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Research Article

Assessment of Rhizopus rot control and quality attributes of manik chaman table grapes after post-harvest ozone and sodium metabisulfite treatment

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Abstract

An important fruit crop, grapes are vulnerable to fungal degradation, at different points, together with harvesting, post-harvest management, and storage. The effectiveness of ozone at concentrations 4416.6 L L^{-1}, 6624.9 L L^{-1}, & 8833.2 L L^{-1} in combination with sodium metabisulfite (SMB), at concentrations of 50 mg, 70 mg, and 90 mg per 100 ml of potato dextrose broth (PDB) was investigated to solve this issue. Fumigation, and food poisoning techniques, were used in these treatments to stop *R. stolonifer* (Ehrenberg) Vuillemin's radial growth.

In a different trial, SMB in the forms of Indian, and African grape guards, as well as SMB powder packaged in pouches (at a rate of 0.5g per 500g of fruit), was examined, for its potential to combat Rhizopus rot in table grapes subjected to cold storage conditions (52 °C, RH-85-95%), over 49 days. The results showed that sodium metabisulfite at 90 mg/100 ml PDA, and ozone at 8833.2 l L^{-1} strongly reduced the radial growth of *R. stolonifer* by 94.82% and 98.14%, respectively. While the inoculation control registered 0.89 Disease Severity (DS), fruits treated with O_3 and SMB showed, no symptoms of disease severity. Ozone at 7274.4 L L^{-1} & 5455.8 L L^{-1} demonstrated, greater firmness retention of the berries (85.09 N; 84.82 N), as well as higher concentrations of ascorbic acid (3.90; 3.88 mg/100 g). Additionally, these treatments reduced, the levels of Total Soluble Solids (TSS) (18.68 N; 18.72 N), Percentage of Loss in Weight (PLW) (7.49; 7.55), and TSS/acid ratio (20.57 N; 21.52 N). In the sensory evaluation, these therapies received the highest overall acceptance ratings (8.04; 7.70). When combined with ozone, the application of SMB powder at a rate of 0.5g per 500g of grapes showed promising results, among the fruits that had been treated with SMB. In addition, compared to ozone, SMB treatments produced significantly higher *L** and *b** values. The highest *L** value (40.53), highest *b** value (23.77), and lowest *a** value (-1.92) were all found with SMB powder at 0.5g/500g grapes. In conclusion, ozone shows promise in regulating Rhizopus rot effectively and keeping, the qualitative characteristics of table grapes, during cold storage, offering a feasible substitute, for sulfur dioxide treatments in conventional grape production.

Introduction

The non-climacteric nature of grapes (*Vitis vinifera* L.), and their propensity for fast degradation are well recognized. This vulnerability is ascribed to elements that jointly shorten their shelf life, including firmness loss, berry detachment, rachis discoloration, desiccation, and fungal rot [1]. In the Bagalkot district of Karnataka, India, Rhizopus rot poses a significant threat to grape crops, especially during harvest, transportation, and marketing; resulting in significant crop losses. Tropical and subtropical regions are frequently affected by Rhizopus rot, a rapid and destructive decay, brought on by the disease caused by *R. stolonifer*.

In the past, SO_2 has been used as the go-to treatment for table grape rot [2]. However, its usage is limited in many nations due to concerns about sulfite residues, SO_2 discharges, and its detrimental effects on grape quality, including skin

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cracking, berry decolorizing, and rachis browning [3,4]. Ozone has become a viable SO_2 replacement due to its lack of residue and departure from the traditional approach of SO_2 fumigation, according to [5]. Due to regulatory concerns, it has been deemed "organic" by the USDA National Organic Program, making its use largely acceptable as of this point [6]. Additionally, it is become easier and easier to locate high-quality ozone-producing machinery. Producing within the strict decay tolerance criteria is particularly difficult because the standards are so low; for example [72], states that the percentage of berries that are deteriorated during transportation cannot be more than 0.5% in the USA. This presents a problem for "organic" cultivation since sulfur dioxide fumigation and vineyard fungicides are both prohibited. Even while SO_2 is very efficient and cost-effective for conventional farmers, it can cause bleaching damage [8] and a disturbance of the hairline fissure after repeated fumigation [9]. It may also affect the grapes' flavor, according to [10].

While preharvest fungicide applications, as described by [11,12], and cultural practices, as described by [13,14], can significantly lessen postharvest deterioration, they are insufficient to eliminate the need for postharvest sulfur dioxide fumigation. Due to its extremely reactive and oxidizing properties, ozone gas in packinghouses has the ability to cause physiological changes that impact the internal or exterior quality of harvested produce without bleaching grape colors, even at relatively high quantities [15,16]. It has proven successful at preventing mold and germs from growing on surfaces and in the air in cold storage situations. Additionally, it oxidizes and gets rid of ethylene gas. "Ozone application is more effective with higher concentrations of residual ozone at lower storage temperatures," according to studies [17,18]. According to [19,20] "a synergistic effect between ozone treatment and cold temperatures in slowing down ethylene-related physiological changes while maintaining fruit quality without any phytotoxic symptoms" was discovered.

In this research, we examined the impact of sodium metabisulfite and ozone on *the in vitro* radial expansion of *R. stolonifer* (Ehrenb.) Vuillemin, employing techniques relevant to both food preservation and fumigation. Additionally, under cold storage circumstances (52 °C, RH-85% - 95% for 49 days), *in-vivo* assessments of SMB grape guard, SMB powder, and ozone fumigation against Rhizopus rot in table grapes were conducted. The findings suggest that ozone can be a promising replacement for sulfur dioxide treatments in grape production, successfully preventing Rhizopus rot and preserving the quality of table grapes when they are kept in the cold. The introduction, materials and methods, results and discussion, conclusion, and references are the other four components of this research paper in addition to the abstract. In this study, we sought to determine (i) the efficacy of ozone and sulfur dioxide against the radial growth of *R. stolonifer* and (ii) the effect of ozone and sulfur dioxide on Rhizopus rot and quality in grape cv. Manik Chaman over 49 days of storage in cold conditions (52 °C, RH-85-95%).

