Research Article

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Response of antioxidant system to postharvest ozone treatment in 'Soreli' kiwifruit

Valentina Goffi,[a] Anna Magri,[b] Rinaldo Botondi[a] and Milena Petriccione[b*]

Abstract

BACKGROUND: Among the challenges for postharvest researchers is that of understanding the physiological and biochemical pathways associated with postharvest fruit decay. Fruit senescence directly affects sensorial and nutritional quality during postharvest life. It has been clarified that reactive oxygen species and oxidative damage are responsible for fruit senescence. Some cultivars of yellow-fleshed kiwifruit can be stored for a short period compared with green-fleshed kiwifruit. Postharvest performance is affected by the physiological state of the fruit at harvest, associated with its postharvest management. Among several postharvest applications, ozone treatment is considered as a cost-effective and eco-friendly food-processing technology to preserve the fruits' quality during cold storage. In this study, we investigated the influence of ozone, after gradual cooling treatment, on the antioxidant defense system in *Actinidia chinensis*, 'Soreli'.

RESULTS: Bioactive compound content decreased during cold storage, and ozone treatment enhanced the activities of superoxide dismutase and catalase during cold storage. This treatment preserved membrane integrity by inhibiting lipoxygenase activity and malondialdehyde accumulation. A multivariate statistical approach, using principal component analysis, provided the global response to the effect of ozone postharvest treatment during cold storage in kiwifruit 'Soreli'.

CONCLUSION: Ozone treatment improves the efficiency of antioxidative system and storability of 'Soreli' kiwifruits.

Keywords: yellow-fleshed kiwifruit; antioxidant system; gradual cooling; cold storage; oxidative stress; ozone treatment

INTRODUCTION

Kiwifruit is appreciated by consumers for its high bioactive compound content with health-beneficial effects. Nowadays, the kiwifruit market is in a transition phase from domination by only one species, *Actinidia deliciosa* 'Hayward', to increasing species diversity.[1] Yellow-fleshed kiwifruit (*Actinidia chinensis* 'Soreli') is a fruit crop that is valued for its good balance between sugar and acidity and its high ascorbic acid (AA) content. The postharvest industry has used several postharvest treatments to improve the quality and safety of kiwifruits during storage and to minimize losses of fruit.[2,3] The main aim of postharvest technology is to slow the ripening process and to delay the onset of senescence. Cold storage, at low temperatures, is widely used by the kiwifruit industry as a postharvest treatment to prolong the storage period. Several studies have demonstrated that different fruit cultivars showed a large disparity in the cold storage period.[4] Several yellow-flesh kiwifruits cultivars are more perishable than green-flesh kiwifruits during cold storage, as demonstrated by several studies.[5] Gradual cooling is a valid tool to adapt fruits to low temperature, increasing chilling tolerance.[6] Ozone is considered as a cost-effective and eco-friendly treatment that can be used at the postharvest stage to delay the ripening process and to preserve fruit quality during cold storage.[7] This sanitizing agent has been used to extend the shelf-lives of different fruits such as apples, grapes, peppers, berries, pears, and kiwifruits.[8–11] Ozone treatment can induce oxidative stress involving enzymatic and non-enzymatic antioxidant system depending on the steady-state reactive oxygen species (ROS) level. Reactive oxygen species and oxidative damage are responsible for fruit senescence, which affects fruit quality during postharvest life.[12] The role of ROS as a protective factor or a damaging factor depends on the equilibrium between ROS production and scavenging. Ozone might be considered as a postharvest elicitor, if applied at the right concentration and time, to increase the enzymatic and non-enzymatic antioxidant system in fruits and vegetables.[13] Several studies have demonstrated that ozone treatment increased the activity of the antioxidant enzymatic system that includes catalase (CAT), guaiacol peroxidase (GPX), superoxide dismutase (SOD), and ascorbate peroxidase (APX) in papaya, grape, and pear fruit.[14] Furthermore, Yang *et al.*[15] demonstrated that gradual cooling treatments improve chilling tolerance and alleviate chilling injuries by enhancing antioxidant enzyme activities, including those of SOD, CAT, GPX, and APX in 'Hongyang' kiwifruit.

The aim of this study was to investigate the effects of gradual cooling and ozone postharvest treatment on bioactive compound

* *Correspondence to: M Petriccione, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia agraria (CREA), Centro di ricerca Olivicoltura, Frutticoltura e Agrumicoltura, Via Torrino 3, I-81100 Caserta, Italy.*
E-mail: milena.petriccione@crea.gov.it

a *Department for Innovation in Biological, Agro-Food and Forest systems (DIBAF), Università degli Studi della Tuscia, Viterbo, Italy*

b *Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia agraria (CREA), Centro di ricerca Olivicoltura, Frutticoltura e Agrumicoltura, Caserta, Italy*

content, antioxidant enzymes, and membrane oxidative damage in 'Soreli' kiwifruit during cold storage.

