Biological Forum – An International Journal 16(12): 154-158(2024)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of Ozonated Water on Post Harvest Quality Attributes in Grapes cv. Muscat Hamburg

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(Received: 06 September 2024; Revised: 01 October 2024; Accepted: 15 October 2024; Published: 14 December 2024)
(Published by Research Trend)

ABSTRACT: The present study aimed to know the influence of ozonated water on post harvest attributes in grapes cv. Muscat Hamburg during 2023-2024. The study involved treatments of 0.3 ppm of ozonated water at different minutes *viz*., 3, 6, 9 and 12 min. The harvested berries were immersed in the ozonated water and air dried before storage. Observations were recorded on titratable acidity, total phenols, physiological loss in weight, shelf life and rachis dehydration. Significant differences were observed among the control and treated berries. The titratable acidity (0.34 & 0.29), total phenols (119.55 & 107.38), physiological loss in weight (10.1 & 7.52), shelf life (29.25 & 34.50) and rachis dehydration (2.8 & 3.0) were observed during season (1 & 2) and indicating the possibility of improving post harvest attributes and life of grapes berries.

Keywords: Grapes, ozone water, phenols, shelf life.

INTRODUCTION

Grapes are a significant fruit crop recognized for their substantial nutritional content. It comprises an array of nutrient components, including vitamins, minerals, carbs, and phytochemicals. Grapes are consumed either fresh or processed into wine, raisins, juice, concentrate, jam, and seed oils. Grey mould infection (*Botrytis cinerea*) is the primary postharvest illness (Cappellini *et al*., 1986; Shen *et al*., 2021). Rachis browning is the second most significant issue in table grapes, leading to consumer rejection and fruit degradation during postharvest storage for long-distance markets (Lichter, 2016). *Botrytis cinerea* poses significant challenges since it can proliferate among berries even at -0.5 °C, whereas rachis browning acts as an indicator of freshness and significantly influences customer preference. Fumigation with sulfur dioxide (SO_2) is frequently employed to preserve the postharvest quality of table grapes. The issues related to SO_2 fumigation include unwanted bleaching damage to the berries (Smilanick *et al*., 1990). Persistence of sulfite residues in grapes (Austin *et al*., 1997). Consumer awareness regarding the potential health risks associated with persistent toxicants has generated a need for more environmentally friendly food additives. Ozone (O_3) is an innovative, environmentally friendly technology that serves as a powerful oxidant and effective cleaning agent, applicable in both gaseous and aqueous states. The proliferation of grey mould was impeded in the table grape cultivar Thompson. Seedless subjected to 0.3 ppm gaseous ozone at 5°C for 7 weeks. No phytotoxic lesions were seen on the berries and rachis (Palou *et al.*, 2002). Excess ozone decomposes swiftly to generate oxygen, leaving no remains in the treated food sample. Utilizing ozone bubbles in water is more pertinent than employing gaseous ozone due to the delicacy of table grapes (Heleno *et al.*, 2015). This study was conducted to assess the impact of ozonated water on prolonging the shelf-life of grape cultivars. Muscat, Hamburg.

MATERIALS AND METHODS

The experiment was conducted twice, from April 2024 to May 2024 (season I) and in September 2024 to October 2024 (season II) in the Post Harvest Laboratory of SRM College of Agricultural Sciences, Baburayanpettai, Chengalpattu.

Experimental Materials. The fully mature fruits of grape cultivar Muscat Hamburg, taken from a farmer's field in Surilipatti village, Cumbum Block, Theni, during the summer season (April 2024) and winter season (September 2024), were utilized for the current study. The fruits were sourced from the farmer's field to guarantee uniformity of the testing material. The farmer maintained the vines in accordance with the practices outlined in the Crop Production Guide (TNAU, 2013). Pruning for the summer crop occurred in the second half of April 2024, while harvesting took place in the first half of September 2024.

Ozonated water treatment. Ozone was generated using ozone generator (OFS-400 MG model manufactured by Ozone Feed systems) by high frequency corona discharge technology using oxygen as feed gas that was supplied by the oxygen generator. The ozone gas produced was diffused into deionized water to produce ozonated water with the help of a bubble diffuser.

