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Effects of ozone treatment on storage quality and antioxidant capacity of fresh-cut water fennel [*Oenanthe javanica*]

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Abstract

Water fennel is vulnerable to a loss in quality during storage. To improve postharvest quality, fresh-cut water fennel was treated with 37.04 mg m^{-3} ozone for 15 minutes every 5 days, and then stored at 5 °C for 20 days, and the related quality and antioxidant capacity indexes were determined. The results showed that compared with the control group, the ozone-treated water fennel had better sensory quality, color (L*, a*, ΔE, Chroma) and firmness after 20 days of storage, and the weight loss decreased by 47%, malondialdehyde content decreased by 41%, soluble solids content decreased by 20%.The ozone treatment also effectively inhibited the increase of respiratory rate and phenolic substances during storage of fresh-cut water fennel. Meanwhile, ozone treatment maintained the content of ascorbic acid, inhibited the activity of polyphenol oxidase and induced an increase of peroxidase, catalase, ascorbate peroxidase and superoxide dismutase, but reduced the content of reduced glutathione. Water fennel after ozone treatment maintained the appearance, texture characteristics and high commercial value during storage. Therefore, ozone treatment may be used as an effective preservation technology for postharvest storage and circulation of water fennel.

Keywords: water fennel; ozone; preservation; antioxidant capacity.

Practical Application: Important method to prolong the postharvest storage and improve the quality of water fennel vegetables.

1 Introduction

Water fennel (Oenanthe javanica) is a perennial aquatic herb with a distinctive aroma and taste that is widely grown in regions such as China, Korea and Japan (Ai et al., 2016). It is not only an excellent source of vitamins, minerals, volatile oils and dietary fiber, but also rich in fructose, glucose, amino acids, apigenin, caffeic acid and other bioactive components (He et al., 2019). In traditional Chinese medicine, water fennel can also be widely used to prevent certain diseases, such as jaundice and various types of chronic and acute hepatitis, and it is also recommended as a treatment for hypertension, diabetes, allergies, arrhythmia and cardiovascular diseases (Choi et al., 2011; Huang et al., 2001). However, fresh-cut water fennel due to its high water content, fragile tissue, exuberant respiration, such as color changes, tissue softening, weight decline, microbial infections and other overall quality decline phenomenon during storage (Yang et al., 2016). These negative effects can result in a decline in overall quality and increasingly amount of water fennel discarded, which finally causes economic losses to local industries. Therefore, it is necessary to develop new and practical fresh-cut water fennel preservation technology.

For better food sensory quality and nutrient, many non-thermal technologies have been used for food preservation, such as cold plasma, high-pressure processing (HPP), oscillating magnetic fields, ultrasound, pulsed electric field processing (PEF), irradiation, pulsed light and ultraviolet light (Abdilova et al., 2022; Aljahani et al., 2022; Rocha et al., 2022). However, some factors limit the use of non-thermal processing technologies in the industry, such as high investment costs and consumer concerns about food safety and so on (Rocha et al., 2022). Ozone treatment as a non-thermal technology is less costly and does not leave any residue on the products (Cao et al., 2022). Moreover, Ozone can act on many saturated and unsaturated organic matter, and effectively reduce the content of ethylene, ethanol and acetaldehyde in ripe fruits and vegetables (Xu et al., 2019). Because of its oxidative ability on proteins, lipids, enzymes, nucleic acids, membranes and other cellular components, ozone usually causes various physiological changes in fruits and vegetables to improves stress resistance of plants (Pinto et al., 2020). Studies have shown that ozonated water can inhibit the activity of polyphenol oxidase and respiration rate of fresh-cut celery, and the sensory quality is better than that of the control group (Zhang et al., 2005). (Wang et al., 2021a) pointed out that ozone with ultrasound assisted aerosolization sanitizer can increase the 3,4-dihydroxybenzoic acid and vanillin phenolic compounds content in fresh-cut lettuce and had no negative effect on its color properties. In addition, ozone has a strong bactericidal effect. It has been reported that ozone can significantly reduce the number of escherichia coli, yeast and mold on the surface of toona sinensis and maintain the storage quality (Lin et al., 2019). Ozone treatment is an effective method for post-harvest treatment of fruits and vegetables, which has

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achieved good preservation effect in fruits and vegetables such as kiwifruit (Cao et al., 2022), cantaloupe (Chen et al., 2020), button mushrooms (Wang et al., 2021b), toona sinensis (Lin et al., 2019), winter jujube (Zhang et al., 2022), etc. However, there are few reports on gaseous ozone treatment of fresh-cut water fennel. As a result, the goal of this research is to investigate the effects of ozone on storage quality of fresh-cut water fennel and measure the activities of enzymes related to antioxidant capacity, and finally determine whether ozone use is an effective method for maintaining postharvest quality of water fennel.

2 Materials and methods

2.1 Raw material

Water fennel was purchased in Chizhou, Anhui Province, China, transported by cold chain and returned to the laboratory in time. Fresh water fennel without mechanical damage, pests and diseases were selected for the experiment.

2.2 Ozone treatment

In the preliminary experiment, fresh-cut water fennel was treated with 18.52, 37.04, 55.56 and 74.07 mg m^{-3} ozone for 15 min at the same time. The preliminary results showed that 37.04 mg m^{-3} ozone could prolong the shelf life of fresh-cut water fennel more effectively than the control group and other concentration treatment groups. Therefore, we select ozone with 37.04 mg m^{-3} to treat water fennel for 15 min.

The parts of the water fennel stem with approximately the same thickness were cut into fresh sections of about 16 cm, and then pre-chilled in an incubator (SHP-150, Shanghai Jinghong Test Equipment Co., LTD, Shanghai) for 2 hours at 5 °C. The fresh cut water fennel was randomly divided into two groups. One group was ozone treatment group: The outlet of an ozone generator (Gn-S2S, Anhui Jieyu Electronic Technology Co. LTD, Anhui, China) was then passed through the inlet port at the head of the box (54 cm × 40.5 cm × 31 cm), and the water fennel in the box was subjected to an ozone treatment of 37.04 mg m^{-3} for 15 min. The other group was the control group: no ozone exposure, other conditions were the same as the OZ. After treatment, Allotments of 120 g water fennel were packed into 0.12mm thick polyethylene film bags, folded and then stored in the incubator at 5 °C (40-50% relative humidity). We repeated above operations every five days, water fennel samples after storage were determined for the evaluation of physicochemical index, antioxidant enzymes every five other days. Three replicates from each treatment group were analyzed.