MATERIALS AND METHODS

Fruit samples

'Soreli' kiwifruit were harvested at commercial ripening (8 °Brix and 12 N mm^{-1}) and placed in plastic boxes by 'AgricolliBio S.r.l' (Cisterna di Latina, Italy) (41° 30′ 33,84′′ N, 13° 4′ 33.17′′ E, 334 m above sea level), and were transported to the Postharvest Laboratory, Dibaf, Università degli Studi della Tuscia (Viterbo, Italy). In this laboratory, fruits were selected on the basis of uniform size and absence of disease, and were packed individually.

Treatments and storage conditions

Fruits were divided into two lots (approximately 150 fruits each lot). Both lots were subjected to gradual cooling treatment. Kiwifruit were stored for 24 h at room temperature and they were subsequently gradually cooled from 26 to 2 °C for 8 days (decreasing 3 °C from 26 to 23, 20, 17, 14, 11, 8, 5, and 2 °C after 1, 2, 3, 4, 5, 6, 7, and 8 days of storage, respectively). After gradual cooling, the first lot was maintained at 2 ± 0.05 °C with 85 ± 5% relative humidity (RH) (control). The second lot was treated with ozone gas in a continuous flow at 300 ppb, as previously indicated,[10] using a C32-AG ozone generator (Industrie De Nora Spa, Milan, Italy) and maintained at 2 ± 0.05 °C with 85 ± 5% RH. The gas was carried in the cold room and continuously estimated using a BMT 146 ultraviolet (UV) photometric ozone analyzer (Messtechnik Gmbh, Remscheid, Germany). All kiwifruit lots were stored for 60 days. Three biological replicates were realized per treatment and each contained 10 fruit. All analyses were performed at harvest, after gradual cooling, and every 15 days of cold storage.

Bioactive compounds content

Bioactive compounds, such as total phenolic and flavonoids, were extracted from kiwifruit flesh (2.5 g) in methanol solution (80% v/v). The extract was centrifuged (14 000×*g* for 5 min) and filtered through Whatman No. 41 paper and used for analysis.

Total phenolic (TP) and flavonoid (TF) content was determined by the Folin–Ciocalteu[16] and aluminum chloride colorimetric methods,[17] respectively. The TP content was expressed as milligrams of gallic acid equivalent (GAE) per 100 g^{-1} fresh weight (FW) and TF as milligrams of catechin equivalent (CE) per 100 g^{-1} fresh weight (FW).

The AA content from kiwifruit was determined following the method described by Goffi *et al.*[10] and expressed as mg AA per 100 g^{-1} (FW). All analyses were performed in triplicate.

Enzyme extraction and activity assays

Total soluble proteins were extracted by homogenizing 1.5 g of frozen fruit flesh powder in 3.5 mL of a buffer containing 0.1 mol L^{-1} potassium phosphate (pH 7), 1 mmol L^{-1} sodium-ethylenediaminetetraacetic acid (EDTA pH 7), polyethylene glycol (PEG), 6.25 mmol L^{-1}, 5% (w/v) polyvinylpolypyrrolidone (PVPP), and 5 mmol L^{-1} AA (only for APX enzyme extraction). The homogenate was centrifuged at 14 000×*g* for 20 min at 4 °C. Surnatant was used to determine total soluble protein content by the Bradford assay,[18] hydrogen peroxide content (H_2O_2), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and superoxide dismutase (SOD) activity.

Catalase and ascorbate peroxidase activity

Catalase (EC 1.11.1.6) activity was assayed according to the method described by Adiletta *et al.*,[19] with minor modifications. The reaction assay contained 100 mmol L^{-1} potassium phosphate buffer (pH 7), 20 mmol L^{-1} H_2O_2, and 100 µL of crude enzyme extract. The reaction was induced by adding H_2O_2, and a decrease in absorbance at 240 nm for 3 min was recorded. Catalase activity was measured in µmol of H_2O_2 per g (FW).

Ascorbate peroxidase (EC 1.11.1.11) activity was determined according to Petriccione *et al.*,[20] with some modifications. The assay mixture contained 100 mmol L^{-1} potassium phosphate buffer (pH 7), 0.33 mmol L^{-1} AA, 0.35 mmol L^{-1} H_2O_2, 0.66 mmol L^{-1} sodium EDTA (pH 7) and 100 µL of crude enzyme extract. The oxidation of AA was determined at 290 nm and APX activity was measured in µmol of ascorbate per g (FW).

Guaiacol peroxidase and SOD activity

Guaiacol peroxidase (EC 1.11.1.7) activity was determined as described by Modesti *et al.*[13] with slight modifications. The reaction assay contained 100 mmol L^{-1} potassium phosphate buffer pH 7, 0.15 mmol L^{-1} sodium-EDTA pH 7.0, 6.60 mmol L^{-1} H_2O_2, 8 mmol L^{-1} guaiacol and 100 µL of crude enzyme extract. Guaiacol peroxidase activity was detected spectrophotometrically at 470 nm, monitoring tetraguaiacol formation. Guaiacol peroxidase activity was expressed in µmol per g (FW).

