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Ozonated water treatment for sustaining quality attributes and prolonging shelf life in grapes

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Abstract

Grapes (*Vitis vinifera*), a subtropical fruit crop, are highly valued for their nutritional composition and global economic importance, both as fresh consumption and processed forms. However, postharvest quality loss, primarily caused by grey mould (*Botrytis cinerea*), microbial decay and physiological weight loss, poses a significant challenge to grape storage and marketability. This experiment was designed to evaluate the efficacy of postharvest ozonated water treatment on quality attributes and extending the shelf life of grapes. Grape bunches were subjected to four treatments: control (no dip) and immersion in 0.3 ppm ozonated water for 5, 10 and 15 min, followed by storage at 4±2°C and 90 % relative humidity (RH). Results indicated significant improvements in key quality parameters for grapes treated with ozonated water, particularly with the 15 min treatment (T_4). These bunches exhibited higher titratable acidity (0.24 %), ascorbic acid content (1.81 mg/100 g) and firmness (4.80 N) while exhibiting 21.82 % lower physiological weight loss compared to the control. Ozonated water treatment also minimized berry abscission (3.77 %) and maintained sensory properties. The sugar-acid ratio was highest (84.72) in 5 min treatment group (T_2) on the 36th day of storage, enhancing flavour attributes. Significantly, T_4 extended the shelf life of grapes to 39.84 days, nearly five days longer than the untreated grapes. This study demonstrated the potential of ozonated water as an eco-friendly, residue-free technology for postharvest preservation, offering a safer alternative to chemical fumigation. The findings support the integration of ozonated water treatments into grape postharvest management practices to enhance quality, storability and consumer acceptability.

Keywords

grapes; ozonated water; postharvest; quality; shelf life

Introduction

Grapes (*Vitis vinifera)* serve as a potential source of vitamins (A, C, B_6 & folate), minerals and antioxidants and provides 75 cal/100 g of fruit (1). Antioxidants, mainly flavonoids and phenolic compounds, help to neutralize harmful free radicals in the body. By reducing oxidative stress, these antioxidants are crucial in protecting cells from damage and supporting overall health. Regular consumption of grapes has been linked to improved cardiovascular health. Grapes are used as a dessert fruit and for processed product preparation, such as wine, raisins, juice, jam, concentrate and seed oils. In India, the area under grape cultivation is estimated to be around

175000 ha, with an annual production of 3910000 MT and productivity of 22.2 MT/ha (2). Grapes from India have a good export potential. The country has exported 343982.34 MT of grapes to the world, worth around 3460.70 crores (417.07 USD Million) during 2023-24, according to the second advance estimate of APEDA (3). For both local and export markets, the bunches and berries of grapes must be free from foreign matter and any damage caused by pests, diseases, or extreme temperatures. They should also be free from abnormal external moisture, foreign smells and visible traces of moulds. According to APEDA, grapes must comply with the pesticide residue levels set by the Codex Alimentarius Commission for exports. About 8.3 percent of grapes experience loss at various stages of postharvest handling in India. Bruises, moisture loss and microbial decay or rotting caused by *Botrytis cinerea* are the significant reasons for quality loss during grapes storage (4).

Periodical fumigation with sulphur dioxide is employed worldwide for controlling grey mould in refrigerated storage of grapes (5). Sulphur dioxide dose and storage temperature are the major factors that determine sulphur dioxide residue in treated grapes (6). Hairline cracking due to reactive oxygen species (ROS) production and sulphur dioxide accumulation in wounded berries occur due to repeated fumigations (7). In recent years, ozone (O_3) has been widely used for postharvest decay management in perishable commodities (8). The broad antimicrobial properties of ozone, without any harmful by-product formation, have expanded an interest in ozone research in recent years. The Energy Power Research Institute (EPRI) recognized ozone usage as safe in 1997 (9). Both gaseous and aqueous ozone are used as sanitizing or disinfecting agents. The ozone decomposition to oxygen in water is due to its unstable nature and has a half-life of 20 to 30 min in water at 20°C (10, 11). Controlling microbial load in grapes will enhance the quality and storability of berries. The increasing regulatory restrictions on the use of harmful chemicals for sustaining quality attributes and prolonging the shelf-life of perishables have necessitated the need for safer alternatives. While significant research has been conducted on the postharvest application of gaseous ozone, little work has been done on using ozonated water. Therefore, this study was undertaken.

