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Evaluation of Ozone and Sodium Metabisulfite Treatments on Postharvest Grapes Quality and Rhizopus Disease Control

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Abstract

Table grapes are important fruit crops subjected to fungal decay during harvesting, post-harvest handling, and storage. An investigation was made on the *in-vitro* evaluation of ozone (O_3) and Sodium metabisulfite (SMB) against radial growth of *R. stolonifer* through fumigation and food poisoning techniques, respectively. In another experiment, *in-vivo* evaluation of O_3 fumigation at various concentrations and SMB at different doses were tested against *Rhizopus* rot in table grapes under cold storage. The results revealed that O_3 at 8833.2 µl L^{-1} and SMB at 90 mg/100ml PDA medium significantly inhibited the radial growth of the pathogen by 94.82% & 98.14 %. Disease severity was zero in both O_3 and SMB treated fruits but it was 0.89 DS in inoculated control, O_3 at 7274.4 µL L^{-1} showed significantly better retention of berry firmness (85.09N) and ascorbic acid level (3.90 mg/100 g) and least TSS (18.68 N), and PLW (7.49) and also scored highest sensory overall acceptability (8.04). SMB powder 0.5g among SMB treated fruits following O_3 showed appreciative results. In addition, SMB treatments showed significantly highest *L**and *b** values highest compared to ozone, and recorded least colour *a** value. Hence, ozone is a good alternative to SO_2 treatments in conventional grape production and it could be a suitable technology to control *Rhizopus* rot disease and maintain quality parameters of grapes in cold storage.

Keywords: Ascorbic acid, Instrumental colour (*L* a* b**), Ozone, Sodium Metabisulfite, *Rhizopus stolonifer*

تر راټولولو وروسته د انګورو په کیفیت او رایزوپس ناروغی کنټرول باندې د اوزون او سودیم میتابای سلفایت درملنې ارزونه

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لنډیز

انګور د مهمو میوه جاتو په ډله کې راځي چې وروسته له را ټولولو د ساتنې او زیرمه کولو پرمهال د فنګسي ناروغیو سره مخ کیږي. په دې تحقیق کې د اوزون ګاز او د سودیم میتابای سلفیټ د *R. stolonifer* د جانبې ودې پر ضد د فومیګیشن او د خوراکي توکو زهرجن تخنیکونو له لارې په لابراتور کې څیړنه شوې. په بله تجربه کې، اوزون او سودیم میتابای سلفایت د رایزوپس خوساکیدنې ناروغی د مخنیوي لپاره په انګورو باندې په سړه خونه کې وڅیړل شو. نتایج څرګندوي چې، اوزون او سودیم میتابای سلفایت د *R. stolonifer* جانبي وده په مؤثره توګه کمه کړی ده. په انګورو کې د ناروغۍ شدت په دواړو اوزون او سودیم میتابای سلفیټ استعمال سره صفر شوی وو، مګر د ناروغۍ شدت په تلقیح شوي کنټرول تریتمنټ کې ۰،۸۹ وو. اوزون د انګورو دانو د سختۍ درجه ۸۵،۰۹ نیوټنه ښودلې، او د اسکوربیک اسید اندازه یې ۳،۹۰ میلی ګرامه تر ټولو ښه ساتنه کړې. همدارنګه، ټول جامد منحل مواد 18.72 بریکسه ، او فزیالوژیکي وزن کمونه 7.49 سلنه ښودلې، او همدارنګه تر ټولو لوړ حسی عمومي منلو ته (۸،۰٤) رسیدلي. د سودیم میتابای سلفایت ۰،۵ ګرامه کڅوړه پوډر ورسته له اوزون مثبتې پایلې ښودلې. او د رنګ د *L** او *b** ارزښتونو په لوړه کچه کې ښودل شوې. اوزون د سلفر ډای اکساید یو ښه بدیل دی او کولی شي په انګوروکې د رایزوپس خوساکیدنې ناروغ کنټرول کړي او د انګورو کیفیت سړه خونه کې وساتي.

کلیدي کلیمې: اوزون، سودیم میتابای سلفایټ، رایزوپس ستولونیفر، اسکاربیک اسید، رنګ (*L* a* b**)

1. Introduction

Grapes (*Vitis vinifera* L.) are non-climacteric fruits that degrade rapidly due to factors like loss of firmness, berry detachment, rachis discoloration, desiccation, and fungal rot pathogens (35). In Bagalkot, Karnataka, *Rhizopus stolonifer* causes significant losses during harvesting, transportation, and marketing (11). Traditionally, sulfur dioxide (SO_2) has been the main treatment; however, it's use is restricted in many countries due to concerns over sulfite residues, emissions, and adverse effects on grape quality, including skin cracking and discoloration (23, 38). Ozone presents a viable alternative, being residue-free and classified as "organic" by the USDA National Organic Program (41). However, strict decay tolerance standards complicate its use, allowing only 0.5% of berries to deteriorate during transport (41). While effective and cost-efficient, SO_2 can also cause bleaching and flavor alterations (14, 45). This study investigates the effects of sodium metabisulfite (SMB) and ozone on the *in vitro* growth of *R. stolonifer* using food preservation and fumigation techniques. *In vivo* assessments of SMB grape guards, SMB powder, and ozone fumigation were conducted against *Rhizopus* rot in table grapes under cold storage. This study focused to determine (i) the efficacy of ozone and SO_2 against *R. stolonifer* and (ii) their effects on *Rhizopus* rot and grape quality in cv. Manik Chaman over 49 days of cold storage.

2 Materials and Methods

2.1 Inoculum preparation

We prepared the fungal inoculum from affected grape samples to test the pathogenicity of *Rhizopus stolonifer*. In January 2020, grape samples exhibiting typical symptoms of *Rhizopus* rot were collected from Honnakatti village in the Bagalkot District of Karnataka, India. The pathogen was isolated using the tissue isolation method. According to the methodology outlined by (33), samples were placed on Potato Dextrose Agar (PDA) in petri dishes and incubated for 96 hours at 25±1°C in an incubator. The isolated culture was subcultured onto PDA slants and allowed to grow for six days at 25±2°C in a BOD incubator, after which the slants were stored at 4°C. Spores were collected from the actively growing culture using 20 ml of sterile distilled water under laminar flow. The resulting suspension was diluted using disinfected distilled water through a serial dilution method to obtain a 10^5 spore suspension for use as inoculum (4, 19, 24).