Superoxide dismutase (EC 1.15.1.1) activity was determined as described by Adiletta *et al.*,[21] with minor modifications. The reaction assay contained 50 mmol L^{-1} potassium phosphate buffer pH 7.8, 0.1 mmol L^{-1} sodium EDTA, 13 mmol L^{-1} methionine, 75 µmol L^{-1} nitro blue tetrazolium chloride (NBT), 0.5 µmol L^{-1} riboflavin, and 25 µL of crude enzyme extract. The reaction was started by adding riboflavin. After 10 min of incubation at room temperature under continuous light, absorbance value was measured at 560 nm. One SOD unit was defined as the amount of enzyme that inhibits the rate of NBT reduction by 50% below the assay conditions. The SOD activity was expressed as U per g (FW).

Hydrogen peroxide content determination

Hydrogen peroxide content was evaluated using the method described by Alexieva *et al.*[22] The reaction mixture consisted of trichloroacetic acid 0.1%, 1 mol L^{-1} potassium iodide and 300 µL of crude enzyme extract. The H_2O_2 content was expressed as nmol per kg (FW).

Polyphenoloxidase activity

Polyphenoloxidase activity was determined by the method described by Adiletta *et al.*[23] Crude enzyme extract was prepared by homogenizing fruit flesh (1 g) in sodium phosphate buffer (100 mmol L^{-1}, pH 6.4) containing 0.05 g of PVPP. Polyphenoloxidase (PPO) activity was measured in a buffered substrate (500 mmol L^{-1} catechol in 100 mmol L^{-1} sodium phosphate buffer, pH 6.4) using crude enzyme extract (100 µL) and monitoring the increase in absorbance at 398 nm. Polyphenoloxidase activity was expressed as µmol per g (FW).

Lipoxygenase activity

Lipoxygenase activity was determined by the method described by Petriccione *et al.*[24] with some modifications. Frozen fruit tissue powder (1 g) was homogenized in 3 mL of extraction buffer including 50 mmol L^{-1} potassium phosphate buffer pH 7.8, 1 mmol L^{-1}

sodium-EDTA pH 7, 2% PVPP, and centrifuged at 12 500×*g* for 10 min at 4 °C. The reaction assay contained sodium phosphate buffer 100 mmol L^{-1}, pH 6, 5 mmol L^{-1} linoleic acid sodium salt and crude enzyme extract (50 µL). Lipoxygenase (LOX) activity was measured spectrophotometrically by recording the increase in absorbance at 234 nm. Lipoxygenase activity was expressed as nmol per g (FW).

Malondialdehyde content determination

Malondialdehyde (MDA) content was measured using the method described by Pasquariello *et al.*[25] with some modifications. Frozen fruit flesh powder (1 g) was re-suspended in 10 mL of 10% trichloroacetic acid (w/v), and 0.5% thiobarbituric acid dissolved in HCl 0.25 mol L^{-1}. The solution was kept in a boiling water bath at 95 °C for 30 min and immediately cooled. The homogenate was centrifuged at 12 000×*g* for 10 min and the absorbance of supernatant was read at 450, 532, and 600 nm. The MDA content was expressed as nmol per g (FW).

Statistical analysis

All results were expressed as the mean ± standard deviation. Statistical significance among untreated and treated kiwifruit was analyzed by two-way analysis of variance (ANOVA) and the Tukey test at 5% level was calculated to compare differences between means. Differences at $P < 0.05$ were considered significant and were indicated with different letters. Correlations among the evaluated parameters were analyzed using Pearson's correlations ($P < 0.05$ and $P < 0.01$). A principal component analysis (PCA) was applied to describe the influence of ozone treatment on different antioxidant systems and to identify the components responsible for the main variations in the dataset. All analyses were performed using the SPSS software package, version 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Effect of ozone postharvest treatment on non-enzymatic and enzymatic antioxidant system

Ozone is a strong and natural oxidant agent that has many potential applications in the food industry. Indeed, ozone-based treatments have been tested during storage and processing to extend shelf life and delay the microbial growth of bacteria and other microorganisms in several fruits.[8,26–28]

Kiwifruit is considered a functional fruit with a high content of non-enzymatic antioxidants (AA, polyphenol, flavonoids) that are able to counteract the high concentration of the ROS, alleviating the biochemical imbalances.[29] Nowadays, many natural compounds such as chitosan, jasmonates, and some volatile compounds like ozone, nitric oxide, and ethylene have been tested as postharvest treatments and some of them can alter the antioxidant system's status.[30,31] Oxidative damage to the membranes caused by the failure of the antioxidant system is considered an early signal of a fruit's senescence.[32,33]

The bioactive compound content decreased during cold storage and no significant differences were registered ($P < 0.05$) between ozone-treated and untreated samples (Table 1). Several studies have reported that AA is an unstable compound that can be degraded during kiwifruit storage due to the ripening and senescence processes caused by the loss of cell integrity.[34] This AA decrease could be due to scavenging by the free radicals formed during the decomposition of ozone or, due to the activation of ascorbate oxidase involved in the degradation of AA to dehydroascorbic acid.[35]

The polyphenol content, which includes several compounds such as phenolic acids, flavonoids, stilbenes, and lignans, is influenced by ozonation as demonstrated by different studies.[34] In our previous study no significant effect was observed of ozone treatment of the yellow 'Soreli' kiwifruit on polyphenol content during cold storage.[10] The degradation of polyphenols during ozonation can be due to several chemical reactions. Those reactions may be direct reactions of ozone with polyphenols due to hydroxyl radicals produced through ozone decomposition catalyzed mainly by the hydroxide ion (OH) or mainly catalyzed by polyphenol oxidase.[34]

Postharvest oxidative stress is involved in potential storability and susceptibility to development of physiological disorders during storage of several fruits.[10] Superoxide dismutase is the first enzyme involved in antioxidant defense during oxidative stress, that catalyzes the dismutation of radical superoxide (O_2^-) into either hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). Superoxide dismutase activity increased significantly during gradual cooling ($P < 0.05$) and in the first 15 days of cold storage, followed by a decline until the end of storage (Fig. 1(a)). Ozone-treated fruits showed significantly higher values than untreated samples (Fig. 1(a)).