Materials and Methods

The experiment was conducted at the analytical laboratory, TNAU, Coimbatore, from December 2023 to January 2024. The grape bunches cv. Muscat Hamburg required for the study, was procured from a farmers' field in Coimbatore. The bunches were subjected to postharvest treatments viz., No dip (T_1), Dip in 0.3 ppm ozonated water for 5 min (T_2), 10 min (T_3) and 15 min (T_4) and replicated five times. Ozonated water of 0.3 ppm, as approved by the United States Health Administration (12), was produced by diffusing ozone gas generated from an ozone generator adopting high-frequency discharge technology (Model L30G) into deionized water for treating grape bunches. After treatment, the grape bunches were air dried and cold stored (4 ± 2 °C and 90 % RH) in crates and control fruits that received no treatment.

The fruits in the treated bunches were analyzed for quality attributes, berry firmness, berry abscission and physiological loss in weight at seven-day intervals. Total soluble solids were assessed using a handheld refractometer (Make: ERMA) and expressed in degree brix. The titratable acidity was estimated by titrating juice from fruit pulp against 0.1N NaOH with phenolphthalein indicator and expressed as percent tartaric acid equivalents (13). The sugars, viz., total and reducing, were estimated by adopting the procedure outlined by Somogyi (14) and represented in percent. The TSS and sugar: acid ratios were calculated by dividing TSS and total sugars with acidity, respectively. The Total phenol content, ascorbic acid content and anthocyanin content were determined by the standard methods (15, 16, 17).

A standard penetrometer was used to determine berry firmness. The plunger was pressed into the berry between the plunger and the holder up to a specified mark on the plunger. The readings were recorded in Newtons (N). Berry abscission was calculated using the following Equation 1 and was expressed in percent.

$$\text{Berry abscission (\%)} = \frac{\text{Number of berries abscised from the rachis}}{\text{Total number of berries in a bunch}} \quad \text{(Eqn. 1)}$$

Physiological loss in weight was calculated by adopting the formula given by the Association of Official Analytical Chemists and expressed in percent (18). The shelf-life of the fruits was calculated by taking the mean of the storage life of each replication, beyond which the fruits lost their marketability and consumer preference. It was expressed in days. Sensory evaluation was conducted with a panel of ten members using a 9-point hedonic scale on the last day of each treatment's shelf life.

The experiment was laid in a Completely randomized design and treatment means were compared using a one-way analysis of variance (ANOVA) and the Least Significant Difference (LSD) analysis of ANOVA was used to determine the significance across treatments. Using the appropriate R studio packages (version 4.3.1), the statistical analysis was performed in R software. Data significance was established at $p \leq 0.05$.

Results and Discussion

An increasing trend in the TSS content was noticed from the first day till the last day of sampling in all the treatments, as shown in Table 1 and the results are in line with earlier findings in grapes treated with ozone gas (19). Titratable acidity is a key factor determining the flavour of table grapes along with sugars. A declining trend was noticed for the parameter from the 1st to the last day of storage (Table 1). The highest acidity (0.24 %) was registered in T_4, compared to the control, which recorded 0.18 % on the 36th day of

Table 1. Effect of ozonated water treatment on total soluble solids and titratable acidity in grapes