Materials and methods

In this investigation, we made inoculum from affected grape samples and ran tests on *R. stolonifer* pathogenicity. Both ozone and sodium metabisulfite were tested by fumigating in controlled laboratory settings (*in vitro*) and actual storage situations (*in vivo*) against *R. stolonifer* at varying doses. Ascorbic acid content (mg /100 g), total soluble solids (°Brix), TSS to acid ratio, inhibition of radial growth of *R. stolonifer*, berry firmness, disease severity (DS), instrumental color values (L^* a^* b^*), and sensory evaluation using a 9-point hedonic rating scale were among the parameters for which we recorded results. Using the Web Agri Stat Package (WASP) Version 2 and Operational Statistics (OPSTAT), the acquired data underwent statistical analysis.

Inoculum preparation

From the Honnakatti village in the Bagalkot District of Karnataka, India samples displaying the typical symptoms of Rhizopus rot were obtained in January 2020. The tissue isolation method was used to isolate *R. stolonifer*. According to the [21] methodology, these sections were placed on Potato Dextrose Agar (PDA) in petri dishes and incubated there for 96 hours at 25±1 °C. The isolated culture was allowed to grow at 25±2 °C for six days in a BOD incubator after subculture on PDA test tubes. These test tubes were then stored in a refrigerator at 4 °C. The spores were gently removed from an actively growing culture with 20 ml sterile distilled water per each petri dishes in a safety chamber. After that resultant suspension was diluted with disinfected distilled water by serial dilution method to get 10^{-5} spore suspension which was used as inoculum [21-23].

Pathogenicity test for *Rhizopus stolonifer*

In the Karnataka village of Honnakatti in the Bagalkot District, samples displaying the typical symptoms of Rhizopus rot were taken in January 2020. Using the tissue isolation method, *R. stolonifer* was isolated. This method involves immersing minute fragments of diseased samples in one percent sodium hypochlorite solution for 30 seconds, followed by three rinses in distilled water. These sections were then placed on PDA in petri dishes, where they were cultured for 96 hours at 25 °C following the [21] methodology. The isolated culture was sub-cultured on PDA test tubes before being cultivated at 25 °C for six days in a BOD incubator. These test tubes were then chilled to 4 °C in a refrigerator. Spores were carefully removed from an actively growing plant to create the spore suspension.

Ozonation system

To create ozone, we used the SEONICS Ozone Generator. For coronavirus discharge to take place, which separates regular oxygen molecules into individual atoms, it is necessary to introduce purified oxygen from an oxygen concentrator into the ozone generator. These atoms subsequently interact with additional O_2 molecules in the surrounding air to generate O_3, which has a mass per cubic meter measured in grams. Converting this figure to microliters per liter is necessary. 0.5 to 5 liters per minute (LPM) of the purified oxygen passes via the oxygen concentrator and into the ozone generator. 51.96 grams of O_3 per cubic meter per minute were produced for this investigation using one liter of concentrated oxygen per minute (51.96 g/m³/min = 51.96 mg/liter/min = 51.96 l L^{-1}

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min^{-1}). In the experiment, we applied ozone treatments to nine airtight LDPE boxes that were connected to the ozone generator by a 6 mm Outer Dia tube connector. Each box in the *in-vitro* investigation held petri dishes and had a volume of 0.5 liters. Each box containing fruits in the *in-vivo* trial had a volume of two liters. In the *in-vitro* trial and the *in-vivo* investigation, this left 8.5 liters and 7 liters of space, respectively, for ozone. We multiplied the 8.5-liter volume of each box by 51.96 mg/liter/minute for different periods (i.e., 10 minutes, 15 minutes, and 20 minutes), resulting in final ozone concentrations of 4416.6 l L^{-1}, 6624.9 l L^{-1}, and 8833.2 l L^{-1}, respectively, to determine the total ozone concentration in the *in-vitro* study. Similar calculations were made in the *in-vivo* study, where we multiplied each box's 7-liter volume by 51.96 mg/liter/minute for periods of 10, 15, and 20 minutes, respectively, to arrive at final ozone concentrations of 3637.2 l L^{-1}/10 minutes, 5455.8 l L^{-1}/15 minutes, and 7274.4 l L^{-1}/20 minutes.

Sulphur dioxide

In the *in-vitro* phase, we used PDA in combination with sodium metabisulfite (SMB) powder, designated by CAS No. 40180 K05, using a food poisoning technique. We used grape guards that came from both India and Africa throughout the in-vivo phase. We administered SMB powder, which was housed in fabric pouches or bags, at a dosage of 0.5 grams per 500 grams of fruit to comply with a maximum residue limit (MRL) of 10 ppm in 500 grams of grapes.

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

We used a Completely Randomized Design (CRD) with four different treatments that were each reproduced five times in this investigation. We examined how differing ozone and sulfur dioxide concentrations affected *R. stolonifer*'s ability to grow radially. The experiment was set up by pouring 20 ml of PDA medium into sterile petri dishes and letting it set. A 5 mm disc from an *R. stolonifer* culture that had been growing for six days was then added to the center of each petri dish's medium. Except for the control petri dish, infected petri dishes were exposed to ozone at concentrations of 4416.6 l L^{-1}, 6624.9 l L^{-1}, and 8833.2 l L^{-1} every day during the ozone exposure experiment.