Catalase is involved in the detoxification of H_2O_2, produced by SOD activity.[25] Catalase activity increased after gradual cooling compared to harvest, followed by a decrease during cold storage, with significant higher values in ozone-treated samples (Fig. 1(b)). Another scavenger of ROS is APX, an important key enzyme of AA metabolism. Gradual cooling and cold storage induced a decrease

Table 1. Antioxidant non-enzymatic content, ascorbic acid (AA-mg/100 g (FW)), polyphenol content (POL-mg GAE/100 g (FW)); flavonoid content (FLAV-mg CE/100 g (FW)) in ozone-treated (Ozone) and untreated (CTRL) 'Soreli' kiwifruit at harvest (T0-H), after gradual cooling (T0-GC), and after 15 days (T1), 30 days (T2), 45 days (T3), and 60 days (T4) of cold storage at 2 °C

Traits	Treatments	T0-H	T0-GC	T1	T2	T3	T4
AA	CTRL	159.27 ± 0.93 (hA)	140.74 ± 0.84 (gA)	132.51 ± 0.51 (fB)	114.33 ± 0.40 (eA)	109.37 ± 9.23 (cA)	102.82 ± 0.13 (aA)
	Ozone	159.27 ± 0.93 (hA)	140.74 ± 0.84 (gA)	106.98 ± 0.6 (bA)	112.30 ± 0.13 (dA)	110.43 ± 0.21 (cA)	114.73 ± 0.11 (eB)
POL	CTRL	133.92 ± 8.73 (dA)	101.35 ± 1.91 (cA)	80.63 ± 0.58 (abB)	74.91 ± 0.31 (aA)	81.93 ± 0.24 (abB)	79.39 ± 0.34 (abA)
	Ozone	133.92 ± 8.73 (dA)	101.35 ± 1.91 (cA)	75.97 ± 0.73 (aA)	80.76 ± 0.27 (abB)	80.34 ± 0.24 (abA)	85.68 ± 0.30 (bB)
FLAV	CTRL	5.38 ± 0.23 (eA)	4.13 ± 0.17 (cdA)	3.58 ± 0.10 (aA)	4.33 ± 0.11 (dB)	3.98 ± 0.02 (bcA)	4.11 ± 0.01 (cdB)
	Ozone	5.38 ± 0.23 (eA)	4.13 ± 0.17 (cdA)	3.83 ± 0.03 (abB)	3.62 ± 0.04 (aA)	4.26 ± 0.08 (cdB)	3.75 ± 0.04 (abA)

Capital letters reflect comparisons between different treatments. Small letters reflect comparisons between different times for the same treatment. Means followed by the same letter in the same column or line do not differ from each other according to the Tukey test ($P < 0.05$).

