International Journal of Horticultural Science and Technology
Journal homepage: https://ijhst.ut.ac.ir

Assessment of Flavonoid Biosynthesis and Activity of Antioxidant Enzymes in Two Sweet Orange Cultivars under Foliar Treatments and Water Stress

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ARTICLE INFO

Article history:

Received: 9 March 2025,
Received in revised form: 25 May 2025,
Accepted: 26 May 2025,

Article type:

Research paper

Keywords:

Antioxidant activity,
Citrus fruit quality,
Drought stress,
Post-harvest storage,
Pre-harvest treatments

ABSTRACT

The post-harvest deterioration of orange fruit quality, including weight loss and changes in biochemical attributes, which is exacerbated by drought stress and can be influenced by pre-harvest treatments and storage conditions. This two-year study (2021-2022) analyzed the contribution of pre-harvest foliar applications of chitosan (500 ppm) and melatonin (100 µM), individually and in combination, on the post-harvest quality of ten-year-old 'Valencia' and 'Thomson Navel' orange trees under well-watered and water-stressed (40% FC) conditions. The experiment, conducted at a commercial citrus orchard in Sari, Iran, employed a factorial experiment based on RCBD design with irrigation levels and foliar treatments as pre-harvest factors. During the post-harvest stage, fruits were stored for 0, 30, 60, and 90 d under either traditional or modified atmosphere packaging (MAP: 10% CO_2, 5% O_2, 85% N_2) at 5 °C. Results indicated a significant increase in weight loss of fruit, reaching a minimum of 118.1 g for 'Valencia' and 175 g for 'Thomson Navel' after 90 d. Additionally, total soluble solids increased during storage, while MAP effectively mitigated weight loss. Drought stress further decreased fruit weight and TSS. However, chitosan and melatonin, particularly when used together, reduced weight loss. The combined treatment resulted in the smallest weight reduction (3.08%) in 'Valencia' oranges after 30 d. Antioxidant enzyme activities (DPPH, APX, SOD, POX, PPO, and PAL) generally increased with storage, but MAP decreased DPPH, SOD, and POX activities, while increasing APX and PAL. The highest DPPH activity (52.64%) was observed after chitosan application. Drought stress and chitosan/melatonin increased antioxidant enzyme activity. During storage, total phenolic content increased. MAP application reduced this increase, while chitosan/melatonin application promoted it. The highest phenolic content was recorded in 'Valencia' (0.93 mg g^{-1}) treated with chitosan under drought stress and conventional packaging after 60 d. Total flavonoid content varied across treatments and storage durations, indicating complex interactions. For example, the highest flavonoid content in 'Valencia' (948.6 µg g^{-1} FW) was observed under drought stress, MAP, and combined chitosan/melatonin application after 60 d. Correlation analysis revealed negative correlations between some antioxidant enzymes/flavonoids and fruit weight/quality traits and positive correlations between phenolics/flavonoids and certain antioxidant enzymes. These findings highlight the complex interactions among pre-harvest treatments, storage conditions, and their combined effects on orange fruit quality and antioxidant capacity.

Introduction

Citrus fruits are among the most economically important horticultural crops worldwide. In Iran because of widespread cultivation, citrus is exposed t to various environmental restricts, including drought and cold stresses. Given the economic importance of citrus production, understanding the mechanisms underlying plant responses to water stress and identifying strategies to enhance their tolerance are critical research priorities (Ahluwalia et al., 2021; Sadeghi and Jabbarzadeh, 2025). Water stress is a major constraint on agricultural productivity in arid and semi-arid regions and has been the focus of extensive research. Under water-deficit conditions, plants undergo physiological and biochemical modifications, including alterations in flavonoid composition and antioxidant enzyme activity, to adapt and mitigate stress effects (Roussos et al., 2019).

Several studies have reported that water stress leads to a decline in chlorophyll content and photosynthetic activity in citrus. However, the extent of these reductions varies depending on rootstock and scion type, as well as the severity of drought stress, with different citrus species and rootstocks exhibiting distinct drought tolerance behaviors (Zaher-Ara et al., 2016; Amiri et al., 2024). Reactive oxygen species (ROS) are naturally produced during aerobic metabolism; however, environmental stressors can disrupt metabolic homeostasis, resulting in excessive ROS accumulation (Garcia-Caparros et al., 2021). Abiotic stresses, such as reduced CO_2 availability due to stomatal closure, exacerbate ROS accumulation. While ROS, including hydrogen peroxide (H_2O_2), function as essential signaling molecules (Ahmad et al., 2025), excessive levels can cause cellular damage and impair photosynthesis (Hasanuzzaman et al., 2020). To counteract oxidative stress, plants activate antioxidant defense mechanisms, which include enzymatic ROS scavengers such as superoxide dismutase (SOD), peroxidase (POX), and ascorbate peroxidase (APX) (Garcia-Caparros et al., 2021). Among these, SOD serves as a primary defense against superoxide radicals, converting O_2^- into H_2O_2, which is subsequently detoxified by APX and catalase (CAT) (Dumanović et al., 2021; Singh et al., 2023). APX plays a crucial role in maintaining cellular redox balance by utilizing ascorbic acid (AsA) as an electron donor to reduce H_2O_2, a process dependent on the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) (Garcia-Caparros et al., 2021). Multiple studies have demonstrated that drought stress enhances antioxidant enzyme activity in citrus species (Gonçalves et al., 2016; Hussain et al., 2018).

Chitosan and melatonin have been identified as promising compounds for improving plant tolerance to environmental stressors, including water stress, through diverse mechanisms. Chitosan enhances plant defense by activating immune responses and promoting the biosynthesis of flavonoids and antioxidant enzymes. Melatonin, a potent antioxidant, mitigates oxidative damage by scavenging free radicals and improving the antioxidant capacity of plants. Ahmad et al. (2020) highlighted melatonin's role in mitochondrial electron transport and the regulation of antioxidant enzyme activity. Jafari and Shahsavar (2021) reported that under severe drought conditions, citrus species treated with melatonin exhibited significantly higher levels of total flavonoids and phenolics compared to untreated plants. Furthermore, their analysis confirmed hesperidin as the dominant polyphenol in both citrus cultivars.

Chitosan, a naturally derived biopolymer obtained through the deacetylation of chitin from crustacean shells, has demonstrated beneficial effects on citrus fruit quality. Tadayon et al. (2023) reported that foliar application of chitosan improved fruit quality characteristics in ‘Valencia’ orange trees. Similarly, Ahmed et al. (2016) found that pre-harvest application of chitosan enhanced growth parameters, physiological attributes, and biochemical properties in ‘Washington Navel’ orange trees. These researchers further observed improvements in pomological traits, including increased fruit weight, firmness, and total soluble solids (TSS), following chitosan application.