In a second experiment, we exposed sulfur dioxide to four treatments with five replications each. The food poisoning method described by [24] was used to include sodium metabisulfite at doses of 50, 70, 90, and 0 mg per 100 ml of PDA. After being prepped, disinfected, and cooled to 45 °C, the PDA was used. The indicated therapy was then followed by the addition of SMB. After being put into clean Petri dishes, the mixture was left to set. Following the procedure outlined by [25], a 5 mm mycelial disc from a six-day-old culture of *R. stolonifer* was then positioned in the middle of each petri dish containing PDA and various concentrations of SMB.

Fruit

We used a two-factor completely randomized design (CRD) in this study, with eight different treatments that were each reproduced three times. Table grapes (*Vitis vinifera* L.) from the Main Horticultural Research and Extension Centre, University of Horticultural Sciences Bagalkot, were the subject of our study in March 2020. The fruits underwent pre-cooling at 5 °C for 12 hours after being harvested before being immediately transported to the cold storage facility of the postharvest technology department. After that, thorough physico-chemical analyses were performed at the Postharvest Technology department's lab.

Each sample was made up of 0.5 kilograms worth of bunches. To disinfect these bunches, they were submerged in a one percent sodium hypochlorite solution for 2 minutes, rinsed with distilled water, and then dried. Following an application of *R. stolonifer* spore suspension (at a concentration of 10^{-5}), the bunches were put in a laminar airflow setting for 20 minutes to allow excess water to evaporate. They were then divided into eight different treatments, which included being exposed to ozone for 10 minutes at 3637.2 l/L, 15 minutes at 5455.8 l/L, and 20 minutes at 7274.4 l/L. Additionally, grape guards from India and Africa, SMB at 0.5g packaged in cotton pouches, and control samples that had been inoculated and not been inoculated were included. After that, these samples were put in cold storage. Over a storage period of 49 days, the first three treatments received ozone exposure every 24 hours at intervals of three days.

Observations 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 color values (L^*, a^*, b^*), and a sensory evaluation using a 9-point hedonic rating scale were all factors that were studied in this study.

Radial growth inhibition (%) of *Rhizopus stolonifer*: The study 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 the 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 under a method developed by [26]:

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

Disease severity (DS): Using a 0–5 scale, disease severity was determined: No disease, 5% disease, > 5% - 15% disease, > 15% - 30% disease, > 30% - 60% disease, > 60% disease, and > 60% disease [27] 500g in CFB box

Berry firmness: Using a texture analyzer provided by UK-based Stable Micro Systems and the piercing test method, fruit hardness was measured. Table grape berries were pierced with a cylindrical 2 mm probe following predetermined guidelines. The greatest force needed to pass the test-first stated in

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kilogram-force (kgf) and afterward in Newtons (N)-was used to determine the firmness.

Physiological Loss in Weight (PLW): The initial weight of each pair of table grape bunches was measured and recorded at the start of the storage period to calculate the physiological loss in weight. The fruits were reweighed as the storage period wore on, precisely every two days, and these readings were recorded as the final weight. The PLW was then determined using the specified methodology and expressed as a percentage.

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

Total soluble solids (°Brix): To calculate the total soluble solids, juice that was obtained by smashing table grape pulp and filtering it through muslin fabric was used. A FISHER Digital Refractometer was used to conduct this assessment. The outcomes, more specifically Brix, were noted in degrees.

TSS to the acid ratio: Total soluble solids to titratable acidity were calculated to determine the TSS to Acid ratio.

$$\text{TSS to Acid ratio} = \frac{\text{Total soluble solids}}{\text{Titratable acidity}}$$

Ascorbic acid content (mg 100 g^{-1}): The 2,6-dichlorophenol indophenol titration method was used to measure the ascorbic acid content. 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 color was discernible. The ascorbic acid concentration was calculated and represented in milligrams per gram of fruit.

$$\text{Ascorbic acid} = \frac{\text{The ascorbic acid content in standard } (\text{mg}) \times \text{Total sample volume} \times \text{TV}_2 \times 100}{(\text{mg / 100 ml of juice}) \text{ ml of aliquot} \times \text{Weight of sample} \times \text{TV}_1}$$

Instrumental colour value (*L* a* b):** A Hunter color meter (Model: Colour Flex® EZ Standard Box) with an 8 mm diameter aperture was used to assess the samples' color. The black and white tiles that were provided were used to calibrate the instrument. The units used to express color values are *L**, *a**, & *b**. The intensity of a color is indicated by the *L** value, which ranges from *L** = 100 for pure white to *L** = 0 for deep black. When positive, the * number signifies redness; when zero, neutrality; and when negative, greenness. In contrast, the *b** value denotes blueness when it is negative, neutrality when it is zero, and yellowness when it is positive. Grape fruit samples were placed over the aperture of the color meter. Each sample underwent three measurements, with the results being averaged.

Sensory evaluation (9-point hedonic rating scale): A group of semi-skilled judges from the College of Horticulture, Bagalkot, including members of the teaching staff and graduate students, conducted the sensory evaluation of table grapes. The modified score card below shows how they evaluated different sensory qualities using a 9-point Hedonic scale, including color and look, ease of detachment, freshness of the rachis, incidence of disease, and overall attractiveness.

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.

Results and Discussions

The infected table grape samples, which had classic Rhizopus rot signs, were gathered from several Bagalkot locales. These harmed grape samples produced three *R. stolonifer* isolates, which were then identified as *R. stolonifer* after close inspection of the colony, mycelia, and spores using morphological and microscopic methods. The colonies first expanded quickly, forming a dense cottony white mass that subsequently changed color to a reddish-gray-brown shade. When they sporulated, they had a fluffy texture and black heads. The mycelia were also cultivated on PDA for 96 hours along with its numerous parts, including rhizoids, stolon's, sporangiophores, columella, sporangia, and sporangiospores. The sporangiospores were seen to be globose in shape and brownish-black in color. These morphological traits were in line with earlier discoveries of *R. stolonifer* made by [23,28].