2.2 Pathogenicity test for *Rhizopus stolonifer*

Using the tissue isolation method, we further confirmed the pathogenicity of *R. stolonifer* on fresh grape samples collected from Honnakatti village. The process involved immersing small fragments of diseased samples in a 1% sodium hypochlorite solution for 30 seconds, followed by three rinses in sterilized distilled water. The fragments were then placed on PDA in petri dishes and cultured for 96 hours at 25°C, following the methodology outlined by (24). The isolated culture was sub-cultured in test tubes containing PDA slants and incubated at 25°C for six days in a BOD incubator before being cooled to 4°C. Spores were carefully collected to create a spore suspension for the pathogenicity test.

2.3 Ozonation system

We utilized the SEONICS Ozone Generator to produce ozone by introducing purified oxygen from an oxygen concentrator, facilitating Corona Discharge, which separates oxygen molecules into individual atoms. These atoms then react with O_2 molecules to form O_3, with a concentration measured in grams per cubic meter. Specifically, 0.5 to 5 liters per minute (LPM) of purified oxygen were fed into the generator, yielding 51.96 grams of O_3 per cubic meter per minute (51.96 g/m³/min = 51.96 mg/liter/min).

For the experiment, ozone treatments were applied to nine airtight LDPE boxes connected to the generator via a 6mm outer diameter tube. Each box for the *in-vitro* trial contained petri dishes with a volume of 0.5 liters, leaving 8.5 liters for ozone. Final ozone concentrations were calculated based on exposure times of 10, 15, and 20 minutes, yielding concentrations of 4416.6, 6624.9, and 8833.2 l μL^{-1}, respectively. In the *in-vivo* trial, each box held 2 liters of fruit, leaving 7 liters for ozone, resulting in ozone concentrations of 3637.2 l μL^{-1} for 10 minutes, 5455.8 l μL^{-1} for 15 minutes, and 7274.4 l μL^{-1} for 20 minutes.

2.4 Sulphur dioxide Treatment

In the *in-vitro* phase, PDA was combined with sodium metabisulfite (SMB) powder (CAS No. 40180 K05) using a food poisoning technique. During the *in-vivo* phase, grape guards from India and Africa were employed, with SMB powder applied at 0.5 grams per 500 grams of fruit, contained in fabric pouches to comply with a maximum residue limit (MRL) of 10 ppm in 500 grams of grapes.

2.5 *R. stolonifer* on *in-vitro* radial growth inhibition

We utilized a Completely Randomized Design (CRD) with four treatments, each replicated five times, to assess the effects of varying ozone and sulfur dioxide concentrations on the radial growth of *R. stolonifer*. The experiment commenced by pouring 20 ml of PDA medium into sterile petri dishes and allowing it to set. A 5 mm disc from a six-day-old *R. stolonifer* culture was placed in the center of each dish. All infected dishes, except for the control, were exposed daily to ozone at concentrations of 4416.6, 6624.9, and 8833.2 l µL/L. Additionally, the impact of sulfur dioxide was examined through four treatments with five replications. Following the food poisoning method outlined by (36), SMB was incorporated at doses of 50, 45, 90, and 0 mg per 100 ml of PDA. After preparation, disinfection, and cooling to 45°C, the PDA was treated with SMB before being poured into clean petri dishes. Finally, a 5 mm mycelial disc from a six-day-old *R. stolonifer* culture was positioned in the center of each dish.

2.6 Fruit Preparation

This study employed a two-factor completely randomized design (CRD) with eight treatments, each replicated three times, focusing on table grapes (*Vitis vinifera* L.) from the Main Horticultural Research and Extension Centre, University of Horticultural Sciences Bagalkot, in March 2020. After harvesting, the grapes were pre-cooled at 5°C for 12 hours before being

transported to the cold storage facility of the postharvest technology department for physico-chemical analyses. Each sample consisted of 0.5 kilograms of bunches, which were disinfected by soaking in a 1% sodium hypochlorite solution for 2 minutes, rinsed with distilled water, and air dried. Following an application of *R. stolonifer* spore suspension (10-5 concentration), the bunches were placed in a laminar airflow setting for 20 minutes and then assigned to eight treatments, including exposure to ozone for 10, 15, or 20 minutes at varying concentrations, grape guards from India and Africa, SMB at 0.5g in cotton pouches, and control samples (both inoculated and uninoculated). These samples were stored for 49 days, with the first three treatments receiving ozone exposure every 24 hours at three-day intervals.

2.7 Observations recorded

The study recorded the inhibition of *Rhizopus stolonifer* radial growth, disease severity, berry firmness, physiological weight loss, total soluble solids (°Brix), the ratio of total soluble solids to acidity, ascorbic acid content (mg per 100 grams), instrumental colour values (*L**, *a**, *b**), and sensory evaluation using a 9-point hedonic rating scale.

2.7.1 Radial growth inhibition (%) of *Rhizopus stolonifer*

We calculated the percentage of growth inhibition for each treatment to assess the inhibitory effects of sodium metabisulfite and ozone on mycelial growth in PDA. The average diameter of mycelial growth, when it reached the edges of the control plate's petri dishes, was measured (in millimeters). The information was then converted to a percentage using calculations made in accordance with a method developed by (46):

Radial growth inhibition (%) = DC- DT/ DC × 100

Where: DC = Radial growth of fungus colony (cm) in control,

DT = Radial growth of fungus colony (cm) in treatment

2.7.2 Disease severity (DS)

Disease severity was determined using a 0–5 scale: No disease, 5% disease, >5%–15% disease, >15–30% disease, >30–60% disease, and >60% disease (13) in 500g in CFB box.

2.7.3 Berry firmness

Berry firmness was measured using a texture analyzer provided by UK-based Stable Micro Systems and the piercing test method. Table grape berries were pierced with a cylindrical 2 mm probe. The greatest force needed to pass the test—first stated in kilogram-force (kgf) and then in Newton (N)—was used to determine firmness.

2.7.4 Physiological loss in weight (PLW)

The initial weight of each pair of table grape bunches was measured at the start of the storage period. The fruits were reweighed every two days throughout the storage period, and the final weight was recorded. The PLW was calculated as follows:

$$\text{Physiological loss in weight (\%)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Initial weight (g)}} \times 100$$

2.7.5 Total soluble solids (°Brix)

To determine total soluble solids, juice obtained from smashing table grape pulp was filtered through muslin fabric. A FISHER Digital Refractometer was used to conduct this assessment, and the outcomes were noted in degrees Brix.