Given the significance of citrus in the agricultural economy and the detrimental impact of water stress on production, further research is essential to enhance citrus tolerance to environmental stressors. Investigating the effects of foliar application of chitosan and melatonin on flavonoid composition and antioxidant enzyme activity across different citrus cultivars may provide effective strategies to enhance productivity and improve fruit quality. Proper storage and packaging play a crucial role in preserving the quality and extending the shelf life of citrus fruits (Naserzadeh and Mahmoudi, 2025). The application of Modified Atmosphere Packaging (MAP) alongside traditional storage methods has been shown to reduce post-harvest losses while maintaining the nutritional value of stored produce (Roppolo et al., 2025). MAP is particularly effective in prolonging the quality of agricultural products by creating a controlled gaseous environment distinct from ambient air, often in combination with polyethylene films that exhibit superior gas barrier properties (Wang et al., 2025). This approach minimizes enzymatic degradation, reduces respiration rates by limiting oxidative reactions, and ultimately extends the shelf life of fresh produce (Paulauskienė et al., 2020; Tinebra et al., 2021).

When used in conjunction with cold storage, MAP further decreases respiration rates in horticultural products by restricting gas and moisture exchange through packaging (Naserzadeh and Mahmoudi, 2025).

Numerous studies have demonstrated the effectiveness of MAP technology in preserving fruit quality over extended periods. For instance, MAP has been successfully applied to maintain the post-harvest quality of berries (Tinebra et al., 2021), jujube (Moradinezhad and Dorostkar, 2021), and 'Kinnow' tangerines (Baswal et al., 2020). In this study, the effects of foliar application of chitosan and melatonin on flavonoid composition and antioxidant enzyme activity in two economically important citrus cultivars ('Valencia' and 'Thompson Navel') were examined under water stress conditions and across different storage durations. The study employed both MAP and traditional storage methods to assess post-harvest quality retention. The primary objective was to elucidate the mechanisms by which chitosan and melatonin enhance plant resilience to water stress, ultimately improving fruit quality, tolerance to environmental stressors, and shelf life during storage.

Materials and Methods

Experimental design and plant materials

This study examined effects of pre-harvest foliar applications of chitosan and melatonin on post-harvest fruit quality of two sweet orange cultivars grown under water stress conditions. The research was conducted during the 2021 and 2022 growing seasons in Dasht-e-Naz Company, Sari, Iran (36°33′50″ N, 53°00′25″ E). The laboratory analyses were conducted at the Citrus and Subtropical Fruits Research Institute in Ramsar and the Islamic Azad University, Science and Research Branch, Tehran. A factorial experimental design was employed, incorporating two irrigation levels (90 and 40% field capacity [FC]) and four pre-harvest foliar treatments: control (water spray), chitosan (500 ppm in 0.5% acetic acid), melatonin (100 µM), and a combined chitosan-melatonin application. Post-harvest, fruit storage treatments included four durations (0, 30, 60, and 90 d) and two storage conditions (traditional packaging (5 °C and 85%) and Modified Atmosphere Packaging [MAP]).

The study utilized ten-year-old 'Thompson Navel' orange trees grafted onto 'Citrange Carrizo' rootstock and 'Valencia' orange trees grafted onto citrumelo rootstock, both maintained under a drip irrigation system. Foliar treatments were applied three times a week for 60 d, starting with the onset of drought stress. A manual pump was used to deliver 30 mL of solution per tree. The irrigation protocols were based on the methodology of Jafari and Shahsavar (2021). The weight method was used to manage stress treatments and calculate water amounts as a percentage of FC. Dry soil was prepared by oven-drying 4 kg of soil at 103 °C for 48 h. Water stored at FC was determined by subtracting the dry soil weight. The chitosan foliar application (500 ppm in 0.5% acetic acid) followed the procedure outlined by Ahmad et al. (2016), while the melatonin application (100 µM) was conducted as described by Jafari and Shahsavar (2021). To enhance solution spreading and penetration, Tween-20 (0.1%) was added to all foliar sprays. Chitosan and melatonin were sourced from Sigma-Aldrich.

Pest and disease management strategies, along with overall tree maintenance practices during the growth period, were implemented according to the protocols established by Dasht-e-Naz Company and under the supervision of the management team. Fruits were harvested at commercial maturity (based on the ratio of TSS to titratable acidity, of 6.5) and handled in accordance with standard hygiene protocols before being immediately transferred to cold storage. Sweet orange fruits designated for MAP treatment were packaged in polyethylene bags using a Multivac A300 packaging machine, with the desired gas composition established and maintained via a WITT KM100-3M gas-mixing controller (Witten, Germany). Two atmospheric conditions were used: ambient air (control) and a modified gas mixture consisting of 10% CO_2, 5% O_2, and 85% N_2. All packaged fruits were stored at 5 °C and 85% relative humidity (RH). Fruit quality assessments were conducted at 0, 30, 60, and 90 d of storage.

Assessment of fruit weight loss

Fruit weight loss, used as an indicator of moisture loss (Lin and Zhao, 2007), was assessed by measuring the weight of five randomly selected fruits from each treatment. Initial weights were recorded before storage, with subsequent measurements taken at 30, 60, and 90 d to evaluate weight reduction over time.

Biochemical analyses

Biochemical analyses were performed on two randomly selected fruits per replicate from each cultivar, following established protocols. Whole fruit samples, including both flesh and peel, were utilized for these measurements. TSS content was determined using a refractometer (HRH30, Kruss Company, Germany), as described by Arzani et al. (2008).

Antioxidant enzyme activity assays

To assess antioxidant enzyme activity, ground tissue (0.5) was homogenized in 1 mL of liquid nitrogen and potassium phosphate buffer, followed by centrifugation at 14,000 rpm for 15 min at 4 °C. The resulting supernatant was collected for further

analysis (Hammerschmidt et al., 1982). DPPH radical scavenging activity was measured by a spectrophotometer via adding 0.1 mL of extract to 3.9 mL of the DPPH solution, with absorbance values recorded at 515 nm (Park et al., 2011). APX activity was determined according to Nakano and Asada (1981) using a reaction mixture containing enzyme extract, potassium phosphate buffer, ascorbic acid, and hydrogen peroxide. Absorbance was monitored at 290 nm over a 2 min interval.

Superoxide dismutase (SOD) activity was measured by a spectrophotometer following the method of Giannopolitis and Ries (1977). The reaction solution contained EDTA, phosphate buffer, methionine, nitro blue tetrazolium (NBT), riboflavin, and enzyme extract. After exposure to light followed by dark incubation, absorbance was measured at 560 nm. Peroxidase (POX) activity was assessed at 470 nm over a 3 min interval using guaiacol, hydrogen peroxide, and enzyme extract (Nakano and Asada, 1981). Polyphenol oxidase (PPO) activity was determined based on catechol oxidation, following the method of Pizzocaro et al. (1993). Clarified supernatant was combined with phosphate buffer, and enzyme extract was added to a buffer solution containing sodium phosphate and catechol. Absorbance changes were monitored at 420 nm over a 3 min period. Phenylalanine ammonia-lyase (PAL) activity was evaluated by measuring cinnamic acid production, following Wang et al. (2006). A reaction mixture containing L-phenylalanine, distilled water, and enzyme extract was incubated before termination with HCl. After extraction, evaporation, and dissolution in NaOH, cinnamic acid concentration was measured at 290 nm.