Pathogenicity test

Table grape clusters were submerged for two minutes in a spore suspension (10^{-5}) to assess the pathogenicity of *R. stolonifer* isolates. The infected grape berries showed a delicate, extremely watery rot after 96 hours. The berries' base showed signs of initial infection before developing longitudinal fissures. First, a yellowish mold formed, then along the crevices it turned gray-black. The berry's surface turned a pale gray color. Re-cultures of *R. stolonifer* isolates from the infected berries were then made, and these were examined under a microscope to establish their similarity to the original culture, supporting Koch's postulates.

The results showed that *R. stolonifer* successfully invaded the host through the base of the berry, causing longitudinal fissures and pathogenic infection. Re-isolation of *R. stolonifer* from the infected grapes further demonstrated that it resembled the initial culture, supporting Koch's hypotheses. These findings are in line with other studies conducted by [23,29].

Significant differences in pathogenicity indices, including virulence, disease severity, and physical traits of *R. stolonifer* isolates, were found in this investigation. The illness manifested as a soft, dripping rot that started at the base of the berries and caused longitudinal fissures. The berry's skin turned a light gray color due to these fractures, which were coated in a gray-black mold. These discoveries are consistent with those

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made by [30], who noted the same Rhizopus rot symptoms brought on by *R. stolonifer*. The skin appeared light gray, and there were longitudinal fissures with black mold growing along them. Infections were frequently linked to damaged berries, especially during warm weather harvesting conditions. By keeping grapes below 4 °C, Rhizopus rot can be completely avoided. Furthermore, *R. stolonifer*-caused Rhizopus rot was described by [31,32], in which infected fruits immediately became wet, mushy, and subsequently perished. The symptoms began as sores or fissures that appeared during harvest. They also provided information regarding the ideal temperature for mycelial growth, measures of sporangiophores, sporangia, columella, and sporangiospores, and other specifics of the fungus.

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

In this work, we looked examined how gaseous ozone affected *R. stolonifer's* radial development in an in-vitro environment. The results showed a considerable reduction in radial growth, with the maximum reduction at an ozone concentration of 8833.2 L L^{-1} (94.82%) and the second-highest reduction at an ozone concentration of 6624.9 L L^{-1} O_3 (92.22%). After 12 hours of inoculation, both concentrations were significantly more effective than 4416.6 l L^{-1} O_3 (63.33%). The radial growth inhibition, however, decreased after 24 hours of inoculation with the same ozone concentrations (64.07%, 20.37%, and 9.26%), indicating a potential rise in ozone demand for inhibiting radial growth. In comparison to the control group, ozone treatment generally produced positive outcomes (0.00%).

In addition, mycelial development changed noticeably after ozone treatment from thick-fluffy to thin-flat, and the color of sporangia changed from black to whitish. The oxidation of vital cellular components, disruption of microbial cell membranes, inactivation of enzymes, and destruction of microbial biofilm caused by ozone fumigation are all thought to contribute to the inhibition of fungal spore production, according to observations of fungal colonies on PDA. These results are in line with those of [33], who found that ozone-induced extensive oxidation of interior cellular proteins caused microbial biofilms to be destroyed and cells to die off quickly. Similar to this [34], showed that ambient ozone concentrations greatly decreased and higher concentrations completely abolished the generation of *P. digitatum* and *B. cinerea* spores. Studies by [33,35,36] also noted the oxidation of different cell envelope components, including polyunsaturated fatty acids, membrane-bound enzymes, glycoproteins, and glycolipids, resulting in the leakage of cell constituents and eventual lysis, emphasizing the deadly effects of ozone. In addition, it was discovered that ozone oxidized the sulfhydryl groups of enzymes and the double bonds of unsaturated lipids, altering cell permeability and speeding up cell death.

In a different experiment, we tested sulfur dioxide's (SO_2) ability to inhibit *R. stolonifer*'s radial growth in an *in-vitro* investigation. Notably, 70 mg SMB/100ml PDA and 90 mg SMB/100ml PDA both showed the maximum radial growth inhibition (98.14%). 50 mg SMB/100 PDA demonstrated the least amount of radial growth inhibition (46.67%) when compared to other SMB dosages. According to [4,37] this action of SMB may be linked to the disruption of mycelia caused by SO_2 diffusing through membranes and building up in microorganisms, which results in an ionization entrapment mechanism that prevents fungal radial growth. According to [38] *R. stolonifer* and *B. cinerea* were significantly resistant to the fungicidal effects of the essential oils of black caraway and fennel (74.76% and 76.07%, respectively). The 400 l L^{-1} concentration of black caraway essential oil fully prevented the growth of *B. cinerea*. Fennel oil reduced the radial growth of *R. stolonifer* fungal colonies in PDA medium at concentrations greater than 600 l L^{-1}.

Disease Severity (DS)

In this study, different gaseous ozone and sulphur dioxide concentrations were applied to purposefully infected table grapes. The results showed that during cold storage (at 5°C - 2 °C, with relative humidity at 85% - 95%), both ozone and sulfur dioxide treatments resulted in the lack of illness signs. The disease severity was specifically measured as 0.00 DS for the ozone and sulfur dioxide treatments as well as the uninoculated control, in contrast to 0.89 DS for the inoculation control. This can be explained by the fact that *R. stolonifer* spores are sensitive to cold temperatures since they cannot survive below 5 °C. Some spores can start to grow at 2 °C, but they cannot continue to thrive at such low temperatures [39,17].

Additionally, the low temperatures and ozone treatments worked together to lessen the severity of the illness by oxidizing vital pathogen cellular components [35,36]. These results are consistent with those of [40], who found that grapes infected with *Rhizopus stolonifer* decayed well with an ozone supply of 8 mg per minute for 30 to 40 minutes. Additionally [37], suggested that, for Thompson Seedless grapes, 2500 or 5000 L/L h of O_3 fumigation during pre-cooling equally efficiently decreased gray mold by about 50% after 7 days of storage at 15 °C.