2.3 Sensory quality assessment

Sensory quality evaluation was modified according to the evaluation criteria of (Han et al., 2014). A professionally trained group of 10 people rated the water fennel for appearance, texture and fragrance. Nine-point scale for scoring: 9, excellent (Fresh green, glossy, crisp texture, with normal water fennel fragrance; edible); 7, good (Slightly dark yellow at the incision of water fennel stem, crisp texture, water fennel fragrance; edible); 5, moderate (further loss of freshness, crisp texture, and water fennel fragrance, tissue softened; rarely eaten); 3, poor (Stem segments appear a small part of the water-soaked rot, light color, texture wilting; Inedible) and 1, extremely poor (Stem serious water-soaked rot, the color is basically dark; inedible).

2.4 Determination of color

The color was measured on the intermediate stem segment surface of the water fennel using a handheld colorimeter (CR-400; Konica Minolta, Japan), with direct recording the L*(luminosity: black - white axis), a*(green - red axis), b*(blue - yellow axis) values. The color saturation (chroma) and the color difference (ΔE) calculation reference (Souza et al., 2018)

2.5 Determination of total chlorophyll, chlorophyll-a and chlorophyll-b contents

Chlorophyll contents were determined following (Yang et al., 2016) with modification, the specific method is to weigh 2 g sample into a mortar, add 95% ethanol grinding extraction, filtered to 15 mL, the filtrate was measured by UV spectrophotometer(UV-1800, Shimadzu Company, Japan) at 665 nm and 649 nm absorbance value. Chlorophyll contents was expressed as μg g^{-1} fresh weight (FW).

2.6 Determination of weight loss and firmness

The weight loss was calculated as a percentage according to the method of (Souza et al., 2018).

Firmness determination: Randomly select 5 water fennel stem segments, and the middle firmness of stem segments was measured by GY-5B digital display fruit hardness tester (Hangzhou Aipu Instrument Equipment Co., Ltd, Hangzhou, China). The result was expressed as Newton (N).

2.7 Determination of soluble solid content

Soluble solid content (SSC) was determined by Abbe refraction apparatus (2WAJ, Shanghai Optical Instrument No.5 Factory, Shanghai, China). A total of 5 g sample was ground in a mortar and filtered through gauze. The juice was read directly with an Abbe refractometer and expressed as mass fraction (%)

2.8 Determination of respiration rate

The respiration rate of fruits and vegetables can be expressed by CO2 production rate (Fonseca et al., 2002). The 50 ± 1 g fresh-cut water fennel of each treatment group was placed in a well-sealed box and then placed in a storage room at 5 °C. After 2 hours, the CO_2 release was measured by Easy Check Two head space analyzer (ADEV company, Italy), and the respiration rate was calculated with the description of (Ghosh & Dash, 2020), expressed as L $kg^{-1}h^{-1}$ FW.

2.9 Determination of malondialdehyde content

The malondialdehyde (MDA) content was measured with the description of (Wang et al., 2021b). Taking 1 g sample and put it into a mortar, add 5 mL 100 g L^{-1} trichloroacetic acid

(TCA) and grind it into slurry, and centrifuged 20 min at 4 °C and 10000g. The 2 mL supernatant was mixed with 2 mL 0.67% (w/v) thiobarbituric acid (TBA), followed by boil water bath for 20 min and then cool down and centrifuged again, then measure the absorbance value of supernatant at 450 nm, 532 nm and 600 nm respectively. The MDA content was expressed as nmol g^{-1} FW.

2.10 Determination of total phenolic and flavonoid substances relative contents

The total phenolic and flavonoid substances relative contents were measured with the description of (Cao et al., 2007). The water fennel tissue (1g) was grinded into slurry with 1% (v/v) hydrochloric acid-methanol solution under ice bath conditions, and then set the volume to 15 mL, and extracted for 20 min at 4 °C in the dark. After filtration, the filtrate was taken to determine the absorbance values at 280 nm and 325 nm. The absorbance values at wavelengths of 280 nm and 325 nm respectively represent the relative contents of total phenols and flavonoids, and the result was presented as OD_{280} g^{-1} and OD_{325} g^{-1} based on FW

2.11 Determination of ascorbic acid and reduced glutathione

Ascorbic acid (ASA) was determined by 2,6-dichlorophenol indiophenol titration (Yu et al., 2021). The 10 g water fennel tissue was ground into slurry with 20 g L^{-1} oxalic acid solution, and the volume was fixed to 100 mL. After filtering, 10 mL was taken and titrated with the calibrated 2,6-dichlorophenol indophenol to a reddish color persisted for 15 s, and the ASA content was calculated by the amount of dye consumed. The ASA content was expressed as mg $100^{-1}g^{-1}$ FW.

Reduced glutathione (GSH) content is slightly modified according to (Xu et al., 2022) method. The 2 g water fennel sample was ground into slurry with 3 mL 50 g L^{-1} TCA (containing 5 mmol L^{-1} ethylenediaminetetraacetic acid disodium salt) in an ice bath and centrifuged at 12000 g for 20 min at 4 °C. 0.5 mL 4 mmol L^{-1} 5,5′-dithiobis-(2-nitrobenzoic acid) and 1.0 mL 0.1 mol L^{-1} phosphate buffer (pH 7.7) were added into 1.0 mL supernatant, After the mixture solution were kept at 25 °C for 10 min, the solution was taken to measure the absorbance value at 412 nm. The GSH content was calculated in μmol g^{-1} FW.