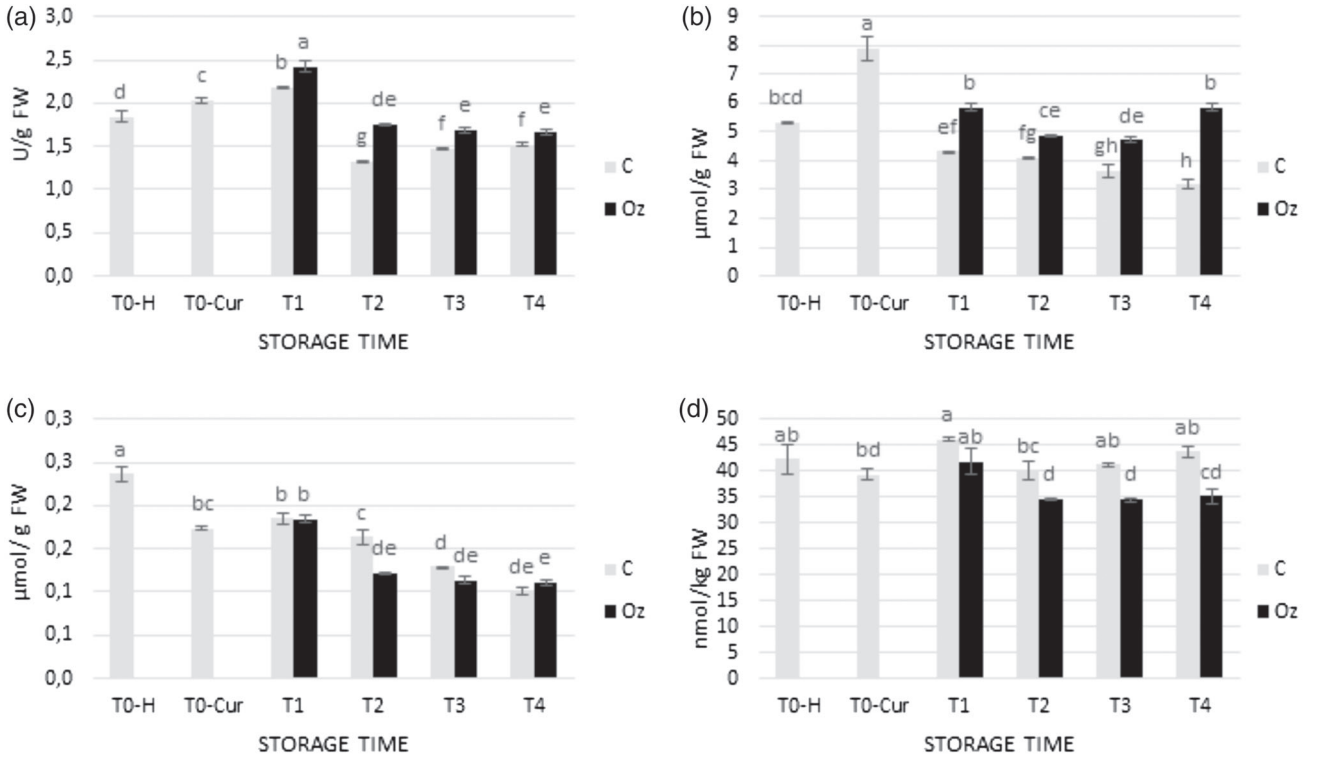

Figure 1. Superoxide dismutase (a), catalase (b), ascorbate peroxidase, (c) activity and H_2O_2 content (d) in untreated (C) and ozone-treated (Oz) kiwifruit at harvest (T0-H), after gradual cooling (T0-GC) and at 15 days (T1), 30 days (T2), 45 days (T3), and 60 days (T4) of cold storage at 2 °C. Error bars indicate standard deviations and values with different letters are significantly different ($P < 0.05$; Tukey test).

in APX activity in untreated and treated samples (Fig. 1(c)), which could be due to the reduction of AA content during storage (Table 1). The H_2O_2 content exhibited a constant trend during storage time. Ozone-treated samples showed a significantly lower H_2O_2 content compared to untreated samples ($P < 0.05$) (Fig. 1(d)). The increase in SOD and CAT activities could enhance both the ability of kiwifruit to convert O_2 into H_2O_2, and detoxification of H_2O_2 in ozone-treated fruits. Catalase and APX are two key enzymes involved in H_2O_2 detoxification with different kinetic features. Catalase shows a high turnover rate and low affinity for substrate while APX is activated at high H_2O_2 dose.[23] Catalase and APX activities showed a high activity during gradual cooling due to cold stress condition and furthermore both enzymes maintain their activity during cold storage to reduce H_2O_2 content. These results have been confirmed by Modesti *et al.*[13] in ozone-treated wine grapes.

Guaiacol peroxidase, using hydrogen peroxide as electron acceptor, catalyzes the oxidation of several antioxidant compounds.[12] Gradual cooling treatment induced a significant increase in GPX activity, whereas lower GPX activity in untreated kiwifruits, and at the end of storage, has been recorded (Fig. 2(a)).

A two-way ANOVA showed the effects of ozone treatment and the storage time and their interaction on the antioxidant system of 'Soreli' during cold storage. Catalase and SOD were affected ($P < 0.001$) by all of the factors considered (PT, D, and PT × D) (Table 2).

Gradual cooling is a process of adaptation to cold storage conditions used to maintain fruit quality and to alleviate chilling injury in kiwifruits industry and to delay *Botrytis cinerea* growth.[36] In 'Soreli' kiwifruit, gradual cooling induced an increase in antioxidant enzyme activity. Our results agree with previous studies that reported that SOD, CAT, APX, and POD activity in 'Hayward' kiwifruit was induced by low-temperature conditioning.[15]

Several studies have demonstrated that postharvest treatment in kiwifruit can improve the antioxidant enzyme system.[3,37] Xu and co-workers[38] demonstrated that lysozyme and 1-methylcyclopropene (1-MCP) treatments prolonged the shelf-life of 'Hayward' kiwifruit, and improved APX, SOD, and CAT activity with higher values in treated kiwifruit than untreated ones. Gradual postharvest cooling, from 15 to 0 °C in 'Hongyang' kiwifruit exhibited higher SOD, CAT, APX, and GPX activity than those treated by direct cooling during storage.[15] Furthermore, nitric oxide postharvest treatment reduced POD activity but increased the CAT and SOD activity in 'Xuxiang' kiwifruit during storage at 4 °C for 80 days.[38] Although many studies have been conducted on ozone postharvest treatments in different fruit species, none of these reported the effects on antioxidant enzymes in kiwifruit.[39,40]