Total phenolic content determination

Total phenolic content was measured using the Folin-Ciocalteu method, according to the procedure outlined by Asami et al. (2003). Briefly, a 0.5 mL aliquot of the extract was mixed with 2.5 mL of Folin-Ciocalteu reagent, and diluted with distilled water (1:10). After a 2 min interval, 2 mL of a 7.5% w/v Na_2CO_3 solution was added, and the mixture was incubated in a water bath at 50 °C for 5 min. The absorbance was measured at 760 nm. Total phenolic content was expressed as mg gallic acid equivalents per 100 g fresh weight (FW).

Flavonoid analysis

Flavonoid compounds, specifically naringin, hesperidin, neohesperidin, catechin, and quercetin, were analyzed via High-Performance Liquid Chromatography (HPLC) (Eibang, 2007). Accordingly, the aliquot (20 µL) of the flavonoid extract became concentrated using a rotary evaporator. Then, it was filtered through a 0.2 µm mesh before injection into the HPLC column. The flavonoid compounds were identified and quantified by comparing their retention times and peak intensities with those of known standards via detection at 280 nm.

Data analysis

Statistical analyses were conducted on data that were confirmed to follow a normal distribution. All statistical procedures were performed using SAS software (version 9.2). Mean comparisons were carried out using the least significant difference (LSD) test. Pearson's correlation coefficients were calculated to assess relationships between traits. Correlation results were visualized. Positive correlations appeared in blue and negative correlations in red, with color intensity indicating correlation level. Non-significant correlations were excluded from the analysis. This assessment was performed using Minitab version 18 and Microsoft Excel 2018.

Results

Fruit weight loss

Analysis of fruit weight revealed a significant decrease during storage in both the 'Valencia' and 'Thompson Navel' cultivars. The highest recorded fruit weight loss, observed after 90 d of storage, were 118.1 g for 'Valencia' and 175 g for 'Thompson Navel' (Table 1). MAP application resulted in a lower weight loss in the average fruit weight compared to conventional storage. Conversely, pre-harvest drought stress decreased the average fruit weight of both cultivars, with a greater reduction observed in 'Valencia'. Pre-harvest application of chitosan and melatonin inhibition of loss of weight in both varieties compared to the control (water-sprayed) treatment. The lowest weight loss in fruit was associated with the combined application of chitosan and melatonin (Table 1). Table 2 presents the extent of fruit weight reduction among the various treatments. In 'Valencia', the smallest weight reduction (3.08%) was observed with combined chitosan and melatonin treatment under normal conditions and with MAP after 30 d of storage. In 'Thompson Navel', melatonin treatment at comparable levels resulted in the lowest weight reduction (4.16%). The largest weight reduction in 'Valencia' (65.43%) occurred with melatonin spraying under drought stress and with conventional packaging after 90 d of storage. In 'Thompson Navel', the largest weight reduction (50.59%) was achieved with melatonin application under well-watered conditions, conventional packaging, and with 90 d of storage.

Total soluble solids

TSS content decreased significantly throughout the 90 d storage compared to fresh fruit in both cultivars.

MAP resulted in higher average TSS values compared to conventional packaging. Pre-harvest drought stress, however, reduced the average TSS values compared to non-stressed conditions. The combined application of chitosan and melatonin performed best in increasing the average biochemical property values, including the TSS (Table 1).

Table 1. Impact of water stress and foliar applications of chitosan and melatonin on fruit weight and TSS in 'Valencia' and 'Thompson Navel' sweet oranges under various storage conditions.

	Fruit weight (g)		Total soluble solids (TSS) (%)	
	'Valencia'	**'Thompson Navel'**	**'Valencia'**	**'Thompson Navel'**
Storage time (d)				
0	224.4±30.4	314.6±36.9	11.98±2.31	13.94±1.95
30	178.2±32.5	263.5±38	10.02±1.83	12.47±1.7
60	131.2±23.9	190.1±30.4	9.99±1.75	12.06±1.84
90	118.1±28	175±35.3	9.46±1.88	12.01±1.88
Storage conditions				
Traditional	159.2±51.2	236.3±66.2	9.81±2.33	12.4±2.04
MAP	166.8±50.5	235.3±67.2	10.92±1.83	12.84±1.93
Water stress				
CK	166.1±50	237.7±64.7	10.38±2.16	12.81±2.05
Stress	159.8±51.7	233.9±68.7	10.35±2.18	12.42±1.92
Foliar nutrition				
Ctrl	147.8±40.8	211.6±46.6	8.93±1.73	11.02±1.36
CH	153.6±42.5	230.1±64.2	10.07±1.51	12.55±1.57
MEL	175.2±55.8	250.5±69.2	10.98±2.43	13.25±2.06
CH × MEL	175.4±57.3	251.1±76.4	11.47±2.02	13.65±1.85
LSD = 0.05	7.92	7.90	0.48	0.37
Interaction effects				
ST × SC	**	ns	**	ns
ST × WS	**	**	**	**
ST × FN	**	**	**	**
SC × WS	ns	*	**	ns
SC × FN	ns	**	ns	**
WS × FN	**	**	ns	ns
ST×SC×WS	*	*	ns	ns
ST×SC×FN	ns	**	ns	**
ST×WS×FN	ns	**	**	**
SC×WS×FN	*	**	*	**
ST×SC×WS×FN	ns	**	ns	**

ns, *, and **: non-significant and significant at the 5% and 1% levels, respectively. Modified atmosphere packaging (MAP), Normal irrigation (CK), Water foliar application (Ctrl), Chitosan (CH), Melatonin (MEL), Storage time (ST), Storage conditions (SC), Water stress (WS), Foliar nutrition (FN).

Antioxidant enzyme activity

The analysis of antioxidant enzyme activity revealed that extending the storage period increased the activity of DPPH and antioxidant enzymes (APX, SOD, POX, PPO, and PAL) in both cultivars. MAP decreased DPPH antioxidant capacity and the activities of SOD and POX compared to conventional packaging, while increasing the activity of APX and PAL (Table 3). Pre-harvest drought stress increased the antioxidant enzyme activity (Table 3).

Foliar application of chitosan and melatonin also increased antioxidant enzyme activity in both cultivars compared with the water spray control. The highest DPPH activity (52.64% and 51.69%, respectively) and APX activity (21.1 and 15.1 U mg

protein^{-1} min^{-1}) were observed with chitosan alone and in combination with melatonin, respectively, in both the 'Valencia' and 'Thompson Navel'. Under drought stress and conventional packaging, the highest SOD activity was detected after 60 d of storage by applying the chitosan spray solution in both 'Valencia' (31.5 U mg protein^{-1}) and 'Thompson Navel' (24.2 U mg protein^{-1}) samples. The lowest SOD levels of activity (5.8 and 4.3 U mg protein^{-1}, respectively) were observed with the chitosan spray solution under non-stressful conditions in MAP after 90 d of storage (Table 4).