Storage / Treatments	Total soluble solids (°Brix)						Titratable acidity (%)					
	1st day	8th day	15th day	22nd day	29th day	36th day	1st day	8th day	15th day	22nd day	29th day	36th day
T_1	15.68	16.16	16.32	17.15	17.88	18.73	0.69	0.56	0.49	0.34	0.27	0.21
T_2	15.54	16.23	16.75	17.58	18.18	19.00	0.67	0.57	0.44	0.32	0.23	0.20
T_3	15.64	16.16	16.45	17.18	17.81	18.60	0.67	0.58	0.47	0.34	0.26	0.22
T_4	15.61	16.26	16.55	17.15	17.44	17.90	0.66	0.62	0.51	0.41	0.36	0.30
SE(d)	0.38	0.48	0.49	0.35	0.44	0.76	0.02	0.02	0.01	0.008	0.007	0.01
CD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	0.03*	0.03**	0.02**	0.01**	0.02**

NS: Non-significant; T_1 - Control (no dip); T_2 - 0.3 ppm ozonated water dip for 5 min; T_3 - 0.3 ppm ozonated water dip for 10 min; T_4 - 0.3 ppm ozonated water dip for 15 min.

storage. A similar outcome was reported in red pitaya fruits. Still, in the contradiction that lower acidity was recorded in treated fruits than in control, the effect was insignificant in oranges and bananas (20, 21). Higher retention in treated fruits might be due to reduced respiratory activity, which can pave the way for better preservation of the fruit quality (22). Reduction in respiratory activity may be caused by inhibiting biosynthetic enzyme activity by aqueous ozone treatment, which is responsible for various metabolic activities, including ethylene biosynthesis (23). TSS:acid ratio exhibited an increasing trend till the last day of storage, as shown in Fig. 1. Significant differences among the treatments for TSS: acid ratio after 8 days may be due to the increase in total soluble solids and decrease in titratable acidity throughout the storage.

Total and reducing sugar content increased irrespective of the treatments throughout the storage (Table 2) and similar results were reported in chestnuts (24). A blend of sugars, organic acids and volatiles is crucial in defining flavour, an intricate quality attribute. The balance between the sugar and organic acid contents chiefly determines the organoleptic quality of table grapes (25). A distinct increase in the sugar:acid ratio was noticed during the storage period irrespective of the treatments (Fig. 2). The maximum value was recorded in bunches treatment for 5 min (84.72) on the 36th day of storage, which was 5.58 %, 13.37 % and 61.98 % higher than in control bunches and those treatment for 10 min and 15 min, respectively. A continuous trend of non-significant increase in total sugar content with profound decline in titratable acidity till the end of storage might be the cause for higher sugar:acid ratio.

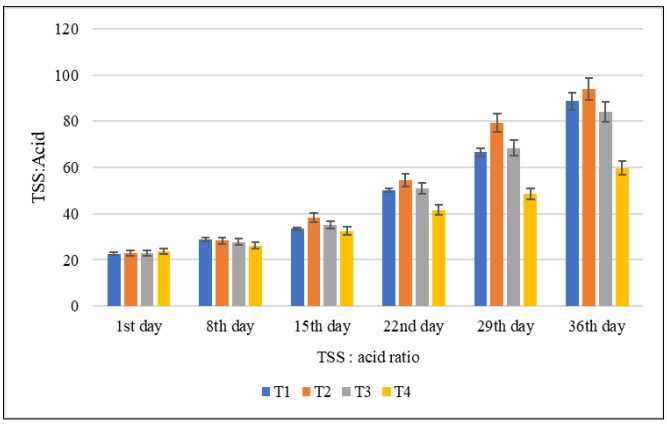

Fig. 1. Effect of ozonated water treatment on TSS: acid ratio in grapes. T_1 - Control (no dip); T_2 - 0.3 ppm ozonated water dip for 5 min; T_3 - 0.3 ppm ozonated water dip for 10 min; T_4 - 0.3 ppm ozonated water dip for 15 min.