Ozone treatment induced an increase in SOD, CAT, and APX activity in papaya and pear fruit.[4,41,42] Modesti *et al.*[13] highlighted that the postharvest treatments by methyl jasmonates and ozone in wine grape induced an initial increase in SOD, APX, CAT, and GPX. Ozone at low concentrations can activate the antioxidant system, reduce intracellular ROS content, and protect membrane lipid peroxidation.[13,43] Ozone treatment (4 ppm) inhibited the decrease in POD and CAT activity, and reduced membrane oxidative damage in strawberry during 20 days of cold storage.[44]

Effect of ozone postharvest treatment on enzymatic browning and membrane damage

Browning in kiwifruit flesh is due to enzymatic oxidation. Polyphenoloxidase catalyzes the oxidation of phenols to quinones, which

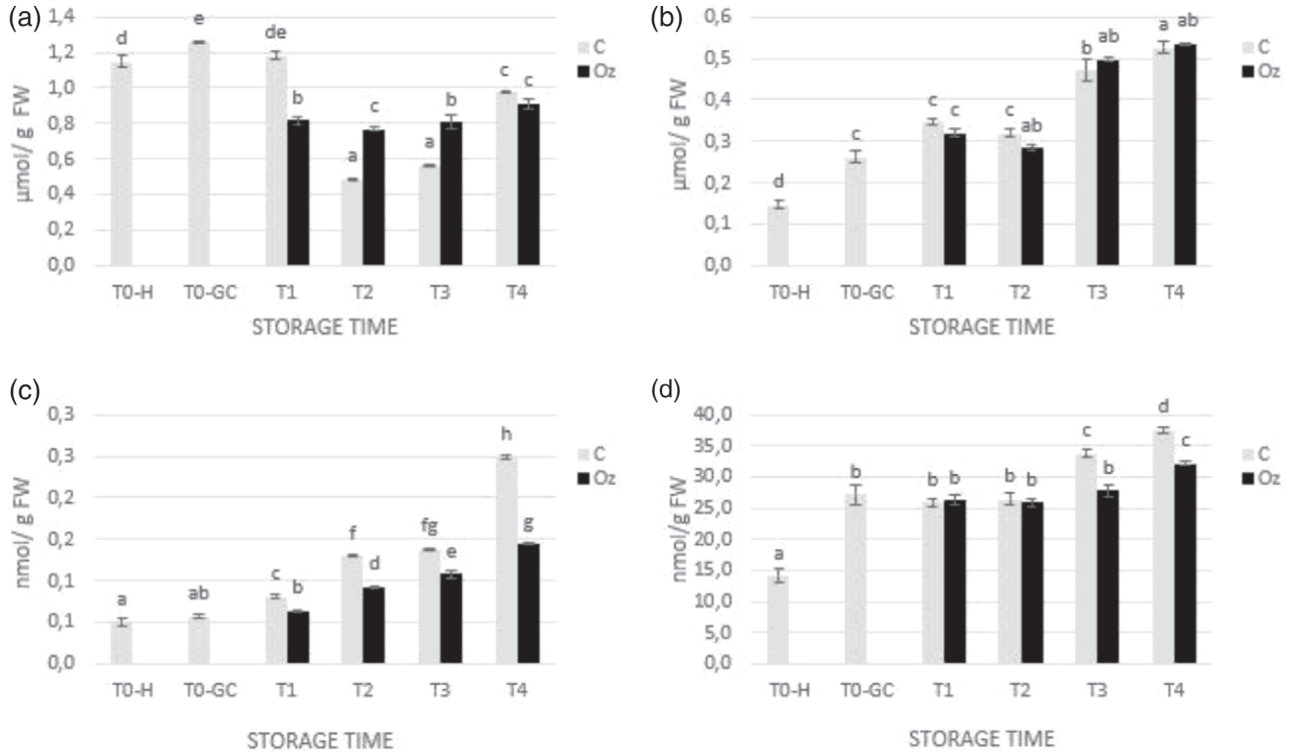

Figure 2. Guaiacol peroxidase (a), polyphenol oxidase (b), lipoxygenase activities (c) and malondialdehyde content (d) in untreated (C) and ozone-treated (Oz) kiwifruits at harvest (To-H), after gradual cooling (T0-GC), and at 15 days (T1), 30 days (T2), 45 days (T3), and 60 days (T4) of cold storage at 2 °C. Error bars indicate standard deviations and values with different letters are significantly different ($P < 0.05$; Tukey test).

Table 2. The *P* values obtained in the two-way ANOVA for each postharvest treatment (PT) during cold storage at 2 °C (D-days) and their interaction (PT × D) in kiwifruit cv 'Soreli' (superoxide dismutase activities (SOD), catalase (CAT), ascorbate peroxidase (APX), H_2O_2 content (H_2O_2), guaiacol peroxidase (GPX), polyphenoloxidase (PPO), lipoxygenase activities (LOX), malondialdehyde content (MDA), ascorbic acid (AA), polyphenol content (POL), and flavonoid content (FLA)

	P-value		
Parameters	Postharvest treatment	Days	PT × D
CAT	<0.001	<0.001	<0.001
APX	0.002	<0.001	0.003
SOD	<0.001	<0.001	<0.001
H_2O_2	<0.001	0.002	0.062
GPX	0.232	<0.001	<0.001
PPO	0.532	<0.001	0.183
LOX	<0.001	<0.001	<0.001
MDA	0.003	<0.001	0.005
POL	0.658	<0.001	0.728
FLA	0.001	<0.001	<0.001
AA	0.409	<0.001	0.040

are responsible for undesirable brown pigments.[20] The PPO activity showed a significant increase after gradual cooling ($P < 0.05$) with no significant differences between ozone-treated and untreated kiwifruit during cold storage (Fig. 2(b)). Polyphenoloxidase uses polyphenols as substrates in the browning reactions and this causes a decrease in polyphenol content during storage (Table 1).