2.7.6 Ascorbic acid content (mg 100 g^{-1})

The ascorbic acid content was measured using the 2,6-dichlorophenol indophenol titration method. A four percent oxalic acid solution was used to dilute the initial 5 ml of fresh fruit juice to a predetermined level. The volume was increased to 100 ml using the same four percent oxalic acid solution after being filtered through muslin cloth to achieve a clear juice. Then, 5 ml of this aliquot was titrated against a dye solution until a pink colour was discernible. The ascorbic acid concentration was calculated and represented in milligrams per gram of fruit.

$$\text{Ascorbic acid} = \frac{\text{Ascorbic acid content in standard (mg)} \times \text{Total sample volume} \times TV_2 \times 100}{\text{(mg/100 ml of juice) ml of aliquot} \times \text{Weight of sample} \times TV_1}$$

2.7.7 Instrumental colour value (*L* a* b**)

A colorimeter was used to assess colour attributes using the CIE *L**, *a**, *b** parameters,

- ***L****: This represents **lightness**. The value of *L** ranges from 0 (black) to 100 (white), indicating the brightness or darkness of a color.
- ***a****: This represents the **green-red axis**. Positive values of *a** indicate a shift toward red, while negative values indicate a shift toward green.
- ***b****: This represents the **blue-yellow axis**. Positive values of *b** indicate a shift toward yellow, while negative values indicate a shift toward blue.

2.7.8 Sensory evaluation

Sensory evaluations were conducted by a panel of 10 trained individuals who assessed color, texture, taste, and overall acceptability using a 9-point hedonic scale, where 9 indicated "like extremely" and 1 indicated "dislike extremely."

2.8 Statistical analysis

Physico-chemical characteristics and disease severity were measured and evaluated using a two-factor completely random design. Statistical analysis was conducted using OPSTAT and WASP Version 2. Significant differences between the means were identified at a significance threshold of $P \leq 0.01$ for *in-vitro* experiments and $P \leq 0.05$ for *in-vivo* investigations. Post hoc analyses were performed using Duncan's multiple-range test.

3. Result and Discussion

Infected table grape samples with typical *Rhizopus* rot were collected from various Bagalkot locations, resulting in three *R. stolonifer* isolates. Morphological and microscopic examination revealed fast-growing colonies that transitioned from a dense cottony white mass to a reddish-gray-brown hue. The mycelia, cultivated on PDA for 96 hours, exhibited typical structures,

including rhizoids and sporangiospores, which were globose and brownish-black. These characteristics align with previous findings of *R. stolonifer* made by (9,19).

3.1 Pathogenicity test

To assess the pathogenicity of *R. stolonifer* isolates, table grape clusters were submerged in a spore suspension (10^{-5}) for two minutes. Infected berries exhibited a watery rot after 96 hours, with initial infection signs at the base leading to longitudinal fissures. A yellowish mold developed, which later turned gray-black, and the berry's surface became pale gray. Re-cultures of *R. stolonifer* from infected berries were microscopically examined to confirm their similarity to the original culture, supporting Koch's postulates. The results indicated that *R. stolonifer* effectively invaded the host, causing fissures and pathogenic infection, consistent with findings by (24,47). Significant differences in pathogenicity indices, including virulence and disease severity, were observed. Symptoms included soft, dripping rot starting at the berry base, leading to light gray skin and black mold along fissures, aligning with observations by (21). Infections were often associated with damaged berries, particularly in warm harvesting conditions. Maintaining grapes at temperatures below 4°C can effectively prevent Rhizopus rot.

3.1 *R. stolonifer* on *in-vitro* radial growth inhibition

This study investigated the effects of gaseous ozone on the radial growth of *R. stolonifer in vitro*. The results showed significant growth inhibition, with the maximum reduction at 8833.2 µL L^{-1} (94.82%) and the second-highest at 6624.9 µL L^{-1} (92.22%). After 12 hours, these concentrations were more effective than 4416.6 µL L^{-1} (63.33%). Radial growth inhibition decreased after 24 hours, indicating an increased demand for ozone (64.07%, 20.37%, and 9.26%). Ozone treatment resulted in a shift from thick-fluffy to thin-flat mycelium and sporangia color change from black to whitish, attributed to oxidative damage and disruption of cell membranes, consistent with findings from (48).

In a separate experiment, SO_2 was tested for its inhibitory effects on *R. stolonifer*. The highest inhibition (98.14%) occurred at 70 mg SMB/100 ml PDA and 90 mg SMB/100 ml PDA, while the lowest (46.67%) was seen with 50 mg SMB/100 ml PDA. This inhibition may result from SO_2 disrupting mycelial membranes, as noted by (17, 38).

3.2 Disease Severity (DS)

In this study, varying concentrations of gaseous ozone and SO_2 were applied to infected table grapes. During cold storage at 5–2°C and 85–95% relative humidity, both treatments prevented disease symptoms, recording a disease severity (DS) of 0.00, compared to 0.89 in the inoculation control. This is due to the sensitivity of *R. stolonifer* spores to cold temperatures, which cannot survive below 5°C (12).

The combination of low temperatures and ozone also reduced disease severity by oxidizing vital cellular components of the pathogen (16,49). Similar results were reported by (37) with an ozone supply of 8 mg per minute preventing decay in infected grapes. Additionally, (17) showed that fumigating Thompson Seedless grapes with 2500 or 5000 µL L^{-1} h of ozone reduced gray mold by 50% after 7 days at 15°C.

Furthermore, (2) found dual SO_2 pads effectively prevented gray mold in 'Italia' grapes during 50 days of cold storage. The control group showed disease symptoms by day seven (DS of 0.83), worsening to 1.22 by day 49. Notably, after an additional week at room temperature (33°C, 37.5% RH), treated grapes remained healthy, while the control deteriorated.

3.3 Berry firmness

Table grape firmness is crucial for marketability, with water loss being the primary cause of postharvest softening (29). The softening process is attributed to changes in the chemical composition of cell walls (32). During the storage period, berry firmness significantly declined from an initial 96.15 N to 68.15 N by day 49 (Table 1). This decline, seen in grapes infected with *R. stolonifer* and treated with ozone and sodium metabisulfite, is likely due to alterations in cell wall polysaccharides.

Ozone treatments at concentrations of 7274.4 µL L^{-1}, 5455.8 µL L^{-1}, and 3637.2 µL L^{-1} resulted a higher firmness values (85.09 N, 84.82 N, and 82.90 N, respectively) compared to the inoculation control (77.48 N). This increase in firmness may relate to varying degrees of cell wall composition changes, such as pectin breakdown and hydrolysis of cellulose and hemicellulose. While ozone helps maintain grape firmness, it also inhibits microbial growth.