Table 2. Extent of weight loss in two cultivars of sweet oranges, 'Valencia' and 'Thompson Navel', under different storage treatments, water stress, and foliar spray treatments compared to the control.

ST	SC	WS	FS	'Valencia'	'Thompson Navel'
ST2	SC1	WS1	FS1	-39.75	-12.68
			FS2	-22.82	-28.88
			FS3	-25.91	-12.04
			FS4	-27.45	-21.26
		WS2	FS1	-18.14	-25.73
			FS2	-29.88	-13.11
			FS3	-23.55	-15.31
			FS4	-21.27	-10.64
	SC2	WS1	FS1	-5.07	-7.62
			FS2	-7.95	-11.45
			FS3	-12.8	-4.16
			FS4	-3.08	-13.65
		WS2	FS1	-25.64	-22.01
			FS2	-13.98	-15.95
			FS3	-22.5	-17.67
			FS4	-25.16	-22.95
ST3	SC1	WS1	FS1	-57.82	-14.98
			FS2	-34.26	-44.45
			FS3	-43.16	-29.22
			FS4	-54.79	-49.46
		WS2	FS1	-11.24	-50.29
			FS2	-40.92	-27.64
			FS3	-37.29	-37.22
			FS4	-45.04	-49.36
	SC2	WS1	FS1	-33.53	-32.8
			FS2	-40.25	-35.7
			FS3	-42.99	-23.58
			FS4	-51	-56.8
		WS2	FS1	-34.15	-32.44
			FS2	-37.12	-45.57
			FS3	-29.57	-42.31
			FS4	-56.7	-49
ST4	SC1	WS1	FS1	-40.76	-37.45
			FS2	-34.05	-57.13
			FS3	-57.75	-60.59
			FS4	-45.04	-39.94
		WS2	FS1	-38.49	-32.38
			FS2	-57.06	-27.58
			FS3	-65.43	-52.43
			FS4	-40.78	-35.19
	SC2	WS1	FS1	-35.03	-37.43
			FS2	-41.63	-57.05
			FS3	-38.72	-46.44
			FS4	-47.71	-56.05
		WS2	FS1	-39.03	-33.6
			FS2	-56.56	-46.52
			FS3	-51.95	-37.25
			FS4	-59.23	-43.69

ST2, ST3, and ST4: 30, 60, and 90 d of storage; SC1 and SC2: traditional packaging and MAP; WS1 and WS2: non-stress and water stress conditions; FS1, FS2, FS3, and FS4: foliar application of water, chitosan, melatonin, and chitosan+ melatonin.

Table 3. Influence of water stress, chitosan, and melatonin on antioxidant enzyme activity in two sweet orange cultivars during storage.

	DPPH (%)		APX (U mg protein^{-1} min)		SOD (U mg protein^{-1})		POX (U mg protein^{-1} min)		PPO (U mg protein^{-1} min)		PAL (µ g^{-1} FW min)	
	'Valencia'	'Thompson Navel'	'Valencia'	'Thompson Navel'	'Valencia'	'Thompson Navel'	'Valencia'	'Thompson Navel'	'Valencia'	'Thompson Navel'	'Valencia'	'Thompson Navel'
Storage time (d)												
0	39.01±13.98	33.78±14.37	9.1±4.3	6.3±2.8	11±3.1	7.2±1.9	5.6±1.3	7.9±5.4	2.5±1.3	1.01±0.5	4.15±1.2	3.01±0.8
30	46.06±13.45	47.01±15.05	18±6.4	12.9±4.4	10.1±2.3	7.3±1.3	12.3±5.7	8.7±3.6	3.44±4.8	1.85±2.6	4.97±0.7	3.89±0.4
60	53.17±13.97	51.1±16.99	25.9±12.4	18.2±9	14.4±8.5	10.2±6.3	14.2±5.4	10.4±3.8	4.56±2.4	2.33±1.2	6.2±2.1	4.72±1.6
90	60.62±18.32	60.13±14.98	26.8±15.1	19±9.9	14.9±5.4	11±3.9	13.3±3.3	9.7±1.6	5.11±2.5	2.69±1.1	6.85±2.1	5.42±1.3
Storage conditions												
Traditional	50.38±18.44	49.3±18.98	17.4±12.3	13±9.2	13.4±6.7	9.9±4.9	12.5±5.6	8.8±4.1	3.89±2.9	2.07±1.9	4.81±1.5	3.9±1.3
MAP	49.04±15.45	46.71±16.92	22.5±12.5	15.2±8.1	11.8±4.5	7.9±3.1	10.2±5	9.5±3.8	3.92±3.4	1.87±1.4	6.27±2.1	4.62±1.4
Water stress												
CK	44.63±13.63	44.4±15.91	17±10.6	12±7.2	11.5±4.3	8.2±2.9	9.8±3.2	9.2±3	3.73±3.4	1.98±1.9	5.15±1.9	4.05±1.5
Stress	54.79±18.49	51.61±19.25	22.8±13.8	16.2±9.7	13.7±6.7	9.6±5.2	12.9±6.7	9.2±4.7	4.08±2.9	1.96±1.4	5.93±1.9	4.47±1.3
Foliar nutrition												
Ctrl	44.73±18.73	42.58±18.28	17.5±10.4	12.5±7	11.4±3.9	8.2±2.8	10.6±4.5	9±3.3	3.55±2.8	1.8±1.4	5.32±2.1	4.14±1.5
CH	52.64±16.73	51.69±18.83	20.1±12.9	14.2±9.1	12.4±6.2	9±4.7	11.4±5	8.9±3.7	3.63±3.7	1.85±1.9	5.42±2.2	4.48±1.3
MEL	50.05±14.65	49.02±15.67	21.1±12	14.6±8.2	12.9±6.5	9.5±4.3	11.6±5.5	9.2±3.9	4.03±3.2	2.01±1.5	5.66±1.8	4.15±1.5
CH × MEL	51.44±17	48.74±18.28	21.1±14.9	15.1±10.5	13.7±5.9	8.9±4.9	11.8±6.7	9.7±4.7	4.4±3	2.22±1.7	5.77±1.7	4.27±1.4
LSD = 0.05	3.17	4.37	2.20	1.51	1.16	0.63	0.78	0.68	0.41	0.18	0.29	0.18
Interaction effects												
ST × SC	**	**	**	**	**	**	**	**	**	**	**	**
ST × WS	ns	ns	**	**	**	**	**	**	**	**	**	**
ST × FS	**	*	ns	ns	**	**	**	**	**	**	**	**
SC × WS	ns	ns	**	**	**	**	*	ns	**	**	*	**
SC × FS	**	**	ns	ns	ns	ns	**	**	*	**	ns	*
WS × FS	**	**	ns	ns	ns	*	ns	*	*	**	ns	ns
ST×SC×WS	**	**	**	**	**	**	**	**	**	**	**	**
ST×SC×FS	**	**	**	**	*	**	**	**	**	**	**	**
ST×WS×FS	**	**	**	**	**	**	**	**	*	**	**	**
SC×WS×FS	*	ns	ns	ns	ns	ns	**	ns	*	**	**	**
ST×SC×WS×FS	ns	ns	ns	ns	**	**	**	**	**	**	**	**

^{ns}, *, and **: non-significant and significant at the 5% and 1% levels, respectively. Modified Atmosphere Packaging (MAP), Normal irrigation (CK), water foliar application (Ctrl), Chitosan (CH), Melatonin (MEL), Storage time (ST), Storage conditions (SC), water stress (WS), Foliar spray (FS).