2.12 Enzyme activity measurement

The determination of polyphenol oxidase (PPO) activity was modified according to the method of (Xu et al., 2019). The 2 g water fennel sample was ground into slurry under ice bath conditions with 3.0 mL 0.1 mol L^{-1}, pH5.5 acetic acid-sodium acetate buffer (containing 1 mmol polyethylene glycol 6,000, 4% (w/v) of polyvinylpolypyrrolidone (PVPP), 1% (v/v) of Triton X-100), and centrifuged at 4 °C and 12000g for 20 min. The supernatant was the crude enzyme solution. 0.2 mL crude enzyme solution was added into 3.0 mL 50 mmol L^{-1}, pH 5.5 acetic acid-sodium acetate buffer and 1.0 mL 50 mmol/L catechol solution. The absorbance changes at 420 nm within 3 min were recorded. One PPO activity unit is defined as a change in absorbance per minute per gram of water fennel sample (FW), expressed as U g^{-1}.

The activity of peroxidase (POD) was carried out by guaiacol method (Xu et al., 2019) and slightly modified. The procedure of enzyme extract is the same as that of PPO. 0.2 mL 0.5 mol L^{-1} H_2O_2 solution were added into 3 mL 25 mmol L^{-1} guaiacol solution and 0.5 mL crude enzyme solution to start the reaction. The absorbance change value at 470 nm within 2 min was recorded, and the increase of absorbance change value by 1 per gram of water fennel sample (FW) per minute was considered as one POD activity unit U, expressed as U g^{-1}.

Catalase (CAT) and superoxide dismutase (SOD) activity was slightly modified according to the method of (Azam et al., 2021). The 2 g water fennel sample was ground into a slurry under ice bath conditions with 5 mL 0.1mol L^{-1}, pH 7.5 sodium phosphate buffer (containing 5 mmol L^{-1} dithiothreitol, 5% (w/v) PVPP) and centrifuged at 4 °C, 12000g for 20 min. The supernatant was the crude enzyme solution. 2.9 mL 20 mmol L^{-1} H_2O_2 solution was added into 0.1mL enzyme solution to start the reaction, and the absorbance change value at 240 nm within 2 min was recorded to calculate CAT activity. The change value of absorbance decreased by 0.01 per minute per gram of water fennel sample (FW) as one CAT activity unit U, expressed as U g^{-1}. The 1.7 mL 50 mmol L^{-1}, pH 7.8 phosphate buffer, 0.3 mL 130 mmol L^{-1} methionine, 0.3 mL 750 μmol L^{-1} nitroblue tetrazolium (NBT), 0.3 mL 100 μmol L^{-1} ethylenediaminetetraacetic acid disodium salt, 0.2 mL enzyme solution and 0.3 mL 20 μmol L^{-1} riboflavin solution were used for SOD activity. The mixtures were illuminated under 4000 LX daylight lamp for 12 min and then the absorbance value at 560 nm was measured immediately. Identical solution held in the dark served as blank. One SOD activity unit U was defined as 50% inhibition of NBT actinic reduction per minute per gram of water fennel sample (FW), expressed as U g^{-1}.

The determination of ascorbate peroxidase (APX) activity was modified according to (Yu et al., 2021). Extract the crude enzyme solution with 3 mL of 0.1 mol L^{-1} and pH 7.5 potassium phosphate buffer (containing 0.1 mmol L^{-1} EDTA, 1 mmol L^{-1} ASA and 2% (w/v) PVPP) as described above. Briefly, 0.1 mL 0.2 mol L^{-1} H_2O_2 solution was added into 2.6 mL 50 mmol L^{-1} and pH 7.5 potassium phosphate buffer (containing 0.1 mmol L^{-1} EDTA and 0.5 mmol L^{-1} ASA) and 0.5 mL crude enzyme solution to start the enzymatic reaction. The change value of absorbance at 290 nm within 2 min was recorded, and the change value of absorbance decreased by 0.01 per minute per gram of water fennel sample (FW) as one APX activity unit U, expressed as U g^{-1}.

2.13 Data statistics and analysis

The measurement of each parameter is repeated three times, and the data were analyzed statistically by one-way analysis of (ANOVA) with SPSS 27.0 software (IBM, USA), presented as means ± standard (SD). Differences were significant at $P<0.05$.

3 Results and discussion

3.1 Effects of ozone treatment on the quality of water fennel

Sensory quality and color

The quality change of water fennel during storage can be directly reflected by the change of stem color and the degree of

wilting. The sensory quality of treated and untreated water fennel showed the same dynamic change trend on the whole, and the scores of both continued to decline (Table 1). The control group had obvious incision browning and wilted on the day 20, most of which were lower than the lowest acceptable limit (5 points); The ozone treatment group, however, obviously slowed down the rate of sensory deterioration, and the sensory quality was generally significantly better than that of the control group after 10 days of storage ($P<0.05$). The sensory quality score of treated water fennel was still 7 ± 0.06 after 15 days of storage, which had certain commercial value (Table 1). From the above analysis, it can be seen that the ozone treatment effectively maintained the appearance quality of water fennel.

Color is a crucial factor affecting the sale of water fennel, which reflects the freshness, and any color change may be considered a symptom of aging (Nunes et al., 2009). Evaluation of color parameters commonly used are L*, a*, b*, where negative to positive values represent dark to light, green to red, and blue to yellow, respectively. Besides, another parameter variable related to color is the chroma, which reflects the color intensity during the process of storage (Souza et al., 2018). All these parameters have been used to assess the color change of various products during storage after ozone treatment. (Wang et al., 2021a) observed that lactic acid combined with ozone treatment had no negative effect on fresh-cut lettuce color properties, such as L*, a*, b*. (Lin et al., 2019) also showed that ozone could delay the color changes of toona sinensis during storage. For the ozone treatment group, the L*, a* and ΔE values used to evaluate whether the treated water fennel changed color were significantly different from those of the control group after 15 days of storage, while the b* value was not significantly different from that of the control group. In addition, the chroma value of ozone treatment group was significantly higher than that of control group after 20 days of storage (Table 1). These results indicated that ozone treatment did not damage the original color characteristics of water fennel, and even had a certain maintenance effect.