Sodium metabisulfite treatments yielded firmness values of 82.88 N, 80.43 N, and 78.52 N for SMB powder at 0.5 g per 500 g of grapes, which were higher than the inoculation control but lower than ozone treatments. Berry cracks were observed in the Indian grape guard (Plate 1), likely due to excessive SO_2 leakage, as high temperatures can cause grapes to absorb too much SO_2 (34).

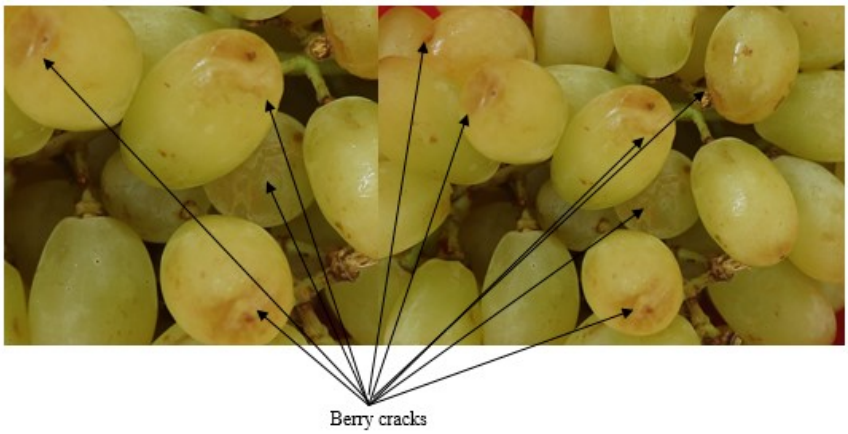

Plate 1. Berry cracks in grape berries after fumigated with sulphur dioxide after 49 days ofstorage under cold condition (5±2°C, RH- 85-95%)

3.4 Physiological loss in weight (PLW)

Physiological loss in weight (PLW) is a key indicator of weight losses in products due to natural processes like transpiration and respiration (36). In this study, PLW gradually increased from 3.83% on the 7th day to 16.11% on the 49th day (Table 2). This increase can be attributed to low storage temperatures, which help maintain the freshness of horticultural produce by slowing down respiration and transpiration rates. Notably, the rate of physiological processes doubles with every 10°C rise in temperature (34,55), contributing to the observed PLW in table grapes during cold storage.

Fruits treated with ozone and sodium metabisulfite exhibited lower PLW compared to untreated (inoculated control) fruits, which had a PLW of 10.58%. Ozone treatments resulted in significantly lower PLW values of 7.49%, 7.55%, and 9.03% for concentrations of 7274.4 µL L^{-1}, 5455.8 µL L^{-1}, and 3637.2 µL L^{-1} O_3, respectively. However, it's important to note that the higher ozone concentrations can lead to increased dehydration, potentially damaging the fruit's cuticle and epidermis (30). While some studies suggest that ozone treatment can reduce PLW in kiwifruit (26), results can vary based on the concentration of ozone used (e.g., 1-10 ppm) and the duration of exposure (28). Higher concentrations, typically around 5-10 ppm, have been found to be more effective in reducing PLW, but prolonged exposure may also lead to undesirable effects such as tissue damage.

While some studies (27, 49) indicate that ozone treatments lead to reduced weight loss, others (15, 30) report increased weight losses. For instance, in an experiment exposing peaches and grapes to gaseous ozone, (30) found that peaches lost significantly more weight during storage than grapes. However, after 49 days of cold storage, all ozone treatments outperformed the infected control, which exhibited a PLW of 10.58%.

Similarly, over the 49-day storage period, SMB powder at 0.5 g per 500 g of grapes (9.29%), Indian grape guard (9.37%), and African grape guard (9.73%) all demonstrated higher PLW than ozone treatments but lower than the infected control.

Table 1: Influence of Ozone and Sodium Metabisulfite on the Firmness of Table Grapes Infected with *Rhizopus stolonifer* during Cold Storage (5±2°C, RH- 85-95%)

Treatments	Firmness (Newtons)									Mean
	Storage duration in days									
	Initial	7	13	19	25	31	37	43	49	
3637.2µl L^{-1} O_3 /10min	96.15	93.31	90.46	87.62	82.66	76.34	76.34	72.52	70.74	**82.90**
5455.8 µl L^{-1} O_3/15min	96.15	93.46	90.78	88.09	85.34	80.19	78.95	75.65	74.73	**84.82**
7274.4 µl L^{-1} O_3 /20min	96.15	93.49	90.83	88.17	85.43	80.34	80.34	77.30	73.75	**85.09**
Indian grape guard	96.15	91.99	87.83	83.67	78.98	70.73	70.73	64.13	62.49	**78.52**
African grape guard	96.15	92.34	88.53	84.72	80.49	73.01	73.01	68.56	67.03	**80.43**
SMB powder 0.5g /500g grapes	96.15	92.97	89.80	86.62	83.80	77.96	76.47	72.11	70.01	**82.88**
Inoculated Control	96.15	91.74	87.32	82.91	77.98	69.37	67.86	62.42	61.57	**77.48**
uninoculated control	96.15	92.09	88.03	83.97	79.31	71.28	71.03	66.20	64.90	**79.22**
Mean	**96.15**	**92.67**	**89.20**	**85.72**	**81.75**	**74.90**	**74.34**	**69.86**	**68.15**	

	Treatments(T)	Days of storage(D)	Interaction (TxD)
S.Em±	0.30	0.32	0.91
CD @ 5 %	0.85	0.90	2.55

The observed PLW reduction in treated fruits may be attributed to the oxidation of respiratory enzymes, which slows down physiological processes. Our findings align with (40), who reported the lowest weight loss (2.9%) in grapes stored with SO_2, compared to a maximum of 3.7% in the control group. Similar observations on PLW have been documented in 'Nagpur' mandarins (55,56) and mangoes (57,58).

3.5 Total Soluble Solids (TSS °Brix)

In fruits and vegetables, the total soluble solids (TSS), primarily consisting of sugars and soluble minerals play a crucial role in determining quality. For table grapes stored under cold storage conditions, TSS concentration significantly increased from 16.45°B on the first day to 21.25°B on the 49th day (Table 3). This rise indicates fruit ripening, attributed to the breakdown of polysaccharides and the accumulation of sugars, along with contributions from water-soluble minerals, acids, vitamins B and C, and some proteins.