Table 4. Combined influence of water stress, chitosan, and melatonin on antioxidant enzyme activity in two sweet orange cultivars during storage.

ST	SC	WS	FS	SOD (U mg protein^{-1})		POX (U mg protein min^{-1})		PPO (U mg protein min^{-1})		PAL (FW µ g^{-1} min^{-1})	
				'Valencia'	'Thompson Navel'	'Valencia'	'Thompson Navel'	'Valencia'	'Thompson Navel'	'Valencia'	'Thompson Navel'
ST1	SC1	WS1	FS1	11.9±5	8.7±1.4	7.01±0.84	11.39±0.04	1.15±0.12	0.46±0.07	2.2±0.99	1.77±0.42
			FS2	10.6±3	7.2±2.1	7.08±1.05	15.47±1.36	1.11±0.11	0.6±0.04	2.49±0.63	4.12±0.06
			FS3	7.9±3.9	8.3±0.2	6.34±0.23	10.76±1.45	1.28±0.28	0.62±0.17	5.07±0.49	1.81±0.1
			FS4	12.3±2.3	5.3±2.5	5.94±0.9	14.36±1.09	1.81±0.19	0.59±0.06	5.57±0.04	3.69±0.29
		WS2	FS1	11.4±2.5	7.2±1.2	4.39±0.72	3.17±0.36	3.8±0.28	1.61±0.19	4.76±0.5	3.28±0.23
			FS2	11±1.3	7.7±0.8	4.91±0.76	*2.72±0.81*	3.32±0.79	1.33±0.25	4.44±0.54	3.32±0.23
			FS3	10.2±1.6	7.9±2.8	4.91±0.93	2.78±0.36	3.55±0.83	1.22±0.21	4.37±0.46	3.04±0.33
			FS4	12.7±4.9	6.6±1	4.25±1.12	5.61±4.99	4.01±0.54	1.15±0.65	4.35±0.55	2.69±0.74
	SC2	WS1	FS1	11.9±5	9.5±0.5	7.01±0.84	12.67±2.19	1.15±0.12	0.52±0.12	*2.2±0.99*	2.5±1.41
			FS2	10.6±3	6.8±1.6	7.08±1.05	13.86±3.31	1.11±0.11	0.62±0.08	2.49±0.63	3.37±1.36
			FS3	7.9±3.9	8.3±0.2	6.34±0.23	11.84±2.08	1.28±0.28	0.59±0.16	5.07±0.49	2.47±1.15
			FS4	12.3±2.3	4.5±1.	5.94±0.9	10.95±6.6	1.81±0.19	0.93±0.6	5.57±0.04	3.57±0.3
		WS2	FS1	11.4±2.5	7.9±0.2	4.39±0.72	2.8±0.52	3.8±0.28	1.6±0.19	4.76±0.5	3.15±0.2
			FS2	11±1.3	8.8±2.1	4.91±0.76	2.99±0.74	3.32±0.79	1.13±0.17	4.44±0.54	3.43±0.03
			FS3	10.2±1.6	6.6±1	4.91±0.93	*2.69±0.25*	3.55±0.83	1.32±0.03	4.37±0.46	2.88±0.12
			FS4	12.7±4.9	4±1.4	4.25±1.12	2.86±0.63	4.01±0.54	1.93±0.24	4.35±0.55	3.01±0.24
ST2	SC1	WS1	FS1	9.1±4.3	6.3±1.9	7.78±0.49	6.31±0.91	1.46±0.4	5.66±0.47	5.02±1.29	3.91±0.06
			FS2	9±0.9	6.7±0.6	9.3±1.64	5.47±1.53	1.19±0.27	7.76±1.42	4.51±1.25	3.62±0.55
			FS3	9.5±1	5.6±0.3	9.24±1.84	5.67±0.8	1.08±0.19	4.66±1.19	4.2±0.59	3.56±0.28
			FS4	7.7±2	6.9±0.7	8.02±1.27	5.05±0.58	1.5±0.35	6.15±0.8	4.49±0.68	3.3±0.41
		WS2	FS1	9.8±1.8	7.6±1	19.83±1.29	9.37±1.37	0.58±0.08	0.23±0.12	5.09±0.31	4.33±0.2
			FS2	9±3.4	7.1±2.4	22.58±1.15	9.44±1.23	0.57±0.14	0.33±0	4.93±0.3	4.12±0.2
			FS3	8.4±1.1	7.8±0.4	21.15±0.54	8.84±0.26	0.68±0.13	0.28±0.11	4.5±0.82	4.16±0.27
			FS4	10±0.7	6.6±0.9	21.92±2.69	8.08±0.67	0.93±0.14	0.32±0.04	4.78±0.32	3.81±0.5
	SC2	WS1	FS1	10.8±1.6	7.9±0.9	7.38±2.06	6.83±1.08	10.57±2.07	0.8±0.22	4.88±0.07	3.91±0.16
			FS2	9.5±1.1	7.1±1	8.98±3.16	5.79±0.39	14.15±2.58	0.65±0.13	5.08±0.14	4.01±0.18
			FS3	12.8±2.1	7.2±1.1	7.59±0.65	6.42±1.18	8.87±3.71	0.56±0.1	4.5±0.19	3.85±0.16
			FS4	10.3±1.5	9±1.4	6.97±0.75	5.59±0.78	11.46±1.26	0.77±0.17	4.96±0.32	3.42±0.13
		WS2	FS1	10.3±1.6	6.8±1	11.89±1.27	14.94±0.93	*0.39±0.22*	0.28±0.04	5.65±0.48	4.07±0.41
			FS2	11.6±1.3	7.7±0.9	11.92±0.95	13.11±0.81	0.56±0.03	0.28±0.07	5.28±0.27	4.41±0.29
			FS3	12.4±3.7	8.3±1.6	11.44±1.14	14.57±0.43	0.5±0.22	0.35±0.07	5.45±0.17	3.95±0.09
			FS4	12±2	8.1±2.2	10.33±0.43	14.25±1.57	0.56±0.08	0.45±0.06	6.12±0.4	3.86±0.04
ST3	SC1	WS1	FS1	10.6±1.9	7.2±0.7	11.59±0.68	8.1±0.56	2.94±0.45	1±0.12	2.78±0.52	2.05±0.17
			FS2	15.4±4.6	10.6±3.1	13.47±1.14	7.06±0.46	2.58±0.67	0.83±0.18	3.4±0.92	1.89±0.26
			FS3	9.4±3.3	11.8±0.9	13.22±1.43	7.82±0.17	5.16±0.98	0.86±0.21	3.39±0.2	2.62±0.22