Chlorophyll-a, chlorophyll-b and total chlorophyll contents

Chlorophyll degrades will lead to green loss of water fennel and decrease its appearance quality. Chlorophyll breakdown is that chlorophyll -a is catalyzed by chlorophyllase to form chlorophyllide-a, which is then degraded into nonfluorescent chlorophyll catabolites by a series of related degrading enzymes (Hortensteiner, 2013). chlorophyll -a, chlorophyll -b and total chlorophyll in water fennel declined over the course of storage in both groups (Figure 1). Furthermore, the contents of chlorophyll -a and total chlorophyll in ozone treatment group were significantly higher than those in control group ($P<0.05$) after 10 days of storage. However, there was no significant difference in chlorophyll -b content between the two groups except on the 10th day (Figure 1B). The above results indicate that ozone affects the total chlorophyll content mainly by affecting the change of chlorophyll -a content in water fennel. Moreover, ozone treatment can slow down the decomposition rate of chlorophyll in water fennel, which may be related to the effective inhibition of chlorophyll degrading enzyme activity by ozone (Xu et al., 2019).

Weight loss, firmness and SSC

The weight loss is one of the key sensory characteristics affecting the quality evaluation of fresh vegetables. The weight loss of water fennel ozone treated and untreated continued to rise during storage, and the weight loss of water fennel in the ozone treatment group was significantly lower than that in the control group. On the 20th day of storage, the weight loss of the ozone treated water fennel was only about 53% of that of the untreated water fennel (Figure 2A). Due to respiration and transpiration, the weight loss rate changes during storage (Bai et al., 2022), and the water loss by transpiration will reduce the weight. As a result, water fennel became wilted, atrophy and finally unacceptable. Ozone can affect hormone signal transduction in plants and induce stomatal conductance (Hasan et al., 2021), thereby affecting respiration and transpiration, which may be an important reason for the small weight loss of water fennel in ozone treatment group. The results showed that ozone treatment can delay water loss of water fennel during storage, which was similar to research into the storage of toona sinensis (Lin et al., 2019) and button mushrooms (Wang et al., 2021b).

The firmness is an important index reflecting water fennel freshness, which is related to weightlessness and directly affects its commodity value and consumer acceptance. As presented

Table 1. Effects of ozone treatment on sensory quality, L*, a*, b*, ΔE and Chroma values in fresh-cut water fennel during storage at 5 °C for 20 days. Different letters within column mean significant differences between groups ($P < 0.05$). Each value is the mean of three replicates.

Attributes	Treatment	Storage days				
		0	5	10	15	20
sensory quality	Control	8.36 ± 0.05^a	7.54 ± 0.26^a	6.62 ± 0.27^b	5.65 ± 0.29^b	5.04 ± 0.44^b
	Ozone	8.36 ± 0.05^a	7.97 ± 0.15^a	7.25 ± 0.12^a	7 ± 0.06^a	6.39 ± 0.06^a
L*	Control	47.09 ± 2.70^a	53.83 ± 3.27^a	54.73 ± 3.18^a	57.80 ± 2.40^a	60.64 ± 2.76^a
	Ozone	48.21 ± 3.18^a	51.93 ± 2.42^a	54.50 ± 1.90^a	54.91 ± 2.62^b	56.58 ± 2.57^b
a*	Control	-12.37 ± 1.63^a	-11.27 ± 2.07^a	-7.42 ± 0.80^a	-8.41 ± 0.88^a	-7.24 ± 0.62^a
	Ozone	-12.47 ± 1.12^a	-12.51 ± 1.37^a	-8.33 ± 0.91^b	-9.53 ± 0.56^b	-8.66 ± 0.87^b
b*	Control	23.70 ± 2.19^a	25.52 ± 2.55^a	23.47 ± 2.06^a	24.14 ± 1.22^a	20.25 ± 1.98^a
	Ozone	23.85 ± 2.97^a	26.34 ± 2.02^a	23.85 ± 2.40^a	23.90 ± 1.82^a	23.77 ± 2.28^a
ΔE	Control	0	8.35 ± 4.46^a	9.42 ± 2.64^a	11.88 ± 4.16^a	15.20 ± 2.43^a
	Ozone	0	6.07 ± 2.77^a	8.71 ± 4.20^a	8.12 ± 2.55^b	9.87 ± 3.09^b
Chroma	Control	26.76 ± 2.46^a	27.94 ± 2.90^a	24.62 ± 2.10^a	25.58 ± 1.32^a	21.51 ± 2.02^b
	Ozone	26.95 ± 2.77^a	29.20 ± 1.84^a	25.29 ± 2.28^a	25.74 ± 1.74^a	25.32 ± 2.28^a

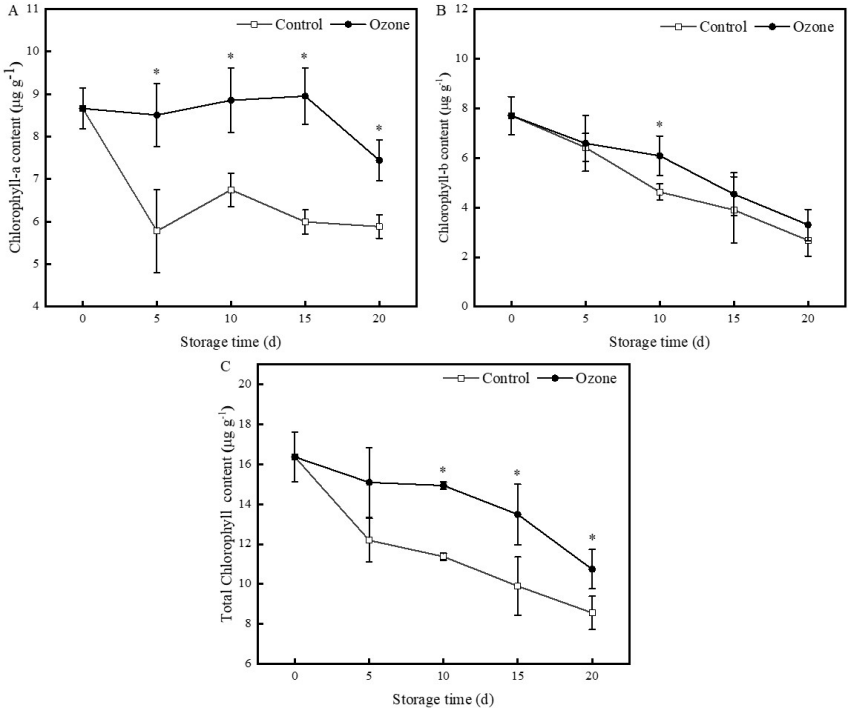

Figure 1. Changes of chlorophyll-a (A), chlorophyll-b (B) and total chlorophyll (C) content of water fennel in ozone treatment group and control group during storage. Vertical bars indicate the standard deviation (±SD). The asterisks mean significant differences between groups ($P < 0.05$).