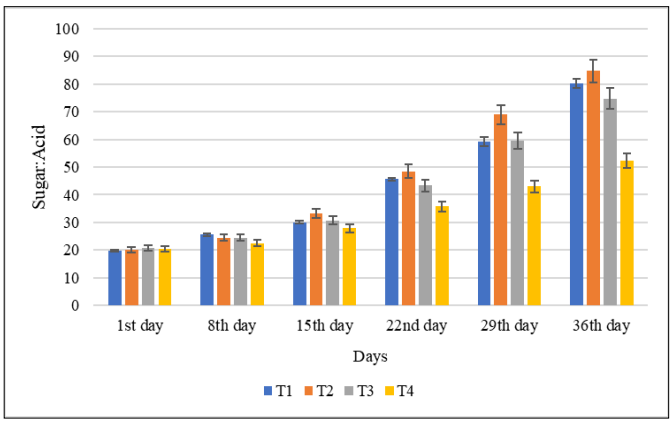

Fig. 2. Effect of ozonated water treatment on sugar: acid ratio in grapes. T_1 - Control (no dip); T_2 - 0.3 ppm ozonated water dip for 5 min; T_3 - 0.3 ppm ozonated water dip for 10 min; T_4 - 0.3 ppm ozonated water dip for 15 min.

Table 2. Effect of ozonated water treatment on total sugars and reducing sugars in grapes

	Total sugars (%)						Reducing sugars (%)					
	1st day	8th day	15th day	22nd day	29th day	36th day	1st day	8th day	15th day	22nd day	29th day	36th day
T_1	13.53	14.01	14.43	15.13	15.78	16.56	13.16	13.65	14.11	14.86	15.54	16.36
T_2	13.41	13.87	14.61	15.28	15.81	16.72	13.02	13.51	14.28	15.01	15.58	16.55
T_3	13.71	14.07	14.27	14.86	15.42	16.15	13.31	13.69	13.92	14.56	15.16	15.93
T_4	13.48	13.75	14.04	14.48	15.15	15.69	13.10	13.34	13.67	14.14	14.84	15.43
SE(d)	0.33	0.40	0.42	0.31	0.38	0.62	0.32	0.38	0.41	0.32	0.38	0.61
CD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS: Non-significant; T_1 - Control (no dip); T_2 - 0.3 ppm ozonated water dip for 5 min; T_3 - 0.3 ppm ozonated water dip for 10 min; T_4 - 0.3 ppm ozonated water dip for 15 min.

Table 3. Effect of ozonated water treatment on total phenol and anthocyanin content in grapes

Storage Period / Treatments	Total phenol content (mg GAE 100g^{-1})						Anthocyanin content (mg 100g^{-1})					
	1st day	8th day	15th day	22nd day	29th day	36th day	1st day	8th day	15th day	22nd day	29th day	36th day
T_1	109.16	112.23	114.84	112.65	109.18	106.49	37.27	37.33	37.48	37.73	37.86	38.03
T_2	109.32	112.05	114.97	113.88	109.91	105.82	37.33	37.41	37.59	37.86	38.00	38.22
T_3	109.21	115.05	116.38	118.92	116.16	111.92	37.29	37.41	37.66	37.88	38.08	38.27
T_4	109.26	116.36	117.87	120.88	119.50	115.48	37.31	37.61	37.54	38.05	38.19	38.46
SE(d)	1.05	1.67	0.62	2.42	2.83	2.14	0.92	1.05	1.08	0.77	0.95	1.31
CD (p=0.05)	NS	3.55*	1.33**	5.13**	6.00**	4.55**	NS	NS	NS	NS	NS	NS

** - Highly significant at 5 % level; * - Significant at 5 % level; NS: Non-significant; T_1 - Control (no dip); T_2 - 0.3 ppm ozonated water dip for 5 min; T_3 - 0.3 ppm ozonated water dip for 10 min; T_4 - 0.3 ppm ozonated water dip for 15 min.