Similar to this [41], showed the high effectiveness of dual sulfur dioxide pads in preventing the incidence of gray mold in clamshell-packed 'Italia' grapes during 50 days of cold storage and then 7 days at ambient temperature. In contrast, the disease started to affect the control group of fruits (with a DS of 0.83) on the seventh day of storage throughout the 49-day research. The DS rose to 1.22 on the 49th day of storage. It's interesting to note that when we maintained the grapes for an additional week at room temperature (332 °C, with a relative humidity of 37.5%), the illness did not develop in the table grapes exposed to ozone and sulfur dioxide. However, the condition considerably worsened in the control group. This shows that when the inoculum was transferred to the market under ambient circumstances, ozone and sulfur dioxide successfully deactivated it and stopped disease progression.

Berry firmness

Table grapes' firmness is a crucial quality factor and a key factor in how marketable they are. Water loss is the main cause

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of the post-harvest softening of berries [42,43]. According to [44], the ripening-related softening process results from changes in the cell walls' chemical makeup. According to [45], it involves the dissolution of the molecules of pectin and xyloglucan as well as a decrease in the amount of cellulose and hemicellulose.

The data on the berry firmness of table grapes shows a significant decline in berry firmness across all treatments, from an initial 96.15 N on the first day to 68.15 N on the 49th day of storage (Table 1). The grapes were intentionally infected with *Rhizopus stolonifer* and subjected to ozone and sodium metabisulfite treatments during cold storage (52 °C, RH-85-95%). Changes in cell wall polysaccharides are probably to blame for this loss of rigidity. These findings are in line with those of [46], who also noted a decrease in fruit firmness during the storage period. Similar tendencies in mangoes were also observed by [47]. The hardness of the grape berries was significantly higher in table grapes treated with ozone at concentrations of 7274.4 l L^{-1}, 5455.8 l L^{-1}, and 3637.2 l L^{-1} ozone (85.09 N, 84.82 N, and 82.90 N, respectively). Comparing all ozone treatments to the inoculation control (77.48 N), a notable increase in firmness was seen. This improvement could be a result of differing degrees of cell wall composition changes, such as pectin breakdown and cellulose and hemicellulose hydrolysis in both the ozone-treated fruits and the control fruits. Additionally, the fruits' declining hardness shows that they are maturing more quickly. While ozone is used to preserve the firmness of grapes, it also inhibits or lessens microbial growth. According to [48,49], the drop in berry softening appears to be related to the decomposition of pectic polymers at low storage temperatures (4.5 and 1 °C). Because endo polygalacturonase, PE, and PME enzyme activity were low or absent in the ozone-treated fruits, the minor firmness loss during storage could be attributable to reduced soluble pectin degradation. According to research by [50], fruits were exposed to aqueous ozone for 2 minutes while being stored at low temperatures, and the firmness of the fruits under control conditions was lowest (1.20 N). They proposed that modifications to the cell wall's structural elements, turgor pressure, and intercellular adhesion might be the main causes of the strawberry fruits' decreased firmness [51]. In contrast [40], claimed that grape firmness was unaffected by an 80-minute ozone treatment at a concentration of 4000 l L^{-1}. Firmness values of 82.88 N, 80.43 N, and 78.52 N, respectively, were noted for SMB-exposed treatments, namely SMB powder at 0.5 g per 500 g of grapes (African grape guard; Indian grape guard). These values were higher than the inoculation control (77.65 N) but much lower than the ozone treatments. In particular, the transformation of insoluble protopectins into soluble pectin, which prolonged stiffness, may be responsible for the slowing of metabolism and a decrease in the breakdown of cell wall components. These findings are consistent with those of [52], who showed that storage of SO_2 and a Controlled Atmosphere (CA) had a substantial effect on berry hardness. Similar to this [53], observed that grapes had a 28.7 N firmness at harvest, which dropped to a 20.00 N firmness. Finally, the lowest firmness (20.00 N) and highest firmness (23.5 N) were found in the control group and SO_2-stored grapes, respectively. Indian grape guard was found to have berry cracks, which may have been caused by an excessive SO_2 leak. According to [54], high temperatures caused grape berries to absorb too much SO_2, which resulted in cracks. Similar to hairline cracks [55], reported that phytotoxicity caused by excessive sulfur dioxide (SO_2) exposure was indicated by the development of tiny, fine, longitudinal, linear cracks that were nearly invisible to the naked eye.

Physiological Loss in Weight (PLW)

According to [56], physiological loss in weight (PLW) is a crucial indicator of the weight loss of produce brought on by natural processes like transpiration and respiration.

Physiological weight loss (PLW) increased gradually over the storage period, increasing from 3.83% on the 7th day to 16.11% on the 49th day (Table 2). This rise can be due to the low storage temperature that is advised, which is essential for maintaining the freshness of horticulture produce for longer, as seen in grapes. At low temperatures, where their rates are slowed, normal physiological processes like respiration and transpiration are important in the steady rise of PLW. It is well known that the rate of physiological processes doubles for

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

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

every 10 °C increase in storage temperature [24,54], which was visible in the steadily rising PLW of table grapes during cold storage.