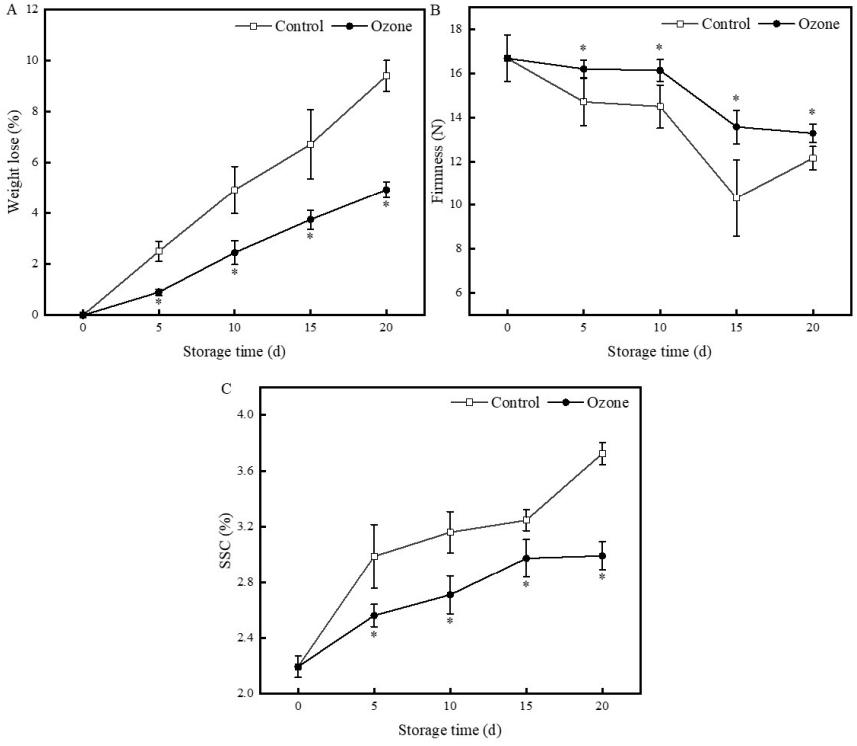

Figure 2. Changes of weight loss (A), firmness (B) and SSC (C) of water fennel in ozone treatment group and control group during storage. Vertical bars indicate the standard deviation (±SD). The asterisks mean significant differences between groups ($P < 0.05$).

in Figure 2B, the firmness of water fennel in ozone treatment group was significantly higher than that in control group during the whole storage period ($P < 0.05$). The firmness of the two groups decreased during storage, which may be caused by the degradation of insoluble protopectin to more soluble pectic acid (Nasrin et al., 2020). The control group and ozone treatment

group reached the minimum on day 15, day20, which were 10.33 N and 13.28N, respectively (Figure 2B), thus ozone can slow the softening of water fennel. Similar researches have shown that ozone can effectively delay softening of kiwifruit (Minas et al., 2014), winter jujube (Zhang et al., 2022), chilli peppers (Glowacz & Rees, 2016), which may be related to ozone can inhibit the activity of cell wall degrading enzymes, such as polygalacturonase (Minas et al., 2014).

The increase in SSC of the two groups during storage may be due to the decrease in water content and the conversion of complex carbohydrates to monosaccharides (Etemadipoor et al., 2020). During the whole storage process, SSC in ozone treatment group was significantly lower than that in control group (Figure 2C), possibly because the control group lost more water (Figure 2A). Studies have shown that in micro-processed fruits and vegetables such as cauliflower (Nasrin et al., 2022), SSC usually increases to cope with water loss during storage (Silva et al., 2009). It has been reported that ozone treatment of carrots can prevent a sharp increase in SSC (Souza et al., 2018), which are similar to the findings. SSC is related to flavor and can reflect the ripening degree and quality of fruits and vegetables. For vegetables such as water fennel, high ripeness is considered as a form of deterioration and not accepted by consumers. All these results indicated that ozone treatment could effectively maintain the quality of water fennel and delay its senescence.

Respiration rate and MDA content

Respiration is a metabolic process that aims to oxidize organic substrates into simple molecules to provide energy for plant biochemical processes (Fonseca et al., 2002). Therefore, reducing respiration rate is an important way for prolonging postharvest life and optimizing postharvest quality. As presented in Figure 3A, the respiration rate of the control group generally increased first and then decreased during storage. Maybe due to water fennel maturing and a respiratory peak. During in 0-20 days, the respiration rate of the ozone-treated group decreased slowly and was lower than that of the control group. This may be because ozone treatment can retard the tissue metabolism, close stomata, cause changes in the ultrastructure of epicuticular waxes (Chen et al., 2020). The results showed that appropriate amount of ozone could inhibit the respiration rate of water fennel, and similar conclusions were also reached on green pepper (Chitravathi et al., 2015; Özen et al., 2021), cantaloupes (Chen et al., 2020), carrots (Chauhan et al., 2011) and celery (Zhang et al., 2005).

MDA is an important product reflecting membrane lipid peroxidation during postharvest senescence of fruits and vegetables (Jiang et al., 2020). As seen in Figure 3B, the MDA content in both groups increased with the prolongation of storage time. However, the MDA content of ozone treatment group was significantly lower than that of control group after 15 days, which indicates that the membrane lipid peroxidation and cell membrane damage was low in ozone treatment group. Ozone treatment can increase the activity of SOD and other antioxidant enzymes, reduce the accumulation of reactive oxygen species (ROS), thereby inhibiting the increase of MDA content (Wang et al., 2021b), which will be confirmed by the results of SOD, CAT etc in section 3.2.2.