Phenols protect the plant tissues from oxidative deterioration and play a significant role in imparting colour, flavour and astringency to grapes (26, 27). Total phenol content increased to a particular stage in control and treated fruits before declining (Table 3). Research indicates similar findings in blueberries and table grapes, respectively (28, 29). An upsurge in the total phenols due to ozone treatment could result from oxidation by polyphenol oxidase enzyme or a change in postharvest metabolic activities like respiration and ethylene production (30, 31). A non-significant increase in the anthocyanin content was noted throughout the storage period in all the treatments, as expressed in Table 3. This contradicts the observations made in research in which grapes are treated with ozone gas (32). A decreasing trend in the ascorbic acid content was noticed irrespective of the treatments with progress in the storage period (Fig. 3). Grape bunches dipped in ozonated water for 15 min (T_4) retained significantly higher ascorbic content of 1.81 mg 100 g^{-1} on 36th day of storage. The profound decline in the ascorbic acid content of control bunches compared to those treated with ozonated water (10 min and 15 min dip) could be due to early senescence (33). Ozone immersion at 0.05 ppm at 10°C increased vitamin C levels, demonstrating a positive effect on ascorbic acid retention in strawberries (34). This might be due to the activation of the ascorbate oxidase enzyme caused by ozone stress, which converts ascorbic acid to dehydroascorbic acid (DHA). Ozone treatment (1 mg L^{-1} for 10 min.) effectively sustained ascorbic acid levels, promoting antioxidant capacity and prolonging shelf life in kiwi fruits (35).

Physiological loss in weight (PLW), as presented in Fig. 4, is an important attribute that aids in determining fruit quality deterioration. Bunches dipped in ozonated water for 15 min (T_4) recorded the lowest PLW, showing 21.82 % and 33.45 % reductions compared to control bunches (T_1). Similarly, in strawberries, ozone treatment delayed senescence and reduced weight loss, indicating its effectiveness in maintaining fruit quality (36). However, ozone at high concentrations can cause irreversible oxidative damage to plants, including fruits' epidermis and cuticle tissues (37). The minimum PLW in citrus fruits treated with ozonated water for 15 min showed reductions of 21.82 % and 33.45 % compared to control (1). The reduced PLW observed in bunches treated with ozonated water may be attributed to inhibiting biosynthetic enzymes responsible for metabolic activities, including respiration and ethylene biosynthesis (38, 39). Higher PLW recorded in the T_2 and T_3 treatments could be due to increased decay incidence, transpiration and respiration. Berry firmness influences the PLW and is a key textural trait that determines the eating quality of table grapes. Grapes with firmer pulp have more consumer preference than those with soft pulp. Berry firmness was significantly affected by the treatments and it declined as the storage period extended regardless of the treatments (Table 4). Firmness was lost due to respiration and water loss during storage. Bunches treated with ozonated water for 15 min recorded a maximum firmness of 4.80 N on the 36th day of storage compared to the control. Similar findings on ozones' positive impact on retaining fruit firmness were reported by earlier workers (40, 41, 42). Grape bunches with greater berry firmness are easy to handle

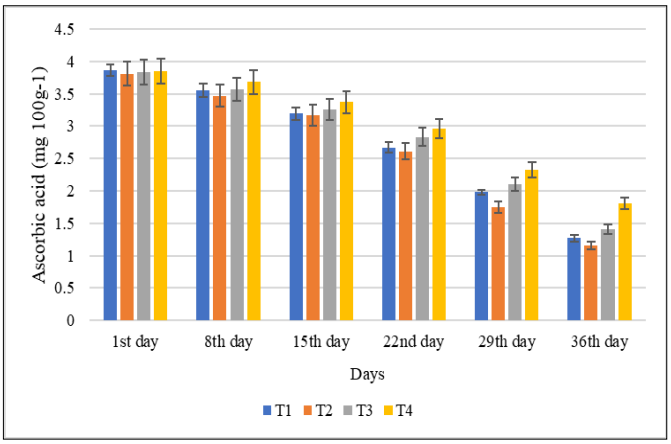

Fig. 3. Effect of ozonated water treatment on ascorbic acid in grapes. T_1 - Control (no dip); T_2 - 0.3 ppm ozonated water dip for 5 min; T_3 - 0.3 ppm ozonated water dip for 10 min; T_4 - 0.3 ppm ozonated water dip for 15 min