Among the treatments, SMB therapies (SMB powder 0.5 g/500 g grapes - 18.79°B, African grape guard - 18.87°B, and Indian grape guard - 18.87°B) and ozone treatments (7274.4 µL L^{-1} O_3 - 18.68°B, 5455.8 µL L^{-1} O_3 - 18.72°B, and 3637.2 µL L^{-1} O_3 - 18.79°B) showed comparable TSS levels. They performed better than both the uninoculated control (19.42°B) and the inoculated control (19.73°B). After 49 days of storage at 5–2°C with 85–95% relative humidity, the inoculated control exhibited the highest TSS (19.73°B), while the treatment at 7274.4 µL L^{-1} O_3 had the lowest (18.68°B).

This increase in TSS may be linked to the respiration of the control fruits (56). Similar findings were noted in pomegranates, where ozone treatment delayed the breakdown of sucrose into glucose and fructose, resulting in a higher TSS compared to untreated fruits (59). In that study, ozonated pomegranates showed a slower rate of sugar metabolism, which contributed to an increase in TSS, similar to the rise observed in the current study with kiwifruit. The slow breakdown of sucrose in ozonated fruits might also contribute to the rise in TSS by preserving the sugars for a longer period. Although (1) reported a significant decrease in TSS in ozonated carrots due to leaching, most studies show no substantial differences in TSS between ozonated and untreated samples. In contrast, (26) found a decrease in soluble solids concentration in kiwifruits in a cold, ozone-rich atmosphere. The increase in TSS in SMB-treated grapes during storage may result from the heightened organic solute concentration due to water loss. Control grapes had the lowest soluble solids, while those stored with SO_2 exhibited the highest after 90 days (40). Similarly, (10) observed that the soluble solids content of table grapes increased during the initial stages of storage but subsequently declined as decay progressed, leading to a drop in pH. Research by (39) also indicated that grapes stored with SO_2 for 90 days exhibited the highest levels of soluble solids, while the control group had the lowest.

3.6 Ascorbic acid (mg/100g)

As storage time increased, ascorbic acid levels significantly decreased, reaching 2.97 mg/100g after 49 days in cold storage (Table 4). The inoculated control had similar ascorbic acid content (3.66 mg/100g) as compared to the uninoculated one (3.71 mg/100g) but lower than all the other treatments. The highest level of ascorbic acid was in the 7274.4 µL L^{-1} ozone treatment (3.90 mg/100g), while the 3637.2 µL L^{-1} ozone treatment showed lower content.

Table 2: Impact of Ozone and Sodium Metabisulfite on Physiological Weight Loss (PLW) in Table Grapes Infected with *Rhizopus stolonifer* during Cold Storage (5±2°C, RH- 85-95%)

Treatments	Percentage of Weight Loss (PLW %)									Mean
	Days of storage									
	Initial	7	13	19	25	31	37	43	49	
3637.2µl L^{-1} O_3 /10min	0.00	4.01	5.67	6.91	9.56	11.43	13.29	14.26	16.12	**9.03**
5455.8 µl L^{-1} O_3/15min	0.00	3.32	4.84	6.19	7.96	9.51	11.05	11.52	13.53	**7.55**
7274.4 µl L^{-1} O_3 /20min	0.00	3.23	4.80	6.13	7.89	9.42	10.95	11.75	13.28	**7.49**
Indian grape guard	0.00	4.04	6.24	7.86	10.48	12.52	14.14	15.17	17.15	**9.73**
African grape guard	0.00	3.82	6.01	7.44	10.02	11.97	13.72	14.72	16.64	**9.37**
SMB powder 0.5g /500g grapes	0.00	3.92	5.83	7.68	9.87	11.79	13.71	14.72	16.06	**9.29**
Inoculated Control	0.00	4.34	6.91	8.87	11.40	13.62	15.31	16.20	18.57	**10.58**
uninoculated control	0.00	3.94	6.16	7.86	10.79	12.89	14.46	15.51	17.54	**9.91**
Mean	**0.00**	**3.83**	**5.81**	**7.37**	**9.75**	**11.64**	**13.33**	**14.23**	**16.11**	

	Treatments(T)	Days of storage(D)	Interaction (TxD)
S.Em±	0.08	0.09	0.24
CD @ 5 %	0.22	0.24	0.67

No significant differences were found among the sodium metabisulfite (SMB) treatments (3.78-3.81 mg/100g), all the treatments were higher as compared with the inoculated control. This might be due to slower physiological processes from enzyme action. Research suggests that controlled atmospheres and SO_2 fumigation do not adversely affect the fruit's phytochemical makeup (54).Ozone interactions with vitamin C may lead to decreases, as seen in studies of pineapple and banana, where ozone scavenges free radicals and may convert ascorbic acid to dehydroascorbic acid (3,22).

Table 3: Impact of Ozone and Sodium M*eta*bisulfite on Total Soluble Solids (TSS) in Table Grapes Infected with *Rhizopus stolonifer* during Cold Storage (5±2°C, RH- 85-95%). NS - Non-Significant

Treatments	Total Soluble Solids (TSS °Brix)									Mean
	Storage timeframe in days									
	Initial	7	13	19	25	31	37	43	49	
3637.2µl L^{-1} O_3 /10min	16.45	17.26	17.76	18.16	18.97	19.47	19.88	20.38	20.78	**18.79**
5455.8 µl L^{-1} O_3/15min	16.45	17.23	17.72	18.11	18.89	19.38	19.77	20.26	20.65	**18.72**
7274.4 µl L^{-1} O_3 /20min	16.45	17.22	17.70	18.08	18.86	19.34	19.72	20.20	20.59	**18.68**
Indian grape guard	16.45	17.44	18.06	18.56	19.54	20.16	20.66	21.28	21.77	**19.33**
African grape guard	16.45	17.28	17.80	18.23	19.06	19.58	20.00	20.52	20.93	**18.87**
SMB powder 0.5g /500g grapes	16.45	17.26	17.76	18.16	18.97	19.47	19.87	20.38	20.78	**18.79**
Inoculated Control	16.45	17.58	18.28	18.85	19.98	20.68	21.25	21.95	22.52	**19.73**
uninoculated control	16.45	17.47	18.11	18.62	19.64	20.28	20.79	21.43	21.94	**19.42**
Mean	**16.45**	**17.34**	**17.90**	**18.35**	**19.24**	**19.80**	**20.24**	**20.80**	**21.25**	

	Treatments (T)	Days of storage (D)	Interaction (TxD)
S.Em±	0.07	0.07	0.21
CD @ 5 %	0.20	0.21	NS

3.7 Instrumental colour *L**and *b** value

Long-term cold storage reduced *L** and *b** values, indicating lower color brightness and yellowness. The infected control group had the lowest values (*L**: 38.25, *b**: 22.06), while SMB powder (0.5g/500g grapes - 40.53; 23.77), African grape guard (40.11; 23.48), and Indian grape guard (39.38; 23.42) had higher values. Water loss contributed to the lower *L** values during storage (6).