Treatment details:

T_1 : Control (no dip)

T_2 : 0.3 ppm ozonated water dip for 3 minutes

T_3 : 0.3 ppm ozonated water dip for 6 minutes

T_4 : 0.3 ppm ozonated water dip for 9 minutes

T_5: 0.3 ppm ozonated water dip for 12 minutes

The berries were immersed in 0.3 ppm ozonated water for a designated duration according to the treatment specifications, air-dried and subsequently stored in crates at 6 ± 2°C and 90% relative humidity (RH). Control fruits, devoid of any prior treatment, were also preserved under identical conditions.

Titratable acidity %. Titratable acidity was assessed by titrating 10 mL of freshly extracted juice, diluted to a specified volume with distilled water, against 0.1 N NaOH, utilizing phenolphthalein as an indicator (Ranganna, 2000). Acidity was measured as a percentage of tartaric acid equivalents in grapes.

$$\text{Titaratable acidity} = \frac{\text{Titre value} \times \text{Normality of NaOH} \times \text{Volume made up} \times \text{Equivalent weight of acid} \times 100}{\text{Volume of sample taken} \times 1000}$$

Total phenolic content (mg GAE 100 g^{-1}). The total phenol content was assessed utilizing the Folin-Ciocalteau method (Singleton and Rossi 1965). Phenols were isolated from the peel with ethanol. The Folin-Ciocalteu reagent and one percent sodium carbonate were included into the extract, followed by the measurement of absorbance at 765 nm. Total phenol content was quantified in Gallic acid equivalents (mg GAE 100 g^{-1}).

Physiological loss in weight (%). The physiological weight loss was determined using the formula provided by A.O.A.C. (2001) and reported as a percentage. Five bunches were collected for each replication from each treatment, and their weights were measured using an electronic balance.

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

Shelf life (days). The shelf life of the fruits was determined by calculating the average storage duration of each replication, after which the fruits diminish in marketability and consumer appeal. It was calculated in days.

Rachis dehydration (1-5 scale). Rachis dehydration was evaluated using a 1-5 scale, with 1 indicating green and fresh, 2 representing green, 3 denoting semi-dry, 4 signifying 50% dry, and 5 indicating completely dry(Xu *et al.*, 2007). Dehydration and browning symptoms were assessed in the primary and secondary rachises.

Statistical analysis. The experimental data were statistically analyzed using the techniques suggested by Panse and Sukhatme (1985). Data analysis was performed using GRAPES software and the critical difference was worked out at 5% level of significance.

RESULTS AND DISCUSSION

Influence of ozonated water on titratable acidity (%). Titratable acidity is a key factor which determines the flavour of table grapes along with sugars. Though organic acids are present in smaller amounts than sugars, they play a vital role in deciding the overall taste. Titratable acidity progressively diminished with the extension of the storage time and exhibited significant variations across all treatments in both seasons (Table 1). On the first day of both seasons, no significant variations were noted among the treatments, with titratable acidity ranging from 0.67% to 0.69% in season I and from 0.37% to 0.41% in season II. The peak acidity was observed in bunches subjected to ozonated water for 12 minutes, measuring 0.34% and 0.29% on the 25th and 30th days of storage in seasons I and II, respectively. In season I, the lowest acidity was seen in the control group (T_1) at 0.30%, followed by bunches immersed in ozonated water for 3 minutes (T_2) at 0.33%, 6 minutes (T_3) at 0.34% and 9 minutes (T_4) at 0.33%. The four treatments were equivalent to each other. In Season II, control (T_1) involved bunches immersed in ozonated water at 0.17% and from 0.23% at 3 minutes (T_2), 0.25% at 6 minutes in (T_3) and 0.29% at 9 minutes in (T_4). Generally, acid levels will decline as they get consumed for respiration during ripening (Ladaniya, 2011). Higher retention of acidity in ozone treated fruits may be due to a reduction in the respiratory activity (Morais *et al.*, 2015) which can pave way for better preservation of the fruit quality. Reduction in respiratory activity may be because of the inhibition of biosynthetic enzyme activity by aqueous ozone treatment that is responsible for a range of metabolic activities including ethylene biosynthesis (Liu *et al.*, 2016).

Effect of ozonated water on total phenolic content in grape berries (mg GAE 100 g^{-1}). Phenols act as antioxidants and protect the plant tissues from oxidative deterioration. It also plays a major role in imparting colour, flavour and astringency to grapes. The data in the Table 2 represents about the impact of ozonated water on total phenol content. On the first day of both seasons, no notable differences were seen in the total phenol content across all treatments.