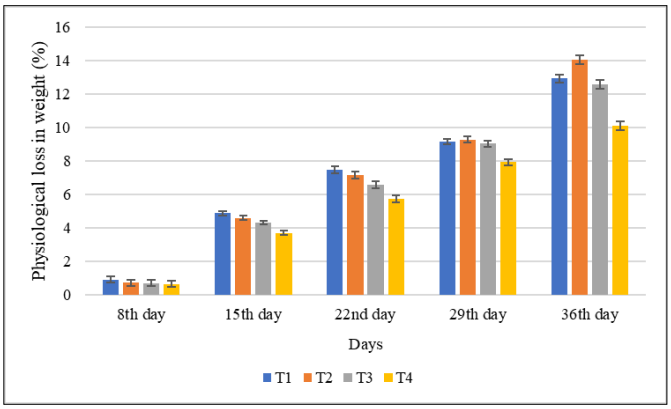

Fig. 4. Effect of ozonated water treatment on physiological loss in weight in grapes. T_1 - Control (no dip); T_2 - 0.3 ppm ozonated water dip for 5 min; T_3 - 0.3 ppm ozonated water dip for 10 min; T_4 - 0.3 ppm ozonated water dip for 15 min.

Table 4. Effect of ozonated water treatment on berry firmness and berry abscission in grapes

Storage Period / Treatments	Berry firmness (N)						Berry abscission (%)					
	1st day	8th day	15th day	22nd day	29th day	36th day	1st day	8th day	15th day	22nd day	29th day	36th day
T_1	8.56	7.33	6.32	5.52	4.69	3.83	0.00 (0.71)	0.00 (0.71)	0.62 (0.98)	2.46 (1.57)	4.20 (1.85)	5.51 (2.13)
T_2	8.63	7.25	6.26	5.33	4.40	3.42	0.00 (0.71)	0.00 (0.71)	1.95 (1.30)	3.91 (2.01)	7.80 (2.71)	9.14 (2.95)
T_3	8.96	7.99	6.96	6.15	5.02	3.94	0.00 (0.71)	0.00 (0.71)	0.79 (1.04)	1.38 (1.30)	2.55 (1.60)	3.95 (1.92)
T_4	8.88	8.10	7.20	6.43	5.71	4.80	0.00 (0.71)	0.00 (0.71)	0.58 (0.97)	1.23 (1.15)	2.49 (1.61)	3.77 (1.87)
SE(d)	0.21	0.22	0.20	0.12	0.11	0.17	0.00	0.00	0.03	0.03	0.05	0.09
CD (p=0.05)	NS	0.48**	0.43**	0.26**	0.24**	0.35**	NS	NS	0.06**	0.06**	0.10**	0.19**

** - Highly significant at 5 % level; * - Significant at 5 % level; NS: Non-significant; T_1 - Control (no dip); T_2 - 0.3 ppm ozonated water dip for 5 min; T_3 - 0.3 ppm ozonated water dip for 10 min; T_4 - 0.3 ppm ozonated water dip for 15 min.

during postharvest and will have longer shelf lives. Better firmness might be due to the reduction in softening of flesh and disintegration of the cell wall during cold storage and inhibition of the activity enzymes, viz., polygalacturonase and pectin methylesterase, responsible for cell wall degradation by ozone (38). Short-term ozone treatments decreased pectin solubilization as pectin methyl esterase activity was reduced. Cross-linking of the cell wall structural proteins may also have occurred in ozone-treated tissues by forming dityrosine associations, strengthening the cell wall and maintaining firmness (43). These complex modifications induced by ozone are yet to be explored.