Color changes were noticeable after 30 days of cold storage exposure, with h values indicating a shift towards brown. SO_2 treatments prevented significant browning, while ozone treatments had lower *L** and *b** values than SMB treatments but were still higher than controls. This may be due to slight declines in phenolic compounds from ozone oxidation.

These results align with those of (6, 8, 44), showing decreased *L** and *b** values at harvest, likely due to increased water loss in control clusters over time.

3.8 Instrumental colour *a** value

During cold storage, the a* value increased. SMB powder (0.5g/500g grapes) had the lowest a* value (-1.92), while the infected control had the highest (-2.39). Among SMB treatments, Indian grape guard (-2.18) outperformed African grape guard (-2.04).

Ozone treatments yielded lower a* values, with 3637.2 l µL L-1 O3 (-2.15) and 5455.8 l µL L^{-1} O3 (-2.19) being less effective than 7274.4 l µL L^{-1} O3 (-2.32).

Table 4: Influence of Ozone and Sodium Metabisulfite on Ascorbic Acid Levels in Table Grapes Infected with *Rhizopus stolonifer* during Cold Storage (5±2°C, RH- 85-95%)

Treatments	Ascorbic acid content (mg per 100 grams)									Mean
	Storage duration in days									
	Initial	7	13	19	25	31	37	43	49	
3637.2µl L^{-1} O_3 /10min	4.51	4.33	4.13	3.99	3.79	3.56	3.46	3.28	3.01	**3.78**
5455.8 µl L^{-1} O_3/15min	4.51	4.38	4.23	4.13	4.02	3.77	3.73	3.20	2.97	**3.88**
7274.4 µl L^{-1} O_3 /20min	4.51	4.37	4.23	4.12	4.01	3.77	3.65	3.41	3.07	**3.90**
Indian grape guard	4.51	4.34	4.14	4.00	3.80	3.50	3.40	3.38	3.02	**3.79**
African grape guard	4.51	4.32	4.13	3.99	3.84	3.56	3.46	3.20	3.01	**3.78**
SMB powder 0.5g /500g grapes	4.51	4.36	4.15	4.02	3.88	3.67	3.58	3.16	2.99	**3.81**
Inoculated Control	4.51	4.28	4.04	3.86	3.69	3.34	3.22	3.16	2.82	**3.66**
uninoculated control	4.51	4.30	4.07	3.91	3.75	3.53	3.40	2.98	2.90	**3.71**
Mean	**4.51**	**4.34**	**4.14**	**4.00**	**3.85**	**3.59**	**3.49**	**3.22**	**2.97**	
	Treatments (T)	Days of storage (D)	Interaction (TxD)							
S.Em±	0.02	0.02	0.06							
CD @ 5 %	0.05	0.06	0.16							

After 49 days, SMB treatments retained color better than ozone and were significantly different from the uninoculated control (-2.37).

Excessive SO_2 emissions may have caused berry cracks, as noted by (46). Similar increases in chroma were observed by (6, 37), while (44) reported a modest rise in chroma. (8) found a consistent increase in a* value. Ozone's effect on browning was noted by (45), while (43) found no effect on cilantro leaves.

3.9 Overall acceptability

Indian grape guard (7.54), SMB powder (0.5g/500g grapes) (7.52), and 3637.2 l µL L-1 O_3 (7.49) had similar organoleptic ratings, while 7274.4 l µL L^{-1} O_3 scored highest at 8.04, followed by 5455.8 l µL L^{-1} O_3 (7.70) and the inoculated control (6.05) after 49 days of cold storage (Table 6). Ozone-treated fruits showed better sensory ratings due to slower physiological changes, resulting in increased flavor and sweetness. Sensory analyses by (5, 9) found no

significant differences between ozonated and non-ozonated fruits. Rachis desiccation scores increased over time, with untreated clusters peaking at 3.5 after 120 days. SO_2-treated grapes performed best, maintaining visual quality and favorable scores for up to 90 days, with noticeable color changes during 4 months of storage (39).

Table 5: Impact of Ozone and Sodium Metabisulfite on General Favorability of Table Grapes Infected with Rhizopus stolonifer under Chilled Storage (5±2°C, RH- 85-95%).

Treatments	Overall acceptability (1-9 scale)				Mean
	Days of storage				
	Initial	25	37	49	
3637.2µl L^{-1} O_3 /10min	8.64	8.56	7.00	5.75	**7.49**
5455.8 µl L^{-1} O_3/15min	8.64	8.61	7.00	6.54	**7.70**
7274.4 µl L^{-1} O_3 /20min	8.64	8.56	8.00	6.96	**8.04**
Indian grape guard	8.64	8.34	7.00	5.62	**7.40**
African grape guard	8.64	8.54	7.00	5.91	**7.52**
SMB powder 0.5g /500g grapes	8.64	8.61	7.00	5.91	**7.54**
Inoculated Control	8.64	7.47	4.08	4.00	**6.05**
uninoculated control	8.64	8.54	6.00	5.50	**7.17**
Mean	**8.64**	**8.41**	**6.64**	**5.77**	

	Treatments(T)	Days of storage(D)	Interaction (TxD)
S.Em±	0.01	0.01	0.02
CD @ 5 %	0.03	0.02	0.06

Overall acceptability hedonic nine-point scale: 9→ like extremely, 8→ like very much, 7→ like moderately, 6→ like slightly, 5→ neither like nor dislike, 4→ dislike slightly, 3→ dislike moderately, 2→ dislike very much and 1→ dislike extremely

4. Conclusion

The study found that high ozone concentrations (8833.2 l µL L^{-1}) and sodium metabisulfite (SMB) (90 mg/100 ml PDA) significantly suppressed *Rhizopus stolonifer* growth. Both treatments eliminated disease severity, while the control showed a severity of 0.89 DS. Ozone at 7274.4 and 5455.8 µL L^{-1} maintained better berry firmness, ascorbic acid, and TSS compared to SMB. SMB powder (0.5g/500g grapes) treated with ozone showed the best quality preservation.
Over 49 days, SMB treatments had higher *L** and *b** values and lower *a** values than ozone treatments. The study concludes that ozone effectively prevents *Rhizopus* rot disease while preserving grape quality, making it a suitable alternative to sulfur dioxide for export.