3.2 Effects of ozone treatment on antioxidant capacity

Total phenols and flavonoids relative contents

Phenolic compounds are secondary metabolites of plants, which are closely related to the flavor, color and quality of fruits and vegetables; besides, flavonoids are a kind of low molecular weight phenolic compounds (Ignat et al., 2011). They inhibit the oxidation of lipids by acting as free radical scavengers and are very important antioxidants in plants (Shezi et al., 2020). During the whole storage period, the total phenol content in the control group increased first and then decreased, while the ozone treatment group showed a fluctuating upward trend; furthermore, total phenol content of water fennel in ozone treatment group was significantly lower than that in control group on day 5, day10 and day15 (Figure 4A). The flavonoid content in both groups also showed a similar trend (Figure 4B). According to existed researches, ozone treatment has an effect on phenolic compounds of agricultural products, such as ozone can increase phenolic compounds in papaya (Ali et al., 2014). However, the

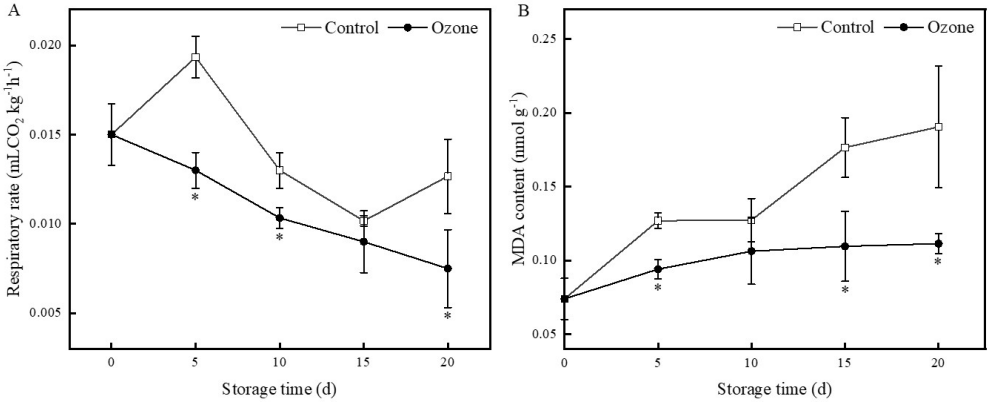

Figure 3. Changes of respiration rate (A) and MDA content (B) of water fennel in ozone treatment group and control group during storage. Vertical bars indicate the standard deviation (±SD). The asterisks mean significant differences between groups ($P < 0.05$).

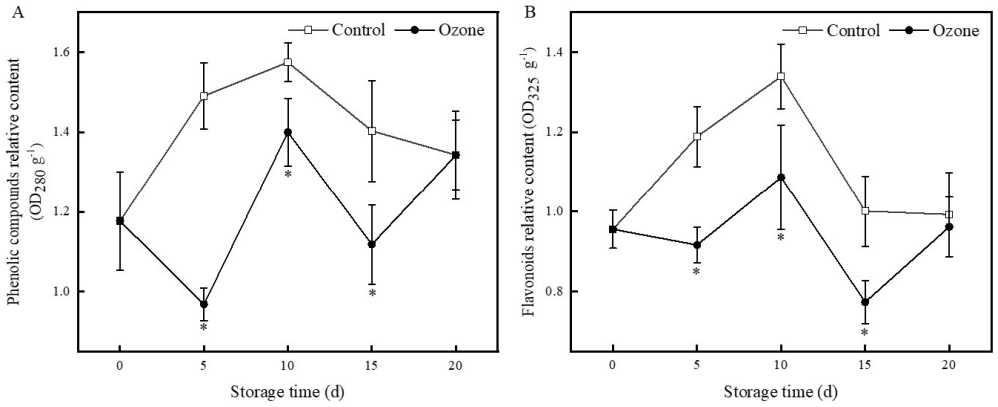

Figure 4. Changes of total phenol (A) and flavonoid (B) relative contents of water fennel in ozone treatment group and control group during storage. Vertical bars indicate the standard deviation (±SD). The asterisks mean significant differences between groups ($P < 0.05$).

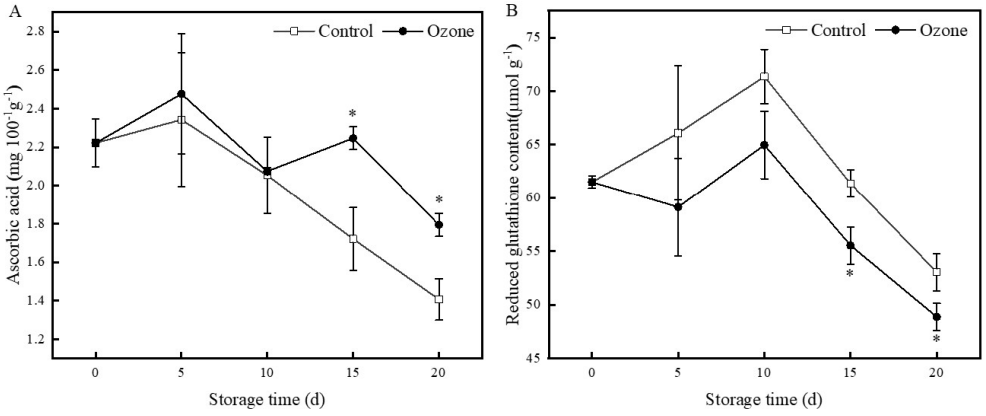

Figure 5. Changes of Ascorbic acid (A) and reduced glutathione (B) contents of water fennel in ozone treatment group and control group during storage. Vertical bars indicate the standard deviation (±SD). The asterisks mean significant differences between groups ($P < 0.05$).

findings showed that ozone treatment will reduce the content of phenolic compounds compared with the control group, which is similar to the results that ozone can cause significant degradation of total phenol content in carrot (Chauhan et al., 2011). This may be because ozone inhibits phenylalanine ammonialyase (PAL) activity that catalyzes the synthesis of phenols from aromatic amino acids phenylalanine (Shezi et al., 2020). In addition, the effect of ozone gas-induced polyphenol biosynthesis also depends on ozone concentration, product fumigation time, application method, fruit and vegetable types and so on (Kaur et al., 2022).