In season I, the total phenol content increased from the 1st to the 15th day in T_1 and T_2, followed by a progressive fall until the 25th day. A comparable trend was noted for T_3, T_4 and T_5, wherein the total phenol level diminished after the 20th day. Marked disparities in total phenol concentration were observed after the first day. On the fifth day of storage, T_4 (115.37mg GAE 100 g^{-1}) and T_5 (116.23 mg GAE 100 g^{-1}) were comparable. On the twenty fifth day of storage, T_4 and T_5 recorded 118.6 mg GAE 100 g^{-1} and 119.55 mg GAE 100 g^{-1}. T_4 exhibited an increase of 8.44% compared to the control (T_1) on the 25th day of storage, marking it as the most effective. T_1 (109.17 mg GAE 100 g^{-1}), T2 (109.95 mg GAE 100 g^{-1}) and T3 (116.18mg GAE 100 g^{-1}) were comparable on the 25th day of storage. The highest phenol concentration was observed in T_5 on the 20th day of storage (121.7 mg GAE 100 g^{-1}).

In season II, the phenol content in T_1, T_2, and T_3 grew until the 20th, 20th, and 25th days, respectively. T_4 and T_5 exhibited an increasing trend from day 1 to day 30, ranging from 89.11 mg GAE 100 g^{-1} to 107.38 mg GAE 100 g^{-1}, and recorded the highest values among all treatments from the 5th to the 30th day of storage. The highest value was seen in treatment T_5 (107.38 mg GAE 100 g^{-1}) on the 30th day of storage, which was 18.61% greater than the control (91.56 mg GAE 100 g^{-1}). In overall, season I exhibited a greater phenolic content (109.17 mg GAE 100 g^{-1} to 119.55 mg GAE 100 g^{-1}) compared to season II (91.56 mg GAE 100 g^{-1} to 107.38 mg GAE 100 g^{-1}). The reason for the accumulation of phenolic content may be attributed to the release of some conjugated phenolic compounds present in the cell wall. This may be due to any cell wall modification that might have taken place during ozone treatment. The decrease in the phenolic content could be due to oxidation by polyphenol oxidase enzyme (Yamaguchi *et al.*, 2003) or change in postharvest metabolic activities like respiration and ethylene production (Shiri *et al.*, 2011).

Impact of ozonated water on Physiological loss in weight during storage (%). Physiological loss in weight (PLW) is an important attribute which aids in determining the fruit quality deterioration. Ozonated water treatment had a significant effect on the PLW (Table 3). The physiological loss of weight was significantly affected by the ozonated water treatments in both seasons during cold storage. PLW increased from the first day to the final day of storage, regardless of the treatments applied in both seasons. PLW increases from 0.68% to 13.95% and from 0.96% to 11.17% in seasons I and II, respectively. On the first day of season I, control (T_1) exhibited a greater PLW than T_2, T_3, T_4 and T_5, with T_2 and T_3 being statistically comparable. On the first day of season II, T_4 exhibited the lowest PLW in comparison to the control (T_1), whereas T_2 and T_3 were statistically equivalent. Bunches subjected to ozonated water treatment for 9 minutes (T_4) exhibited significantly reduced physiological loss in weight (PLW) throughout season I (10.10%) and season II (7.52%). In season I, on the 25th day of storage, treatments T_1 and T_3 exhibited comparable performance, recording lower PLW than T_2. Conversely, in season II, treatments T_2 and T_3 demonstrated similar results on the 30th day of storage. Greater weight reduction was observed in T_1 of season I and T_1 of season II. A reason for lower PLW in bunches treated with ozonated water can be due to inhibition of biosynthetic enzyme that is responsible for a range of metabolic activities including respiration and ethylene biosynthesis (Liu *et al.*, 2016).

Effect of ozonated water on Shelf life (days). Shelf life of the grapes was significantly affected by ozonated water treatments (Table 4). In both seasons, treatments significantly influenced the shelf life of bunches. In season I, T_5 had a maximum shelf life of 29.25 days compared to other treatments. A comparable result was observed in Season II, when treatment T_5 (34.50 days) considerably extended the shelf life of bunches compared to other treatments. Treatments T_1 (26.75 days in season I and 27.25 days in season II) and T_2 (27.65 days in season I and 29.50 days in season II) exhibited comparable results in both seasons.

Influence of ozonated water on Dehydration of rachis (1-5 scale). Dehydration of the rachis was evaluated on a scale of 1 to 5 (Table 5). As the storage duration extended, dehydration intensified regardless of the treatments applied in both seasons.