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5. References

1. Alegria C, Pinheiro J, Goncalves EM, Fernandes I, Moldao M, Abreu M. Quality attributes of shredded carrot (*Daucus carota* L. cv. Nantes) as affected by alternative decontamination processes to chlorine. Innov Food Sci Emerg Technol, 2009; 61–69.
2. Ahmed S, Roberto S R, Domingues A R, Shahab M, Junior O J C, Sumida C H, and De Souza R T. Effects of different Sulphur dioxide pads on Botrytis mold in 'Italia'table grapes under cold storage. Hort, 2018; 29.
3. Alothman M, Kaur B, Fazilah A, Bhat R, Karim AA. Ozone-induced changes of antioxidant capacity of fresh-cut tropical fruits. Innov Food Sci Emerg Technol, 2010; 666–671.
4. Aneja K R. Experiments in microbiology, Plant Pathol and biotech. New Age International, 2007.
5. Artes-Hernandez F, Artes F, Tomas-Barberan FA. Quality and enhancement of bioactive phenolics in Cv. Napoleon table grapes exposed to different postharvest gaseous treatments. J Agric Food Chem, 2003; 5290–5295.
6. Artés-Hernández F, Aguayo E, Artés F. Alternative atmosphere treatments for keeping quality of Autumn Seedless table grapes during long-term cold storage. Postharvest Biol. Technol, 2004; 59–67.
7. Barkai-Golan, R. Postharvest diseases of fruits and vegetables. *development and control*. Elsevier, 2001.
8. Bolin HR, Huxsoll CC. Effect of preparation procedures and storage parameters on quality retention of salad-cut lettuce. J Food Sci, 1991; 60–62.
9. Bullerman LB. SPOILAGE| Fungi in Food–An Overview, 2003.
10. Chen X, Mu W, Peter S, Zhang X. and Zhu Z. The effects of constant concentrations of Sulphur dioxide on the quality evolution of postharvest table grapes. J FOOD NUTR RES, 2016.
11. Crisosto C H, Palou L, Garner D, and Armson D A. Concentration by time product and gas penetration after marine container fumigation of table grapes with reduced doses of Sulphur dioxide. Hort Technology, 2002; 241-245.
12. Dennis C and Cohen E. The effect of temperature on strains of soft fruit spoilage fiingi. Ann. Appl. Biol, 1976; 51-56.
13. Feliziani E, Romanazzi G and Smilanick, J.L. Application of low concentrations of ozone during the cold storage of table grapes. Postharvest Biol. Tech, 2014; 38-48.
14. Fernández-Trujillo J M, Obando-Ulloa J M, Baró R, Martínez J A. Quality of two table grapes guard cultivars treated with single or dual-phase release SO2 generators. J. Appl. Bot. Food Qual, 2008; 1–8.
15. Forney CF, Song J, Hildebrand PD, Fan L, McRae KB. (2007). Interactive effects of ozone and 1-methylcyclopropene on decay resistance and quality of stored carrots. Postharvest Biol Tech- nol., 45(3):341–348.
16. Freitas-Silva O, Venancio A. Ozone applications to prevent and degrade mycotoxins: a review. Drug Metab Rev, 2010; 612–620.
17. Gabler F M, Smilanick J L, Mansour MF and Karaca H. Influence of fumigation with high concentrations of ozone gas on postharvest gray mold and fungicide residues on table grapes. Postharvest Biol. Tech, 2010; 85-90.
18. Gabler F M, Smilanick J L, Ghosoph J M and Margosan D A. Impact of postharvest hot water or ethanol treatment of table grapes on gray mold incidence, quality, and ethanol content. Plant Dis, 2005; 309-316.
19. Hernández-Lauzardo A N, Bautista-Baños S, Velázquez-del Valle M G and Trejo-Espino J L. Identification of *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill., causal agent of Rhizopus rot disease of fruits and vegetables. Revista Mexicana de Fitopatología, 2006; 65-69.
20. Hernández-Lauzardo A N, Velázquez-del Valle M G, Veranza-Castelán L, Melo-Giorgana G E and Guerra-Sánchez M G. Effect of chitosan on three isolates of *Rhizopus stolonifer* obtained from peach, papaya and tomato. Fruits, 2010; 245-253.
21. Latorre B A, Viertel S C and Spadaro I. Severe outbreaks of bunch rots caused by *Rhizopus stolonifer* and *Aspergillus niger* on table grapes in Chile. Plant Dis, 2002; 815-815.
22. Lee SK, Kader AA. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biol. Technol, 2000; 207–220.
23. Lichter A, Mlikota Gabler F, Smilanick J L. Control of spoilage in table grapes. Stewart Postharvest Rev, 2006; 6, 1.
24. Lisker N, Keren-Shacham Z, Sarig P, Zutkhi Y and Ben-Arie R. The biology and pathology of the fungus *Rhizopus stolonifer*, cause of black mould disease of table grapes in Israel. Plant Pathol, 1996; 1099-1109.
25. Luvisi D A, Shorey H H, Smilanick J L, Thompson J F, Gump B H, Knutson J. Sulphur Dioxide Fumigation of Table Grapes, Bulletin 1932. University of California, Division of Agricultural and Natural Resources, Oakland, CA, 1992; 21.

