts with level not exceeding more than 1%. Prolonged storage resulted in greater deviation in sugar composition with reference to harvested value. This increment in soluble solids and sugars is attributed towards rapid conversion of complex starch molecules into simpler sugars [31]. Excess loss of water from the fruiting tissues may also be a valid reason behind this increment [18]. TSS of the fruits also increased during storage mainly due to glycogenesis and metabolism of fruiting tissues that becomes partially inactive due to changes in glucose and fructose. Findings of Samra [32] were in accordance with present study that SSC of fruiting berries were gradually increasing throughout the entire storage period irrespective of the given conditions and treatments.

Titratable acidity (TA)

Consumer preference of grapes relies on the peculiar taste that arises mainly from the organic acids present in the berries responsible for its titratable acidity (TA). The major organic acid present in table

grapes is tartaric acid that declined in all samples with progression in storage days.

Results: Freshly procured grapes at harvest showed a TA value of 0.89% that decreased with prolonged storage varying upon storage conditions and given treatments (Figure 4). Maximum loss of organic acids was recorded in T_0 that developed off flavors at the end of the storage showing TA values of 0.61, 0.52 and 0.40 (%) at 4th, 8th and 12th days of storage respectively. Relatively lower decrease in organic acids concentration was depicted by T_1 with TA of 0.89% at harvest to 0.68% at the end of storage period. Minimum loss in organic acids concentration from the given treatments specifically tartaric acid was observed in T_2 that depicted best results from TA of 0.89% at harvest to 0.82% at termination.

Discussion: Sugars/acids ratio is considered to be the main feature affecting taste of the berries and this ratio is highly affected during stretched storage periods [7]. In present investigation, TA of the berries was continuously decreasing throughout storage and this gradual decrease in acid level was physiologically attributed towards increase in membrane permeability of the fruit allowing the acids to respire in cell vacuoles resulting in transformation of acids into sugars [16]. These outcomes bear a resemblance with Sabir et al. [33] who exposed that TA levels in berries under modified atmosphere, apparently decreased. Randhawa et al. [34] also reported similar results with decreased level of acidity in citrus (non-climacteric) as storage duration was prolonged.

Firmness

Results: 2.74 ± 0.2 N/mm was the force required to the probe of texture analyzer to puncture the skin of the berries at harvest that gradually dropped in all treatments as storage period prolonged. However, CaCl_2 treated grapes stored in modified atmospheric chambers (T_1 and T_2) showed higher degree of firmness as compared to control sample (T_0) as shown (Figure 5). Higher degree of firmness was recorded in T_2 with mean values of 2.73, 2.70 and 2.66 followed by T_1 with 2.69, 2.45 and 2.0 (N/mm) at 4th, 8th and 12th days of storage respectively. Maximum deterioration and minimal degree of firmness was depicted by T_0 where force required to the probe of texture analyzer at 12th day of storage was only 0.7 N/mm indicating maximum quality degradation.

Discussion: Findings of present study are absolutely in accordance with that of Akhtar et al. [20] who treated loquats (non-climacteric) and Yousefi et al. [16] who treated apricots (climacteric) with different concentrations of CaCl_2 and reported that significantly higher degree of firmness was recorded in CaCl_2 treated fruits than untreated ones at the end of storage phase. Cell wall degrading enzymes (β -Glucosidase, Poly-Glacturonase and Pectinmethyl Esterase) may also be a reason for softening of fruit texture as mentioned by Pinzón-Gómez et al. [35].

Total viable count (TVC)

Results: TVC recorded at harvest was 2.73 ± 0.06 log CFU/g that significantly increased in T_0 while reduced microbial load was observed in CaCl_2 treated grapes stored at modified atmospheric chamber (T_1 and T_2) when storage phase terminated, as shown (Figure 6). Control grapes (T_0) had maximum yeast and mold count of 4.95 (log CFU/g) at 12th day of storage indicating severe mold growth caused by *Botrytis cinerea* turning the berries brown in color. No signs of browning were recorded in T_1 and T_2 , however, higher the concentration of CaCl_2 , lower the mold growth was observed. T_1 (1% CaCl_2) had microbial load of 2.79 at 12th day of storage that increased non-significantly from harvested value of 2.73. Likewise, in T_2 (2% CaCl_2), microbial load of

2.6 was recorded at termination that actually decreased from the values at harvest.

Discussion: Among all pathogens of different horticultural produce, *Botrytis cinerea* responsible for gray mold is amongst the list of world's top 10 most threatening fungal pathogenic microorganisms [36].

Increased CO₂ level has been proven to have antimicrobial and fungistatic effects that was the major reason behind lower microbial load in treated berries. Results of this study are in resemblance with the investigations of Romanazzi et al. [4] who reported that mere increased level of CO₂ limited the decay incidence but statistically highly significant results were observed when modified atmospheric conditions were provided. Findings of Nigro et al. [17] also endorses the current study status of minimal microbial load in grapes under calcium chloride dipping prior to storage.

Conclusion

Overall consequences of this study designate that modified atmospheric storage conditions (5% CO₂ level, 80% relative humidity and 10 ± 1°C temperatures) in combination with 2% CaCl₂ pre-storage dipping, successfully escalated the shelf life of grapes up to 12 days' storage by preserving attractive nutritional and safety profile. Inexpensive CaCl₂ salt that is readily available and acceptable due to its non-toxic nature as compared to conventional fungicide sprays, efficiently minimized the incidence of pathogens on berries by retaining maximum firmness and freshness and modified atmosphere storage successfully delayed the postharvest ripening with mean values of quality attributes much closer to the ones at harvest as compared to tap water washed control sample.

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