			FS4	16.4±2	6.2±2.1	12.87±1.27	7.7±0.85	6.45±2.08	1.65±0.65	2.54±0.36	2.45±0.1
			FS1	21.8±3.4	16.4±2.3	17.22±1.72	8.59±0.66	6.91±0.51	3.55±0.45	7.76±0.86	6.31±0.1
		WS2	FS2	31.5±5.3	24.2±1.4	13.91±2.59	8.18±0.61	6.87±1.13	1.22±0.15	7.36±0.59	5.24±0.77
			FS3	29.6±0.8	17.3±4	13.06±1.2	17.75±0.85	8.44±0.62	2.41±0.47	5.47±0.71	5.75±0.44
			FS4	24.5±8	21±0.4	17.8±1.37	20.03±6.38	6.03±1.91	3.1±0.84	6.2±0.88	4.22±0.52
			FS1	7.2±1.4	5.1±0.8	9.78±0.73	10.17±0.35	2.03±0.6	1.56±0.18	8.17±0.7	6.37±0.28
		WS1	FS2	*5.8±0.5*	*4.3±0.2*	11.4±2.22	9.16±0.77	1.63±0.34	1.44±0.39	7.93±0.36	6.07±0.27
			FS3	11.9±4.2	8±3.1	10.39±1.98	9.88±1.24	1.63±0.61	2.7±0.45	6.73±0.89	6.13±0.38
	SC2		FS4	11.5±4.8	8.3±2.8	11.02±1.29	9.41±0.84	3.34±1.24	3.32±1.1	7.43±0.21	5.09±0.66
			FS1	9.1±1	5.8±0.5	10.07±1.52	9.39±1.64	6.42±1.09	3.29±0.32	8.07±0.25	5.65±0.23
		WS2	FS2	8.8±0.9	5.6±0.5	10.94±1.7	11.6±1.02	2.14±0.48	3.25±0.51	6.96±0.05	4.59±0.21
			FS3	6.6±1.3	6.5±0.3	23.48±1.78	9.05±0.92	4.5±0.6	4.1±0.19	8.47±0.24	4.98±0.2
			FS4	9.9±0.1	4.4±1	27.05±9.78	12.38±0.62	5.94±1.87	2.97±1.02	6.58±0.2	6.1±0.22
			FS1	9.4±1.6	7.9±1.5	16.44±1.35	8.18±0.6	3.37±0.12	1.51±0.21	6.01±1.2	5.38±0.52
		WS1	FS2	10.6±0.6	8.6±0.7	13.14±1.9	7.75±0.52	4.81±0.52	1.41±0.21	5.93±1.1	5.26±0.34
			FS3	11±1.1	8.6±1.3	12.79±1.29	10.03±0.9	4.61±1.24	2.92±0.67	5.21±0.1	5.22±0.12
	SC1		FS4	10.8±3.6	9±0.7	16.78±0.75	9.77±0.41	3.89±0.54	3.62±0.87	5.97±0.23	4.62±0.05
			FS1	14.2±1.6	11.4±1.3	14.82±0.33	9.99±1.05	5.89±0.53	2.26±0.23	4.01±0.66	3.54±0.79
		WS2	FS2	11.6±1.1	9.9±0.3	20.12±3.31	8.88±0.57	9.26±1.02	2.04±0.55	3.59±0.32	6.56±0.1
			FS3	18.8±3.5	18.4±0.7	16.99±4.02	9.69±1.23	10.19±2.2	2.45±0.32	6.53±0.83	3.19±0.16
ST4			FS4	22.6±3.1	15.6±3	12.72±1.24	9.13±0.84	8.92±1.14	2.31±0.31	7.04±0.56	5.9±0.47
			FS1	14.2±0.5	9.9±0.9	9.57±0.55	9.16±1.64	2.59±1.1	1.74±0.13	8.69±0.91	6.56±0.44
		WS1	FS2	20.2±0.6	14.1±0.4	10.16±3.04	11.42±0.97	2.35±0.29	2.47±0.28	7.39±0.81	5.99±0.4
			FS3	16.4±2.6	15.8±3.8	12.35±2.2	8.79±0.85	5±2.1	2.34±0.62	7.06±0.65	5.52±0.58
	SC2		FS4	23±5.5	11.7±1.8	11.98±0.26	11.97±0.61	5.99±1.3	2.05±0.26	7.94±0.69	5.48±0.44
			FS1	9.6±0.6	5.9±0.5	10.34±0.14	12.31±2.1	3.82±0.6	2.66±0.25	5.12±0.52	3.4±0.29
		WS2	FS2	12.5±1.9	7.7±1	12.37±0.8	9.15±0.39	3.19±0.67	4.23±0.52	10.5±0.85	5.67±0.23
			FS3	23.3±2.1	6.3±0.3	11.91±2.2	10.83±2.7	4.09±0.87	4.77±0.85	10.16±0.77	7.27±0.67
			FS4	9.9±0.6	15.1±1.4	10.96±0.99	8.22±0.75	3.74±0.43	4.27±0.57	8.44±3.19	7.16±0.58
LSD = 0.05				4.62	2.53	3.08	2.76	1.65	0.75	1.15	0.72

ST1, ST2, ST3, and ST4: 0, 30, 60, and 90 d of storage; SC1 and SC2: traditional packaging and MAP; WS1 and WS2: non-stress and drought stress conditions; FS1, FS2, FS3, and FS4: foliar application of water, chitosan, melatonin, and chitosan+melatonin.

The highest POX activity observed in 'Valencia' (27.05 U mg protein^{-1} min^{-1}) occurred with the combined application of chitosan and melatonin under drought stress and MAP after 60 d of storage. In 'Thompson Navel', POX activities of 17.75 and 20.03 U mg protein^{-1} min^{-1} were recorded after 60 d of storage under drought stress and conventional packaging with melatonin alone and in combination with chitosan, respectively. The lowest POX activities in both cultivars were observed in fresh samples treated with chitosan alone ('Thompson Navel') or the combination of chitosan and melatonin ('Valencia') under drought stress conditions (Table 4). Maximum PPO value occurred in 'Thompson Navel' treated with chitosan under non-stressful conditions and conventional packaging (7.76 U mg protein^{-1} min^{-1}) and 'Valencia' with MAP (14.15 U mg protein^{-1} min^{-1}) after 30 d. The lowest PPO activity was observed with the water spray treatment under drought stress in both cultivars (0.23 and 0.39 U mg protein^{-1} min^{-1}, respectively) (Table 4). The maximum PAL activity in 'Valencia' occurred in response to chitosan and melatonin application individually, and in 'Thompson Navel' with melatonin alone or in combination with chitosan, all under drought stress and MAP after 90 d (10.5, 10.16, 7.27, and 7.16 µg^{-1} FW min^{-1}, respectively). The minimum PAL activity occurred in fresh samples treated with the water spray solution under non-stressful conditions and conventional packaging in both 'Valencia' (2.2 µg^{-1} FW min^{-1}) Navel (1.77 µg^{-1} FW min^{-1}) (Table 4).