26. Minas IS, Karaoglanidis G S, Manganaris G A and Vasilakakis M. Effect of ozone application during cold storage of kiwifruit on the development of stem-end rot caused by *Botrytis cinerea*. Postharvest Biol. Tech, 2010; 203-210.
27. Nadas A, Olmo M and Garcia J M. Growth of *Botrytis cinerea* and strawberry quality in ozone-enriched atmospheres. J. food sci, 2003; 1798-1802.
28. Nayak S L, Sethi S, Sharma R R, Sharma R M, Singh S and Singh D. Aqueous ozone controls decay and maintains quality attributes of strawberry (Fragaria× ananassa Duch.). J FOOD SCI TECH, 2020; 319-326.
29. Nelson K E. Harvesting & handling California table grapes for market. Univ. Calif. Div. Agric. Nat. Resour. Bull. 1913. University of California, Davis, 1985.
30. Palou L, Crisosto C H, Smilanick J L, Adaskaveg J E and Zoffoli J P. Effects of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage. Postharvest Boil. Tech, 2002; 39-48.
31. Pandey K K, Pandey P K and Mishra K K. Bio-efficacy of fungicides against different fungal bioagents for tolerance level and fungistatic behaviour. *Indian Phytopathology*, 2006; 68-71.
32. Robinson S P & Davies C, Molecular biology of grape berry ripening. Aust. J. Grape Wine Res, 2000; 175–188.
33. Rodoni L, Casadei N, Concellon A, Alicia ARC, Vicente AR. Effect of short-term ozone treatments on tomato (*Solanum lycopersicum* L.) fruit quality and cell wall degradation. J Agric Food Chem, 2010; 594–599.
34. Ryall A L and Harvey J M. The cold storage of *vinifera* table grapes (No. 159). US Department of Agriculture, 1959
35. Sabir A and Sabir F K. Postharvest treatments to preserve table grape quality during storage and approaches to find better ways alternative for S (O. sub. 2). Adv. Environ. Biol, (2009; 286-296.
36. Sabir M S, Shah S Z A and Afzal A. Effect of chemical treatment, wax coating, oil dipping and different wrapping materials on physio-chemical characteristics and storage behavior of apple (Malus domestica Borkh). Pakistan J Nutr, 2004; 122-127.
37. Sarig P, Zahavi T, Zutkhi Y, Yannai S, Lisker N and Ben-Arie R. Ozone for control of post-harvest decay of table grapes caused by *Rhizopus stolonifer*. *Physiol. Mol.* Plant Pathol, 1996; 403-415.
38. Smilanick J L, Hartsell P L, Henson D, Fouse D C, Assemi M, Harris C M. Inhibitory activity of Sulphur dioxide on the germination of spores of *Botrytis cinerea*. Phytopathology, 1990a; 217–220.
39. Sortino G, Allegra A, Passafiume R, Gianguzzi G, Gullo G and Gallotta A. Postharvest application of sulphur dioxide fumigation to improve quality and storage ability of" red globe" grape cultivar during long cold storage. Chem. Eng. Trans, 2017; 403-408.
40. Sortino G, Farina V, Gallotta A and Allegra A. June. Effect of low SO_2 postharvest treatment on quality parameters of'Italia'table grape during prolonged cold storage. In VIII International Postharvest Symposium*:* Enhancing Supply Chain and Consumer Benefits-Ethical and Technological Issues , 2016; 695-700.
41. USDA, Sec. 51.886, Tolerances. United States standards for grades of table grapes (European or Vinifera type). Code Fed. Reg, 2009; 388–389.
42. USFDA, Title 21: Food and Drugs. Part 173. Secondary direct food additives permitted in food for human consumption. Subpart D: specific usage additives. Sec: 173.368. Fed. Regist. 2001; 66, 33829.
43. Wang H, Feng H, Luo YG. Microbial reduction and storage quality of fresh-cut cilantro washed with acidic electrolyzed water and aqueous ozone. Food Res Int. 2004; 949–956.
44. Watharkar R B, Burbade R G and Landge K C. Effect of Packaging Material on Shelf Life and Quality Attributes of Grapes (*Vitis Vinifera* L.), 2017.
45. Zhang LK, Lu ZX, Yu ZF Gao X. Preservation of fresh- cut celery by treatment of ozonated water. Food Control 2005; 279–283.
46. Zoffoli J P and Latorre B A. Table grape (*Vitis vinifera* L.). In Postharvest biology and technology of tropical and subtropical fruits (179-214e). Woodhead Publishing, 2011.
47. Fatimoh A O, Moses A A, Adekunle O B and Dare O E. Isolation and identification of rot fungi on post-harvest of pepper. *Aascıt Journal of Biology*, 32017; 24-29.
48. Tzortzakis N and Chrysargyris A. Postharvest ozone application for the preservation of fruits and vegetables. *Food Reviews International*, 2017; 270-315.
49. Ejsmentewicz T, Balic I, Sanhueza D, Barria R, Meneses C, Orellana A, Prieto H, Defilippi B G and Campos-Vargas, R. Comparative study of two table grape varieties with contrasting texture during cold storage. *Molecules*, 2015; 3667-3680.
50. Singh B P and Narayana C K. An integrated approach for storage of mango. *Indian Journal of Horticulture*, 1999; 5-9.
51. Vicente A R, Costa M L, Martínez G A, Chaves A R and Civello P M. Effect of heat treatments on cell wall degradation and softening in strawberry fruit. *Postharvest biology and Technology*, 2005; 213-222.
52. Vicente A R and Sozzi G O. Ripening and postharvest storage of 'soft fruits. *Fruit Veg Cereal Sci Biotechnol 1*, 2007; 95-103.
53. Fraeye I, Knockaert G, Van Buggenhout S, Duvetter T, Hendrickx M and Van Loey A. Enzyme infusion and thermal processing of strawberries: Pectin conversions related to firmness evolution. *Food Chemistry*, 2009; 1371-1379.

54. Rees, D., Farrell, G. and Orchard, J. eds., 2012. *Crop post-harvest: science and technology, Volume 3: Perishables* (Vol. 3). John Wiley & Sons.
55. Nanda S D V S, Rao D S and Krishnamurthy S. Effects of shrink film wrapping and storage temperature on the shelf life and quality of pomegranate fruits cv. Ganesh. *Postharvest Biology and Technology*, 2001; 61-69.
56. Ladaniya M S. Response of Nagpur mandarin fruit to pre-harvest sprays of gibberellic acid and carbendazim. *Indian Journal of Horticulture*, 1997; 205-212.
57. Bender R J, Brecht J K, Baldwin EA and Malundo T M M. Aroma volatiles of mature-green and tree-ripeTommy Atkins' mangoes after controlled atmosphere vs. air storage. *HortScience*, 2000; 684-686.
58. Sudhakar Rao DV and Shivashankara K S. Effect of modified atmosphere packaging on the extension of storage life and quality maintenance of pomegranate (*cv.*'Bhagwa') at ambient and low temperatures. *Journal of food science and technology*, 2018; 2103-2113.
59. Laribi A I, Palou L, Taberner V and Pérez-Gago M B, Modified atmosphere packaging to extend cold storage of pomegranate cv.'Mollar de Elche', 2012.
60. Gundewadi G, Reddy, V R and Bhimappa B B. Physiological and biochemical basis of fruit development and ripening-a review. *Journal of Hill Agriculture*, 2018; 7-21.