The dehydration of the rachis was not substantial from the 1st to 5th day of storage in season I and from the 1st to the 10th day of storage in season II. Significant changes were seen over the remaining storage days in both seasons. Bunches subjected to ozonated water for 12 minutes (T_5) exhibited superior rachis appearance compared to other treatments on the 25th and 30th days of storage in seasons I and II, respectively. Control (T_1) and T_2 were equivalent on the 25th day of storage during Season I. T_2 and T_3 were equivalent on the 30th day of storage during season II.

Table 1: Influence of ozonated water on titratable acidity (%).

TITRATABLE ACIDITY (%)													
	SEASON I						SEASON II						
Treatment/ Storage period	1st day	5th day	10th day	15th day	20th day	25th day	1st day	5th day	10th day	15th day	20th day	25th day	30th day
T_1	0.69	0.67	0.54	0.49	0.42	0.30	0.37	0.33	0.30	0.27	0.24	0.20	0.17
T_2	0.68	0.65	0.57	0.50	0.44	0.33	0.41	0.39	0.36	0.33	0.29	0.26	0.23
T_3	0.69	0.68	0.59	0.53	0.44	0.34	0.38	0.36	0.34	0.32	0.3	0.28	0.25
T_4	0.68	0.66	0.57	0.53	0.45	0.33	0.41	0.39	0.39	0.37	0.35	0.32	0.29
T_5	0.67	0.64	0.58	0.51	0.44	0.34	0.41	0.38	0.36	0.34	0.33	0.31	0.29
Grand Mean	0.68	0.66	0.57	0.51	0.43	0.32	0.39	0.37	0.35	0.32	0.30	0.27	0.24
SE(D)	0.01	0.008	0.009	0.007	0.006	0.004	0.006	0.006	0.004	0.004	0.006	0.004	0.002
CD (0.05)	0.03	0.016	0.019	0.014	0.013	0.009	0.013	0.013	0.009	0.008	0.012	0.008	0.006

Table 2: Effect of ozonated water on total phenolic content in grape berries (mg GAE 100 g^{-1}).

TOTAL PHENOL CONTENT (mg GAE $100g^{-1}$)													
	Season 1						Season 2						
Treatment\storage period	1st day	5th day	10th day	15th day	20th day	25th day	1st day	5th day	10th day	15th day	20th day	25th day	30th day
T_1	109.17	112.13	114.74	114.86	112.66	109.17	87.28	91.51	92.65	96.56	95.02	93.85	91.56
T_2	109.23	112.03	114.86	114.95	113.83	109.95	88.21	93.69	93.54	97.41	99.78	98.65	95.8
T_3	109.34	115.04	115.97	116.36	118.95	116.18	86.98	92.72	94.03	95.56	99.94	101.04	100.27
T_4	109.37	115.37	116.28	117.85	120.85	118.6	88.01	93.98	96.68	100.79	103.15	105.83	106.41
T_5	109.34	116.23	117.77	117.87	121.7	119.55	89.11	94.42	97.76	101.24	104.32	106.54	107.38
Grand Mean	109.29	114.16	115.92	116.38	117.60	114.69	87.91	93.24	94.93	98.31	100.44	101.18	100.28
SE(d)	0.93	1.58	1.94	1.88	1.41	1.86	1.22	1.11	1.58	1.50	2.04	1.26	1.45
CD (0.05)	1.98	3.36	4.13	4.02	3.02	3.96	2.59	2.37	3.38	3.21	4.36	2.70	3.09

Table 3: Impact of ozonated water on Physiological loss in weight during storage (%).

PHYSIOLOGICAL LOSS IN WEIGHT (%)											
	Season 1					Season 2					
Treatment\ storage period	5th day	10th day	15th day	20th day	25th day	5th day	10th day	15th day	20th day	25th day	30th day
T_1	0.95	4.85	7.48	9.38	13.95	1.08	3.15	4.93	6.63	8.35	11.17
T_2	0.75	4.6	7.15	9.25	12.95	1.17	3.57	5.16	7.77	8.97	10.82
T_3	0.72	4.35	6.58	9.01	12.58	1.19	2.93	4.57	6.93	8.32	10.34
T_4	0.68	3.68	5.75	7.93	10.1	0.96	2.12	3.62	5.36	6.24	8.54
T_5	0.7	3.69	5.85	8.25	11.15	1.14	2.25	3.52	5.23	6.13	7.52
Grand Mean	0.76	4.23	6.56	8.76	12.14	1.10	2.80	4.36	6.38	7.60	9.67
SE(d)	0.01	0.06	0.10	0.11	0.12	0.02	0.03	0.07	0.08	0.11	0.15
CD (0.05)	0.03	0.13	0.22	0.23	0.26	0.03	0.07	0.15	0.16	0.22	0.33

Table 4. Effect of ozonated water on Shelf life (Days).