Malondialdehyde content and LOX activity were analyzed to evaluate the oxidative membrane damage after gradual cooling and during cold storage.[38] The MDA content showed a significant increase after gradual cooling; moreover, untreated kiwifruit exhibited higher values after 45 and 60 days of cold storage ($P < 0.05$) (Fig. 2(d)). The LOX activity showed an increase during cold storage with significantly higher values ($P < 0.05$) in untreated samples. No significant differences were highlighted during gradual cooling in kiwifruit. Inhibition in LOX activity was confirmed by low MDA content in ozone-treated samples as demonstrated in other studies.[13] To evaluate the effect of storage time and ozone treatment on enzymatic browning and membrane damage, a two-way ANOVA analysis was performed (Table 2).

Several studies have demonstrated that postharvest treatments such as methyl jasmonates and ozone induced a significant decrease in PPO and LOX activity in wine grape,[13] and nitric oxide reduced MDA and ROS content and LOX activity in kiwifruit.[39]

PCA and Pearson correlation after ozone treatment

Principal component analysis has been used to determine the principal components (PCs) that explained the overall variance in all the analyzed traits (47.1% and 19.0% for PC1 and PC2, respectively) (Fig. 3). ($r^2 = 0.731$), GPX ($r^2 = 0.579$), SOD ($r^2 = 0.715$), and APX ($r^2 = 0.782$) activity were positively correlated with PC1; whereas MDA ($r^2 = -0.829$), LOX ($r^2 = -0.912$), and PPO ($r^2 = -0.840$) were correlated negatively with the first principal component. The H_2O_2 content ($r^2 = 0.916$) was positively correlated with PC2.

The multivariate space of the first two PCs showed that after gradual cooling the average PC scores in kiwifruit shifted from higher values to lower values along positive PC1. After 15 days of cold storage, ozone-treated and untreated fruits showed

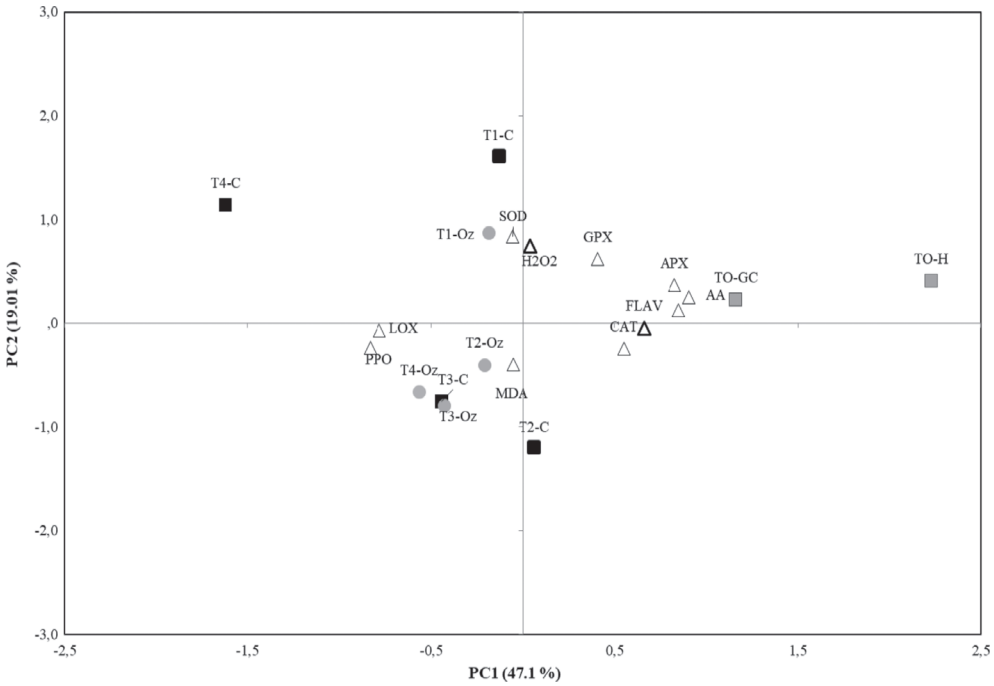

Figure 3. Two-dimensional principal component analysis plot of antioxidant system in untreated (C) and ozone-treated (Oz) 'Soreli' kiwifruit at harvest (T0-H), after gradual cooling (T0-GC), and at 15 days (T1), 30 days (T2), 45 days (T3), and 60 days (T4) of cold storage at 2 °C (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and H_2O_2 content (H_2O_2), guaiacol peroxidase (GPX), polyphenoloxidase (PPO), lipoxygenase activities (LOX), malondialdehyde content (MDA), ascorbic acid (AA), polyphenol content (POL) and flavonoid content (FLA)).

a shift from positive values to negative ones along PC1, with higher scores along PC2. As cold storage progressed, all samples displayed a shift from positive values to negative ones along PC2. After 60 days of cold storage only ozone-treated kiwifruit showed a shift along negative values of PC1 and positive ones of PC2.