Similarly, PLW was lowest in fruits treated with ozone and sodium metabisulfite and significantly higher in untreated (inoculated control) fruits (10.58%). Ozone treatments functioned successfully, showing noticeably lower PLW at 7.49%, 7.55%, and 9.03%, respectively, for 7274.4 l L^{-1} O_3, 5455.8 l L^{-1} O_3, and 3637.2 l L^{-1} O_3. It's crucial to remember that ozone only causes samples to get more dehydrated when used in high concentrations, which could harm the fruit's cuticle and epidermis layers [19]. Regarding the impact of ozone on fruit and vegetable weight loss, the literature has produced contradictory results. Although some academics favor Minas *et al.* (2010) [57], Ozone treatment of kiwifruit has been shown to reduce PLW, although other studies have found mixed outcomes depending on the concentration and length of ozone exposure. (2013); Narciso *et al.* (2014). While some studies: Nadas *et al.*, 2003 [58]; Rodoni *et al.* (2010) [5] found that ozone treatments resulted in less weight loss, others Venta *et al.* (2010) [59], Forney *et al.* (2007) [17], and [19] found that weight loss was increased. Mahapatra *et al.* (2005) [60] even found that ozone treatment had no effect at all. In an experiment where peaches and grapes were exposed to gaseous ozone [19], discovered that peaches lost significantly more weight after storage than grapes did. Nevertheless, following 49 days of cold storage, all ozone treatments outperformed the infected control (10.58%).

Similarly, during 49 days of storage, 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 showed considerably higher PLW than ozone, but lower PLW than the infected control (10.58%). The physiological processes in the preserved fruits were slowed down by the oxidation of respiratory enzymes, which could be the cause of this. Our findings are consistent with [53] who found that grapes stored with SO_2 experienced the lowest weight loss (2.9%) and the maximum weight loss (3.7%) compared to the control group. In addition to [61,62] in pomegranates [63], in 'Nagpur' mandarins, and [64,65] in mangoes, similar findings on PLW have also been reported.

Total Soluble Solids (TSS °Brix)

In fruits and vegetables, there are substances known as total soluble solids (TSS) that are composed primarily of sugars and soluble minerals.

The TSS concentration significantly increased from the first day (16.45°B) to the 49th day (21.25°B) in the case of table grapes maintained under cold conditions (52 °C, RH-85-95%) (Table 3). This increase in TSS is a sign of ripening fruit and is caused by the breakdown or synthesis of polysaccharides as well as the buildup of sugars. A portion of the TSS is provided by the water-soluble minerals, acids, sugars, vitamins B and C, and some proteins found in fresh fruits and vegetables.

Regarding different treatments, SMB therapies (SMB powder 0.5g/500g grapes -18.79°B, African grape guard -18.87°B, and Indian grape guard -18.87°B) and ozone treatments (7274.4 l L^{-1} O_3 - 18.68°B, 5455.8 l L^{-1} O_3 - 18.72°B, and 3637.2 l L-1 O_3 - 18.79°B) were equivalent to one another. In comparison to the unvaccinated control (19.42°B) and the vaccinated control (19.73°B), they performed better. After 49 days of grape storage in cold circumstances (5–2 °C, RH 85–95%), the inoculated control had the greatest TSS (19.73°B), while the 7274.4 l L^{-1} O_3 treatment had the lowest (18.68°B).

According to [61], it might be hypothesized that control fruits used energy through respiration [66]. Found comparable results in pomegranates. The slow breakdown of sucrose into glucose and fructose in ozonated fruits may be responsible for the increase in TSS [67] observed a considerable decrease in total soluble solids content in ozonated carrots, probably as a result of a leaching process, whereas the majority of studies find no significant variations in TSS content between ozonated and untreated samples.

In contrast to our findings [57], found that soluble solids concentration in kiwifruits decreased under a cold, ozone-rich atmosphere. The increase in organic solute concentration brought on by water loss may be what causes the TSS in grapes treated with SMB to rise during storage. Additionally, a variety of anabolic and catabolic activities take place in the fruit, causing it to become senescent [68]. According to [53], control

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

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

NS: Non-Significant

grapes exhibited the lowest soluble solids and the highest soluble solids after 90 days of SO_2 storage. Similar to this [69], found that table grapes' soluble solids content increased during the early stages of storage before declining over the decay process and dropping their pH [70] research also showed that grapes stored with SO_2 for 90 days had the highest levels of soluble solids, whereas the control group had the lowest levels.

Ratio of total soluble solids to acidity

Under cold storage settings (52 °C, RH-85-95%), the TSS/acid ratio showed an increasing trend throughout the storage period, rising from 14.30 on the first day to 31.53 on the 49th (Table 4). This surge is explained by the parallel rise in TSS and fall in acidity. This change may potentially be explained by the conversion of starch and other polysaccharides into soluble sugars, a phenomenon also reported by [71], who explained that starch undergoes hydrolysis into mono and disaccharides, resulting in a higher TSS [72], also noted a comparable rise in the TSS/acid ratio in peach fruits during storage.

In comparison to SMB treatments (SMB powder 0.5g/500g grapes -22.30, African grape guard -22.84, and Indian grape guard -23.51), ozone treatments (7274.4 l L^{-1} O_3 -20.57, 5455.8 l L^{-1} O_3 -21.52, and 3637.2 l L^{-1} O_3 -21.92) showed significantly lower TSS/acid ratios.

As a result, as compared to SMB treatments, ozone treatments showed greater effectiveness with the lowest TSS/acid ratio. Furthermore, after 49 days of storage, both treatments significantly outperformed the infected control (24.83%). This might be explained by the fruits being fumigated with ozone, which prolongs the processes of ripening and senescence while also slowing down the transformation of starch into sugars. Furthermore, it can be caused by the reduction in microbial growth due to the ozone treatment, which helps maintain the quality and extend the shelf life of the fruits. Ozone is a powerful oxidizing agent, which could lead to a decrease in the activity of respiratory enzymes [9,73]. Nevertheless, compared to the infected control (24.83%), all treatments showed a considerable improvement.

To a reduction in respiratory enzyme activity, given that ozone is an effective oxidizing agent [9,73]. Nonetheless, all treatments exhibited significant enhancements over the inoculated control (24.83%).

Ascorbic acid (mg/100g)

As the storage time extended, the levels of ascorbic acid decreased noticeably, reaching their lowest amount of 2.97 mg/100g after 49 days under cold storage circumstances (52 °C, RH-85-95%) (Table 5).