Total phenolic content

In both cultivars, total phenolic content exhibited an increase with increasing storage duration. MAP more decreased the phenolic content than conventional packaging. Pre-harvest spraying with chitosan and melatonin increased the phenolic content compared with water spraying (Table 5). The lowest phenolic content was observed in fresh 'Valencia' (0.11 mg g^{-1}) and 'Thompson Navel' (0.16 mg g^{-1}) samples treated with melatonin under well-watered conditions in both packaging types. The maximum total phenolic content was detected in 60 and 90 d samples under drought stress and conventional packaging: 'Valencia' treated with chitosan (0.93 and 0.90 mg g^{-1}) and 'Thompson Navel' treated with melatonin (1.48 and 1.48 mg g^{-1}) (Table 6).

Table 5. Influence of water stress and chitosan/melatonin on total phenolic and flavonoid levels in stored 'Valencia' and 'Thompson Navel' sweet oranges.

	Total phenolic content (mg g^{-1} GA)		**Total flavonoid content (µg g^{-1} FW)**	
	'Valencia'	**'Thompson Navel'**	**'Valencia'**	**'Thompson Navel'**
Storage time (d)				
0	0.23±0.2	0.32±0.2	509.8±24	398±16.5
30	0.52±0.2	0.79±0.4	546.7±20.6	428.1±12.5
60	0.57±0.2	0.86±0.3	595.1±27.3	480±22.4
90	0.6±0.2	0.86±0.3	579.4±15.1	492.9±14.7
Storage conditions				
Traditional	0.56±0.3	0.76±0.4	535.5±15.4	457.3±13
MAP	0.4±0.2	0.66±0.4	579.9±16.2	442.2±12
Water stress				
CK	0.48±0.2	0.71±0.3	538.9±15.3	433.5±11.9
Stress	0.48±0.3	0.7±0.4	576.5±16.4	466±12.9
Foliar spray				
Ctrl	0.42±0.2	0.61±0.3	581.3±25.7	469.6±17.2
CH	0.5±0.3	0.74±0.4	560.5±13.7	449.7±13.3
MEL	0.51±0.3	0.76±0.5	554.6±28.5	440.2±21.5
CH × MEL	0.49±0.2	0.72±0.3	534.4±19.5	439.5±18.1
Interaction effects				
ST × SC	**	**	**	**
ST × WS	**	**	**	**
ST × FS	**	**	*	**
SC × WS	ns	**	**	**
SC × FS	**	**	**	**
WS × FS	*	**	**	**
ST × SC × WS	ns	*	**	**
ST×SC×FS	**	**	**	**
ST×WS×FS	**	**	**	**
SC×WS×FS	**	**	**	**
ST×SC×WS×FS	**	**	**	**

^{ns}, *, and **: non-significant and significant at the 5% and 1% levels, respectively.

Table 6. Water stress and foliar treatment interactions showing effects on phenolic and flavonoid composition in stored sweet orange fruit.

ST	SC	WS	FS	Total phenolic content (mg g^{-1} GA)		Total flavonoid content (µg g^{-1} FW)	
				'Valencia'	'Thompson Navel'	'Valencia'	'Thompson Navel'
ST1	SC1	WS1	FS1	0.24±0.08	0.38±0.05	349.9±43.6	324.5±6.1
			FS2	0.25±0.1	0.36±0.14	510.2±13.4	414.4±13
			FS3	0.11±0.05	0.16±0.06	690.9±94.9	534.9±4.8
			FS4	0.5±0.31	0.7±0.44	446.7±22.2	336.1±12.4
		WS2	FS1	0.21±0.05	0.28±0.06	680.6±36.5	499±32.2
			FS2	0.13±0.04	0.18±0.05	682±40.3	534.9±22.4
			FS3	0.25±0.08	0.33±0.15	261.4±18.4	*213.2±18.5*
			FS4	0.14±0.03	0.23±0.1	456.4±12.4	345.6±9.7
	SC2	WS1	FS1	0.24±0.08	0.33±0.12	349.9±43.6	341.7±23.3
			FS2	0.25±0.1	0.36±0.14	510.2±13.4	461.5±34.1
			FS3	0.11±0.05	0.16±0.06	690.9±94.9	476.1±61.4
			FS4	0.5±0.31	0.71±0.43	446.7±22.2	386.1±59.4
		WS2	FS1	0.21±0.05	0.25±0.1	261.4±18.4	*241.5±46.8*
			FS2	0.13±0.04	0.23±0.04	682±40.3	449.5±101.2
			FS3	0.25±0.08	0.29±0.19	680.6±36.5	499.8±32.3
			FS4	0.14±0.03	0.23±0.02	456.4±12.4	309.2±30.4
ST2	SC1	WS1	FS1	0.47±0.18	0.68±0.09	362.1±44.8	384.5±20.9
			FS2	0.88±0.23	1.35±0.37	528.7±13.1	414.6±15.1
			FS3	0.7±0.19	1.06±0.1	702±97.2	249.6±23.5
			FS4	0.7±0.04	1.06±0.04	456.7±22.4	376.1±12.5
		WS2	FS1	0.65±0.1	1.06±0.1	675.6±38.2	453.4±17
			FS2	0.49±0.14	0.81±0.23	665.6±44.4	590.3±18.8
			FS3	0.87±0.16	1.4±0.16	284±25.3	487.3±28
			FS4	0.78±0.12	1.27±0.13	475.3±7.7	550.4±32.6
	SC2	WS1	FS1	0.36±0.06	0.55±0.07	492.3±19.4	492.9±21.8
			FS2	0.36±0.06	0.55±0.07	650.1±24.8	385±13.4
			FS3	0.28±0	0.4±0.01	524.3±24.6	515.5±29.7
			FS4	0.51±0.04	0.75±0.05	650.8±44.1	387.7±24.4
		WS2	FS1	0.34±0.08	0.47±0.12	659.5±41.9	389.3±15.1
			FS2	0.45±0.02	0.62±0.04	535.2±12.4	398.4±12.6
			FS3	0.15±0.01	0.22±0.01	720.5±21.1	396.1±21.6
			FS4	0.24±0.09	0.33±0.12	363.8±29.2	378.4±18.3
ST3	SC1	WS1	FS1	0.8±0.19	0.58±0.03	338.5±38.6	264.8±18.5
			FS2	0.8±0.03	0.54±0.05	402.1±10.6	295.7±10.9
			FS3	0.66±0.16	1.01±0.06	357.2±28.5	283.6±12.2
			FS4	0.37±0.06	0.68±0.04	382.9±11.8	283.4±9.3
		WS2	FS1	0.47±0.04	0.57±0.13	665.1±43.2	576.1±6.7
			FS2	0.93±0.11	0.82±0.04	610.1±42	557±6.8
			FS3	0.46±0.04	1.48±0.19	709.3±17.2	587.9±19.3
			FS4	0.85±0.04	0.77±0.05	722.2±32.5	585.9±11.2
	SC2	WS1	FS1	0.34±0.09	1.16±0.27	672.3±28.5	551.1±7.4