The non-enzymatic system (POL, FLAV, and AA) was positively correlated with the samples at harvest (T0-H) and after gradual cooling (T0-GC), whereas the antioxidant enzymes (APX, SOD, GPX, and CAT) were positively correlated with the samples at harvest (T0-H) and after gradual cooling (T0-GC), with the untreated and ozone-treated samples after 15 days of storage (T1-C, T1-Oz), and with ozone-treated sample after 30 days of storage (T2-Oz). Membrane oxidative damage indicators (MDA and LOX) and the main enzyme involving in fruit flesh browning (PPO) were correlated with the sample at the end of storage and significantly with untreated samples after 30 (T2-C), 45 (T3-C) and 60 (T4-C) days of storage, respectively.

The 2-D plot showed that gaseous ozone treatment delayed oxidative stress compared with untreated samples. Cold storage determined the decrease in the bioactive compound content and ozone induced an increase in the antioxidant enzyme activities, which led to a senescence process during long-term storage. Ozone postharvest treatment was effective to preserve the kiwifruit quality during cold storage.[45] Principal component analysis can be a valid tool to evaluate the effect of postharvest treatment on 'Soreli' kiwifruits as demonstrated by other studies in other fruit crops.[11,46,47]

The Pearson matrix underlines the intercorrelations among the antioxidant enzymes. Malondialdehyde is positively correlated with LOX ($r = 0.784$; $P \leq 0.01$) and PPO ($r = 0.748$; $P \leq 0.01$) (Fig. 4). This suggests that, during kiwifruit storage, the lipid peroxidation due to LOX activity leads to oxidative membrane damage, with an

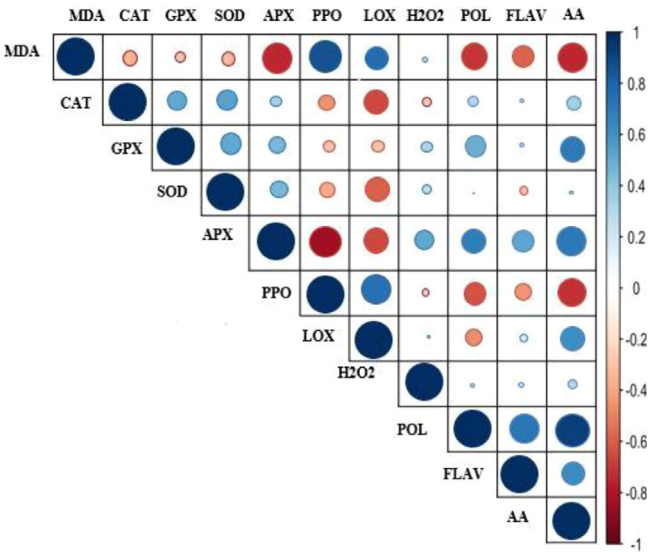

Figure 4. Pearson correlation matrix between superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and H_2O_2 content (H_2O_2), guaiacol peroxidase (GPX), polyphenoloxidase (PPO), lipoxygenase activities (LOX), malondialdehyde content (MDA), ascorbic acid (AA), polyphenol content (POL), and flavonoid content (FLA). The correlation coefficients are proportional to the circular size and color intensity. Positive correlation is displayed in blue and negative in red.

increase in MDA content. Furthermore, the loss in cellular compartmentalization induced an increase in PPO activity and a decrease in polyphenol content, with a negative correlation among them ($r = -0.604$; $P \leq 0.01$). Other studies have demonstrated that lipid peroxidation leads to membrane deterioration and is highly correlated with LOX activity during fruit storage.[33]

Superoxide dismutase (SOD) activity showed a significant positive correlation with APX ($r = 0.458$; $P \leq 0.05$) and CAT activity ($r = -0.545$ $P \leq 0.01$). In kiwifruit the combined action of H_2O_2-generating SOD and H_2O_2-metabolizing APX or CAT is responsible for oxidant resistance during storage. Furthermore, SOD, APX, and CAT are negatively correlated with MDA content and LOX activity, suggesting that oxidative stress is partially responsible for membrane damage during kiwifruit storage. Ascorbate peroxidase was positively correlated to AA content ($r = 0.783$; $P \leq 0.01$) being its reducing substrate of reaction.

Promising, eco-friendly strategies that allow the antioxidant system to improve to maintain cellular compartmentalization and preservation of nutritional components and antioxidants, are valid postharvest tools to improve the storability of fruits.[9,48,49]

CONCLUSION

Ozone postharvest treatments are of great commercial interest due to their flexibility. Our results suggest that gradual cooling and ozone treatment combined in cold storage enhanced the levels of antioxidant enzymes, such as APX, SOD, and CAT, extending the postharvest life of 'Soreli' kiwifruit. Indeed, untreated fruits displayed higher MDA content and LOX activity than ozone-treated kiwifruits. Ozone treatment induced rapid detoxification by reducing oxidative stress.

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