ASA and GSH contents

Figure 5A revealed the changes of ASA in water fennel, showing that the ASA of water fennel decreased generally over the storage period, but the ASA of water fennel treated with ozone was higher than that of the untreated water fennel. When preserved for 20 d, the ASA content of water fennel in control group and ozone group was 1.41 mg 100^{-1} g^{-1} and 1.80 mg 100^{-1} g^{-1}, respectively. Figure 5B illustrated that the GSH content of both treated and untreated water fennel increased first and then decreased, and its content in ozone treated was always at a lower level compared with the untreated water fennel. Both GSH contents in the control group and treated peaked on day 10, and their GSH contents were 71.36 μmol g^{-1} and 64.97 μmol g^{-1}, respectively.

ASA can reduce and neutralize ROS such as hydrogen peroxide. In addition to its direct antioxidant effect, ASA is also the reaction substrate of APX (Lobo et al., 2010), which is particularly important in plant stress resistance. As an important intermediate substance in the ASA-GSH cycle, GSH plays important role in maintaining the cell membrane integrity and inhibiting the cell membrane lipid peroxidation (Meng et al., 2022). There is a certain relationship between ASA and GSH. In the ASA-GSH cycle, ASA and GSH can be indirectly converted to each other under the action of relevant enzymes (Hasanuzzaman et al., 2017), which may be partly responsible for the small changes in ASA and GSH contents in the two groups at the early stage of storage (Figure 5). According to previous studies, ozone treatment can raise the ASA content of fruits and vegetables such as strawberry (Morais et al., 2015) and papaya (Ali et al., 2014), which may be because ozone promotes the ASA-GSH cycle and related antioxidant enzyme activities. Likewise, studies have shown that the appropriate concentration of ozone can increase the GSH content of raspberry, highbush blueberry, strawberry, blackberry

and blackcurrant fruit (Piechowiak, 2021). However, the results of this study showed that ozone reduced the GSH content of water fennel, and a similar phenomenon was also observed in blueberry (Piechowiak et al., 2020), which may be due to the increase of glutathione peroxidase activity or the direct reaction of GSH with ozone and free radicals.

Activities of PPO, POD, APX, CAT and SOD

PPO is related to the color change and aging of fruits and vegetables and can catalyze the formation of o-quinones from polyphenol substrates, leading to tissue browning (Eissa et al., 2006). As depicted in Figure 6A, the activity of PPO in the control group increased as a whole and reached the peak value at 10 d, about 45% higher than that of the treated group. Meanwhile, the activity of PPO in the ozone treatment group ascended continuously during storage and was lower than that in the control group. This indicates that ozone treatment can inhibit PPO activity, possibly because the high oxidation potential of ozone (Botondi et al., 2021). Previous studies have shown that ozone treatment can inhibit coriander PPO activity (Xu et al., 2019), which is consistent with results.

POD, APX, CAT and SOD antioxidant enzymes have special roles in metabolizing reactive oxygen species (Lin et al., 2019; Shezi et al., 2020). SOD can catalyze superoxide radical anion disproportionation reaction to H_2O_2, and then the generated H_2O_2 can be catalyzed by POD, APX and CAT to decompose