Berry abscission is a serious problem during postharvest handling as it affects the shape of the grape bunch. Maximum berry abscission was recorded up to 9.14 % during the 36th day of storage in berries treated with 0.3 ppm ozonated water for 5 min (T_2) (Table 4). Treatments T_4 (3.77 %) and T_3 (3.95 %) had lower abscission on 36th day of storage. The results corroborate the earlier findings in grapes (44, 45). Ozone exposure can alter the expression of genes related to oxidative stress and secondary metabolite production, which may influence berry attachment and abscission processes (44, 46). Applying ozonated water has been linked to reducing fungal diseases, which can also indirectly affect berry health and retention on the vine. The decrease in berry abscission in the present investigation can be attributed to the freshness of the rachis maintained by the ozonated water treatment in T_4.

Sensory properties of the fruits, such as taste, flavour, colour, etc., represented in Table 5, were not altered by ozonated water treatments. Research indicates that ozonation treatment did not adversely affect apples' appearance and odour compounds (47). The shelf life of the grapes was significantly affected by ozonated water treatments. Bunches treated with 0.3 ppm ozonated water for 15 min registered shelf life of 39.84 days. This was nearly 5 days higher than the shelf life of control bunches. Apricots treated with ozone (0.5 ppm) and refrigerated storage registered a shelf life of 12 days compared to 7 days in untreated fruits (48). Research indicates similar outcomes with ozone treatments on mandarins, with gaseous ozone (3.3 to 20 ppm for 10 to 60 min) and ozonated water (2 to 6 ppm) and the results showed up to 97.5 % efficacy for gaseous ozone and 95-97 % for ozonated water, effectively extending the shelf life of mandarins without compromising quality (48). Preservation of quality with prolonged shelf life in grapes treated with 0.3 ppm ozonated water for 15 min may be attributed to higher retention of firmness, reduced decay incidence and limited weight loss compared to control samples.

Conclusion

The study demonstrated the effectiveness of ozonated water (0.3 ppm for 15 min) in maintaining the quality and prolonging the shelf life of grapes. Treated bunches showed reduced physiological loss in weight, higher berry firmness, lower berry abscission and an extended shelf life compared to untreated control. This treatment effectively preserved fruit quality by mitigating decay and maintaining sensory attributes, offering an environmentally friendly and residue-free alternative for postharvest management. Ozonated water, therefore, emerges as a promising solution for improving grape storage and marketability. Scaling up ozonated water treatment for grapes requires developing cost-effective, large-scale systems compatible with commercial operations. Future studies should validate its efficacy under real-world storage conditions, including cold storage, transportation and retail settings. Integration with other postharvest practices like packaging and controlled atmosphere storage should also be explored. Testing across supply chains and export scenarios will ensure robustness.

Table 5. Effect of ozonated water treatment on shelf life and sensory attributes in grapes

Storage Period / Treatments	Shelf life (days)	Sensory Attributes					
		Appearance	Colour	Texture	Flavour	Taste	Overall acceptability
T_1	34.98	6.60	6.80	6.80	6.80	7.00	7.00
T_2	34.94	6.60	6.60	6.80	6.60	7.20	7.20
T_3	36.76	6.80	6.80	7.00	7.00	7.20	7.20
T_4	39.84	7.00	6.80	7.20	6.80	7.20	7.40
SE(d)	0.30	0.36	0.43	0.40	0.41	0.46	0.35
CD (p=0.05)	0.62**	NS	NS	NS	NS	NS	NS

** - Highly significant at 5 % level; NS: Non-significant, T_1 - Control (no dip); T_2 - 0.3 ppm ozonated water dip for 5 min; T_3 - 0.3 ppm ozonated water dip for 10 min; T_4 - 0.3 ppm ozonated water dip for 15 min.

At the same time, environmental sustainability and consumer acceptance studies will highlight its eco-friendliness and safety. Also, standardized protocols and regulatory alignment are essential for uniform adoption. These steps will help establish ozonated water as a viable, scalable alternative for prolonging grape shelf life and quality.

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Authors' contributions

KC carried out conceptualization, methodology, resources, funding acquisition, and original draft writing. AB handled formal analysis and software development. PB was involved in formal analysis and software. SV contributed to methodology, software and writing, review and editing. PK was involved in writing, reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflicts of interest.

Ethical issues: None

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