The ascorbic acid concentration of the inoculated control (3.66 mg/100g) was equivalent to that of the uninoculated control (3.71 mg/100g) despite being much lower than all other treatments. The treatment with 7274.4 L L^{-1} ozone had an ascorbic acid content that was much greater than the other treatments (3.90 mg/100g), but it was comparable to the treatment with 5455.8 L L^{-1} ozone.

The treatment with 3637.2 L L^{-1} ozone among the different ozone concentrations showed considerably less ascorbic acid content (3.78 mg/100g) than the other ozone concentrations. This might be explained by the slowing of biological processes during storage and the effect on ripening. Ozone and vitamin C have been shown to interact in some different ways. In pineapple, banana, and guava treated with ozone, for example [74], noticed a drop in vitamin C content, which they attributed to scavenging free radicals created during ozone decomposition. Additionally, ascorbic acid's conversion to dehydroascorbic acid may have been aided by the activation of ascorbate oxidase [75]. On the other hand [9], concluded that fresh-cut celery might retain more vitamin C when ozone levels were lower. Similar to this [50], discovered a consistent decrease in ascorbic acid during storage in both ozone-treated and untreated "Winter Dawn" strawberry fruits. They hypothesized that the higher ascorbic acid loss in untreated fruits could be explained by its use during respiration as a result of its antioxidative properties, as it may be involved in scavenging free radicals produced during the ozone treatment of strawberry fruits, leading to its reduction [74,76].

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

Treatments	The ratio of Total Soluble Solids (TSS) to acidity									Mean
	Storage duration in days									
	Initial	7	13	19	25	31	37	43	49	
3637.2µl L^{-1} O_3/10min	14.30	16.28	17.70	18.94	21.83	23.97	25.90	28.66	29.70	**21.92**
5455.8 µl L^{-1} O_3/15min	14.30	16.05	17.27	18.62	21.34	23.27	25.53	28.15	29.15	**21.52**
7274.4 µl L^{-1} O_3/20min	14.30	16.07	17.17	18.19	20.48	22.10	23.92	26.02	26.84	**20.57**
Indian grape guard	14.30	16.60	18.27	19.76	23.27	25.90	28.33	31.87	33.24	**23.51**
African grape guard	14.30	16.30	17.73	19.01	21.98	25.26	27.61	31.06	32.29	**22.84**
SMB powder 0.5g /500g grapes	14.30	16.46	18.02	19.42	22.29	24.61	26.72	28.91	29.97	**22.30**
Inoculated Control	14.30	16.84	18.70	20.38	24.40	27.49	30.38	34.67	36.32	**24.83**
uninoculated control	14.30	16.72	18.51	20.12	23.75	26.62	29.30	33.27	34.76	**24.15**
Mean	**14.30**	**16.42**	**17.92**	**19.31**	**22.42**	**24.90**	**27.21**	**30.33**	**31.53**	
.Em± CD @ 5 %	Treatments(T)	Days of storage(D)	Interaction (TxD)							
	0.13	0.13	0.40							
	0.35	0.40	0.06							

Table 5: 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**	
S.Em± CD @ 5 %	Treatments(T)	Days of storage(D)	Interaction (TxD)							
	0.02	**0.02**	**0.06**							
	0.05	**0.06**	**0.16**							

Additionally, there was no discernible change in the ascorbic acid level between the various strengths of sodium metabisulfite (SMB) treatments (SMB powder 0.5g /500g grapes - 3.81mg/100g, African grape guard - 3.78 mg/100g, and Indian grape guard - 3.79 mg/100g). The inoculation control (3.66 mg/100g) was much lower than any of them, yet they were all noticeably higher. This might be explained by the physiological processes becoming slower as a result of the delayed action of the enzymes. According to [52], postharvest techniques such as controlled atmospheres and SO_2 fumigation had no deleterious effects on the fruit's phytochemical makeup or antioxidant activity. However, there were significant differences in polyphenolic concentration and overall antioxidant activity amongst various cultivars.

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

Long-term cold storage (52 °C, RH–85–95%) decreased the *L** and *b** values, which represent color brightness and yellowness, respectively. *L** and *b** values in the infected control group were the lowest (38.25, 22.06). However, compared to all other treatments, the 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) treatments all showed noticeably higher *L** and *b** values [77]. Found in earlier investigations that water loss in berries caused a drop in *L** values during storage. In line with observations on "Autumn Seedless" grapes, whether wrapped or unwrapped in OPP film after two months of cold storage, no appreciable changes in *h** values (showing color hue) were seen. In the current investigation, however, substantial color changes were noted after 30 days of storage. Although these alterations were not immediately apparent to the human eye [78], found increases in h values, indicating a shift in berry color towards brown. In contrast, SO_2 treatment did not cause berry browning to be noticeable throughout the 4-month storage period, and the control group's h values were lower.

They showed noticeably lower *L** and *b** values for ozone treatments than SMB treatments (7274.4 l L^{-1} O_3 - 39.01; 23.30, 5455.8 l L^{-1} O_3 - 39.20; 22.90, & 3637.2 l L^{-1} O_3 - 39.37; 22.74). However, they still had higher values than the control. This might be explained by a modest decline in phenolic compounds during storage as a result of ozone oxidation. Our results are consistent with those of [79-81], who showed a decrease in *L** and *b** values at harvest, albeit with a marginally smaller decrease compared to control fruit, where a more substantial decrease was noted. As the storage time wore on, increasing water losses in control clusters may have played a role in this event.

Instrumental color *a** value

Throughout the cold storage period (52 °C, RH-85-95%),

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the instrumental color parameter *a** value showed a discernible rise.