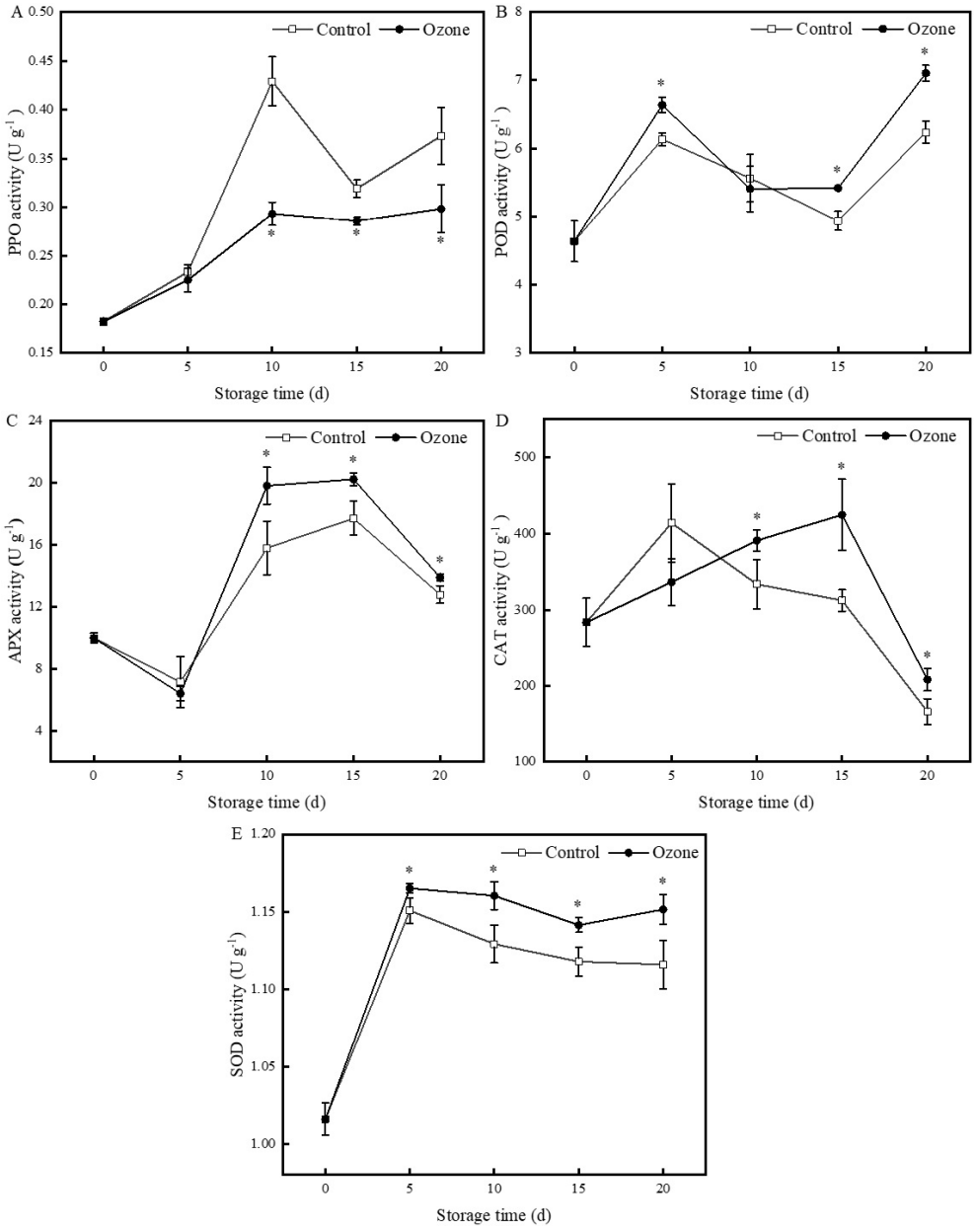

Figure 6. Changes of PPO(A), POD(B), APX(C), CAT(D) and SOD(E) activities of water fennel in ozone treatment group and control group during storage. Vertical bars indicate the standard deviation (±SD). The asterisks mean significant differences between groups ($P < 0.05$).

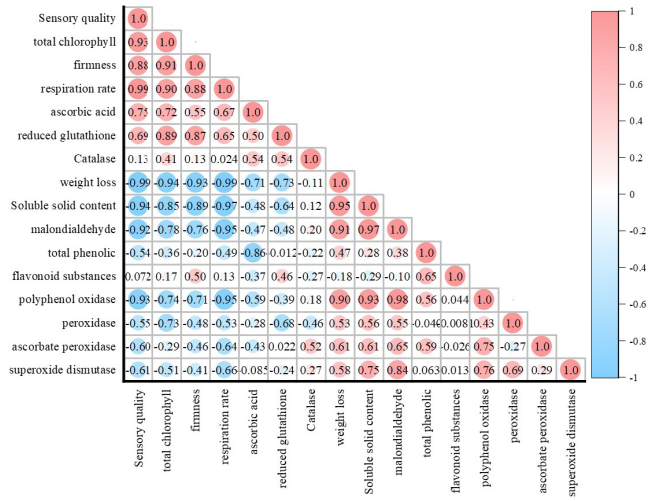

Figure 7. Pearson correlation between different parameters after ozone treatment of fresh-cut water fennel. Pink represents a positive correlation between different parameters, while blue represents a negative correlation.

into water and oxygen (Boonkorn et al., 2012). As shown in Figure 6B and Figure 6C, the activity of POD and APX in two groups of water fennel fluctuated during storage, and the activity of POD in treated group was significantly higher than the untreated group on day 5, day 15 and day 20, while activity of APX in ozone treatment group significantly higher than the control group after 10 days. In general, the activities of CAT and SOD increased first and then decreased, and CAT, SOD activity in water fennel in ozone treatment group was higher than that in untreated water fennel, moreover there was a significant difference between the two groups after 10 days. Ozone is a strong oxidant that activates the antioxidant system in plants, and it can affect ROS metabolism and the plant's antioxidant status. (Xu et al., 2019) showed that ozone could improve the activities of coriander POD, CAT and APX, keep the low H_2O_2 and superoxide anion in coriander, thus maintain the quality of coriander. (Lin et al., 2019) also found that ozone could enhance the effectiveness of the antioxidant defense system of toona sinensis by enhancing the activities of POD, CAT and SOD. These results indicate that ozone can promote the activity of antioxidant enzymes, reduce the oxidative damage caused by H_2O_2 accumulation, and slow down the deterioration of the quality of water fennel.

3.3 Correlation analysis

The Pearson correlation coefficient between different parameters of fresh-cut water fennel after ozone treatment is shown in Figure 7. The total chlorophyll content was highly correlated with firmness ($P<0.05$, $r = 0.90$), GSH content ($P<0.05$, $r=0.90$) and weight loss rate ($P<0.05$, $r= -0.92$), indicating that these parameters had a high influence on the total chlorophyll content. Similarly, firmness is highly correlated with respiration rate, weight loss rate and SSC content. SSC content was highly correlated with sensory quality, weight loss, respiration rate, MDA content and PPO activity. MDA content was correlated with sensory mass, respiration rate, weight loss rate and PPO activity. PPO activity is closely related to sensory mass, respiration rate and weight loss rate (Figure 7). From the above correlation, it can also be inferred that the weight loss, that is, the amount of water loss of water fennel, can determine the storage quality of water fennel.

4 Conclusion

In this paper, the effect of ozone on the preservation and antioxidant ability of water fennel and its possible mechanism were investigated. The quality of fresh-cut water fennel could be maintained by treating it with 37.04 mg m-3 ozone for 15 min every 5 days. For the storage quality, ozone treatment effectively delayed the deterioration of color properties, chlorophyll, weight loss and hardness of fresh-cut water fennel, and inhibited the increase of PPO activity, respiration rate, MDA and SSC. As for the antioxidant capacity, ozone treatment will reduce the content of total phenols, flavonoids and GSH, but could maintain the ASA content, and reduce the effect of ROS accumulation on quality deterioration by increasing the activities of POD, APX, CAT and SOD in water dropwort, which also indicated that ozone could improve the antioxidant capacity of water fennel mainly through enzyme antioxidant system rather than non-enzyme antioxidant system. Finally, ozone treatment has a certain effect on fresh-cut water fennel, which can be expanded further in the field of fresh-cut water fennel, while its functional basis needs further research and exploration.

Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Fan Lin: Investigation and writing of manuscript. Kaiyu Lv: Data curation and writing. Shufeng Ma: Project administration.

Feijie Wang: Formal analysis. Jiangkuo Li: Conceptualization and validation. Liqiang Wang: Project administration, writing – review and editing. All authors reviewed and commented on manuscript.

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