SHELF LIFE (Days)		
Treatments	Season 1	Season 2
T_1	26.75	27.75
T_2	27.65	29.50
T_3	27.98	30.56
T_4	28.50	31.60
T_5	29.25	34.50
Grand Mean	28.03	30.78
SE(d)	0.40	0.50
CD (0.05)	0.85	1.06

Table 5. Influence of ozonated water on Dehydration of rachis (1-5 scale).

DEHYDRATION OF RACHIS (1-5 scale)													
	Season 1						Season 2						
Treatment\storage period	1st day	5th day	10th day	15th day	20th day	25th day	1st day	5th day	10th day	15th day	20th day	25th day	30th day
T_1	1	1	1.7	2.6	3.3	3.4	1	1	1	1.6	2.7	3.1	4.2
T_2	1	1	1.4	2.4	3.2	3.2	1	1	1	1.5	2.5	2.8	3.6
T_3	1	1	1.5	2.1	2.8	3	1	1	1	1.2	1.8	2.7	3.5
T_4	1	1	1.1	1.8	2.7	2.9	1	1	1	1.2	1.5	2.3	3.1
T_5	1	1	1.2	2	2.6	2.8	1	1	1	1.1	1.4	2.1	3
Grand Mean	1	1.00	1.38	2.18	2.92	3.06	1	1	1	1.32	1.98	2.6	3.48
SE(d)	0.03	0.02	0.02	0.03	0.04	0.04	0.01	0.01	0.01	0.02	0.03	0.04	0.05
CD (0.05)	0.01	0.03	0.05	0.06	0.09	0.09	0.03	0.03	0.03	0.04	0.07	0.09	0.11

CONCLUSIONS

In the present study, the treatment of grape bunches with ozonated water for 12 minutes (T_5) was the most effective postharvest treatment in preserving the quality and improving the shelf life of fruits. Thus, ozonated water has got multifaceted applications which can ensure pesticide residue free fruits with better quality and shelf life.

FUTURE SCOPE

1. Comparative Analysis with Other Postharvest Treatments: While ozonated water treatment (T_5) has shown promising results, future studies could involve comparing its efficacy with other common postharvest treatments such as UV radiation, controlled atmosphere storage, or edible coatings. This would help in establishing ozone as a superior or complementary method for enhancing shelf life and quality.

2. Long-Term Shelf Life and Market Studies: It would be valuable to investigate the long-term effects of ozonated water treatment on grape shelf life, particularly in real-world market conditions. The study could involve analyzing consumer acceptance and how ozonated grapes compare with untreated ones in terms of taste, appearance, and overall marketability.

3. Impact on Nutrient Retention and Pesticide Degradation: Future research could explore the effects of ozonated water treatment on the retention of nutrients (such as antioxidants and vitamins) in grapes, as well as its ability to degrade pesticide residues over time. Studies could also investigate whether ozone treatment affects the bioavailability of beneficial compounds in fruits.

4. Exploring Mechanisms of Action: Further studies could investigate the biochemical and physiological mechanisms underlying the beneficial effects of ozonated water on grape bunches. This would include exploring its impact on cellular structures, enzymatic activity, and microbial load, which ultimately contribute to the improved quality and shelf life.

5. Environmental and Economic Sustainability: Given the rising demand for sustainable agricultural practices, a future scope could include evaluating the environmental and economic viability of using ozonated water in large-scale postharvest treatments. Assessing the energy requirements, cost-effectiveness, and environmental impact would be crucial in promoting ozone as an eco-friendly alternative to conventional postharvest practices.

By addressing these areas, future research could enhance the application of ozonated water for improving fruit quality, ensuring safer, longer-lasting, and more sustainable produce for consumers.

Conflict of interest. None.

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How to cite this article: Ajay Surya M., A. Akino, B. Gopu and K. Prakash (2024). Effect of Ozonated Water on Post Harvest Quality Attributes in Grapes cv. Muscat Hamburg. *Biological Forum – An International Journal, 16*(12): 154-158.