In comparison to all previous SMB treatments, SMB powder (0.5 g/500 g grapes) showed the lowest *a** value (-1.92). In comparison, the infected control had the greatest *a** value (-2.39). Indian grape guards in the SMB treatments had a higher *a** value (-2.18) than African grape guards (-2.04).

In contrast to 7274.4 l L^{-1} O_3 (-2.32), concentrations of 3637.2 l L^{-1} O_3 (-2.15) and 5455.8 l L^{-1} O_3 (-2.19) had much lower *a** values. Therefore, after 49 days of grape storage, SMB treatments performed better in terms of a* color retention than ozone, and both were significantly different from the uninoculated control (-2.37).

This could be explained by the occurrence of cracks on the berries brought on by an excess SO_2 emission. Similar to this [55], reported that severe SO_2 exposure caused fruit cracking, which resulted in significant water loss during storage. This is consistent with observations made by scientists including [40,79,82], who linked a rise in chroma to related occurrences [81]. Find that storage results in a modest rise in Chroma (which denotes a decrease in greenness). [80], who observed a constant rise in *a** value with the passage of the storage period, corroborate our findings. Additionally [83], concluded that ozone, in conjunction with oxalic or citric acids, caused a decrease in PPO activity, which in turn resulted in less browning on longan fruit. [84], in contrast, found that ozone had no impact on the color of cilantro leaves for the full storage period. The principal pigment of cilantro leaves, chlorophyll, may have degraded more slowly due to the storage temperature.

Overall acceptability

Indian grape guard (score: 7.54), SMB powder (0.5g/500g grapes) (score: 7.52), and 3637.2 l L^{-1} O_3 (score: 7.49) were discovered to be equally valued in terms of diverse treatments. Over 49 days of cold storage (52 °C, RH- 85-95%), the significant rating for 7274.4 l L^{-1} O_3 was seen to be 8.04, followed by 5455.8 l L^{-1} O_3 with a score of 7.70, and the infected control with a score of 6.05 (Table 6).

Because physical, physiological, and biochemical responses in ozone-treated fruits occur at slower rates than in control fruits, the ozone-treated fruits have higher organoleptic ratings. This is because the ozone-treated fruits are more succulent, flavorful, and sweeter than the control fruits. Results of sensory analyses conducted by [79,85,86] did not reveal any appreciable differences between ozonated and non-ozonated fruits and vegetables. According to [84], cilantro leaves rinsed with ozone maintained their fresh qualities, keeping their green color and odor-free look without yellowing or dryness. According to [53], rachis desiccation scores rose from 0 to 120 days, with the highest value (3.5) being noted in clusters held without treatment, and the lowest value (2.3) being noted in grapes treated with SO_2 after 120 days [53]. noticed that 3637.2 l L^{-1} O_3 (score: 7.49), Indian grape guard (score: 7.54), and SMB powder (0.5g/500g grapes) all obtained comparable ratings across the board for the various treatments. Over 49 days of cold storage (52 °C, RH- 85-95%), the significant rating for 7274.4 l L^{-1} O_3 was seen to be 8.04, followed by 5455.8 l L^{-1} O_3 with a score of 7.70, and the infected control with a score of 6.05 (Table 6). Because physical, physiological, and biochemical responses in ozone-treated fruits occur at slower rates than in control fruits, the ozone-treated fruits have higher organoleptic ratings. This is because the ozone-treated fruits are more succulent, flavorful, and sweeter than the control fruits. Results of sensory analyses conducted by [79,85,86] did not reveal any appreciable differences between ozonated and non-ozonated fruits and vegetables. According to [84], cilantro leaves rinsed with ozone maintained their fresh qualities, keeping their green color and odor-free look without yellowing or dryness.

According to [53], rachis desiccation scores rose from 0 to 120 days, with the highest value (3.5) being noted in clusters held without treatment, and the lowest value (2.3) being noted in grapes treated with SO_2 after 120 days. According to [70], SO_2-treated grapes fared the best during storage. Scores for SO_2 damage and healthy bunches (%) were favorable for up to 90 days, and throughout the 4-month storage period, substantial color changes in the berries were noticed. Up to 105 days of storage, berries treated with SO_2 had superior visual quality.

Table 6: 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**	
S.Em± CD @ 5 %	Treatments(T)	Days of storage(D)	Interaction (TxD)		
	0.01	**0.01**	**0.02**		
	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.

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Conclusion

The study shows that the radial growth of *R. stolonifer* was significantly suppressed by the application of high concentrations of ozone at 8833.2 l L^{-1} and sodium metabisulfite (SMB) at 90 mg /100 ml PDA. The severity of the disease was nil after O_3 and SMB treatments, but the inoculated control showed a severity of 0.89 DS. With decreased PLW and TSS/Acid Ratio compared to SMB and the inoculation control, ozone at concentrations of 7274.4 L L^{-1} and 5455.8 L L^{-1} in particular demonstrated considerably superior retention of berry firmness, ascorbic acid, and TSS. SMB powder (0.5g/500g grapes) treated with ozone showed the best quality preservation of all the SMB treatments, including lower PLW, longer shelf life, better berry hardness, ascorbic acid content, and a minor rise in TSS and TSS to acid ratio. Additionally, SMB treatments received favorable sensory overall acceptability values.

Additionally, throughout the 49 days of cold storage, all SMB treatments showed noticeably higher color L^* and b^* values in comparison to the ozone treatments, as well as lower color a^* values. The study concludes that ozone is a useful method for preventing Rhizopus rot in grapes while maintaining essential quality indicators. In cold storage, it might be an effective replacement for manufactured compounds like sulfur dioxide. The absence of residue post-treatment, which lowers chemical residues and makes ozone an excellent choice for export from India to other nations, is another noteworthy benefit of using ozone.

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Data availability

The data sets used and/or analyzed during the current study are available from the corresponding author upon reasonable request. Further, no experiments on humans and/or the use of human tissue samples were involved in this study.

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