			FS2	0.32±0.02	1.16±0.06	608±34.1	528.1±25.2
			FS3	0.6±0.1	0.94±0.23	801.8±33.1	664.1±9.1
			FS4	0.4±0.02	0.56±0.08	859.7±1.4	689.8±5.1
			FS1	0.34±0.07	0.59±0.05	583.6±20	453.1±12.6
		WS2	FS2	0.47±0.04	1.17±0.11	425.9±9.4	328.8±2.8
			FS3	0.89±0.1	0.6±0.04	434.3±23.2	333.4±21.1
			FS4	0.45±0.01	1.12±0.06	948.6±24.5	697±9.2
			FS1	0.77±0.16	0.58±0.03	653.7±102.1	598.3±4.1
		WS1	FS2	0.74±0.03	0.54±0.05	534.2±12.9	516.6±5.3
			FS3	0.63±0.12	1.01±0.06	557.6±67.3	512.8±15.7
	SC1		FS4	0.38±0.06	0.68±0.04	538.2±14.5	508.9±11.9
			FS1	0.45±0.04	0.57±0.13	593.8±20.6	567.7±7.3
			FS2	0.9±0.11	0.82±0.04	522±35	498.3±22.7
		WS2	FS3	0.45±0.04	1.48±0.19	642.9±33.1	632.6±10.3
			FS4	0.83±0.06	0.77±0.05	679.7±24.9	656±4.3
ST4			FS1	0.42±0.1	1.16±0.27	702.8±28.6	535.3±6.1
			FS2	0.38±0.03	1.16±0.06	468.5±4.8	389.3±0.5
		WS1	FS3	0.68±0.12	0.94±0.23	466.4±32.7	340±22.1
			FS4	0.5±0.02	0.56±0.08	538.9±15.7	430.4±10.5
	SC2		FS1	0.37±0.1	0.59±0.05	475.5±11.6	337.9±7.2
			FS2	0.52±0.07	1.17±0.11	633.4±10.9	433.6±8
		WS2	FS3	1.01±0.11	0.6±0.04	769.5±27.3	574.2±17.2
			FS4	0.53±0.01	1.12±0.06	493.1±22.3	354.8±15
LSWS = 0.05				0.16	0.22	103.66	74.55

ST1, ST2, ST3, and ST4: 0, 30, 60, and 90 d of storage; SC1 and SC2: traditional packaging and MAP; WS1 and WS2: non-stress and drought stress conditions; FS1, FS2, FS3, and FS4: foliar application of water, chitosan, melatonin, and chitosan+ melatonin.

Total flavonoid content

The total flavonoid content in both of 'Valencia' and 'Thompson Navel' orange cultivars varied significantly across different treatments and storage durations (Tables 5 and 6). In 'Valencia', the highest total flavonoid content (948.6 µg g^{-1} FW) was observed after 60 d of storage (ST3) under drought stress (D2), modified atmosphere packaging (MAP), and combined foliar application of melatonin and chitosan (FN4). Conversely, the lowest flavonoid content (261.4 µg g^{-1} FW) was recorded in 'Valencia' after 0 d (ST1) under drought stress (D2), MAP, and foliar water application.

In 'Thompson Navel', the highest total flavonoid content (697 µg g^{-1} FW) was noted after 60 d of storage (ST3) under drought stress (D2), MAP, and a combination of chitosan and melatonin foliar application. The lowest flavonoid content (213.2 µg g^{-1} FW) was observed after 0 d (ST1) under drought stress (D2), conventional packaging (SC1), and melatonin foliar application (FN3). Overall, flavonoid content increased with storage time, although variations were noted depending on the specific combinations of storage conditions, drought stress, and foliar treatments. Drought stress exhibited a complex effect, sometimes increasing and at other times decreasing the flavonoid content, influenced by other factors. Similarly, the effects of chitosan and melatonin were context-dependent. Additionally, MAP played a significant role in influencing flavonoid content. These findings indicated a complex interplay between the factors affecting flavonoid accumulation in these orange cultivars.

Simple correlation results

The findings indicated that increased activity levels of antioxidant enzymes, such as APX and PAL, were significantly and negatively correlated with fruit weight in both cultivars, suggesting an inverse relationship between these attributes. In the 'Valencia' cultivar, phenol content exhibited a negative correlation with fruit weight but showed significant positive correlations with APX and POX. Similarly, in the 'Thompson Navel' cultivar, phenol content was negatively correlated with fruit weight

but demonstrated significant positive correlations with DPPH, APX, PPO, and PAL. Flavonoid compounds displayed negative correlations with pomological and most biochemical traits in both cultivars; however, they were positively correlated with antioxidant enzyme activities (Table 7).

Table 7. Simple Pearson's correlation between total flavonoid content and antioxidant enzyme activities of 'Valencia' and 'Thompson Navel' sweet orange cultivars under the influence of storage treatments, water stress, and foliar spray.

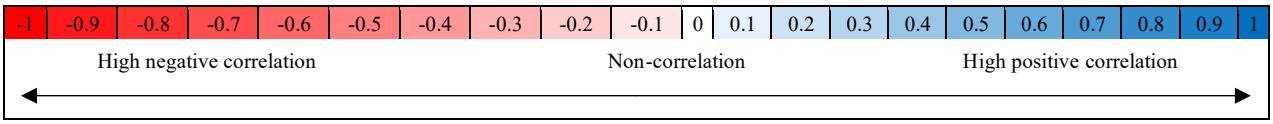
'Valencia'

	1	2	3	4	5	6	7	8	9
2	0.48**								
3	-0.36*	-0.13ns							
4	-0.42*	-0.13ns	0.11ns						
5	-0.20ns	-0.17ns	0.08ns	0.48**					
6	-0.49**	-0.37*	0.42*	0.31*	0.11ns				
7	-0.28*	-0.21ns	0.23ns	0.19ns	0.33*	0.11ns			
8	-0.35*	-0.11ns	0.31*	0.55**	0.25*	0.23*	0.10ns		
9	-0.47*	-0.30*	0.19ns	0.33*	0.24*	0.54**	0.12ns	0.22*	
10	-0.28*	-0.36*	0.15ns	0.06ns	0.08ns	0.18ns	0.23*	0.02ns	0.16ns

'Thompson Navel'

	1	2	3	4	5	6	7	8	9
2	0.39*								
3	-0.38*	-0.06ns							
4	-0.47**	-0.21ns	0.40*						
5	-0.35*	-0.20ns	0.31*	0.64**					
6	-0.23*	-0.03ns	0.06ns	0.37*	0.20ns				
7	-0.36*	-0.02ns	0.17ns	0.26*	0.04ns	-0.12ns			
8	-0.52**	-0.22*	0.35*	0.48**	0.20ns	0.21ns	0.34*		
9	-0.41*	-0.24*	0.32*	0.44*	0.20ns	0.17ns	0.36*	0.43*	
10	-0.22*	-0.21*	0.14ns	0.08ns	0.17ns	0.07ns	0.08ns	0.33*	0.12ns

ns, *, and ** indicate non-significant and significant differences at the 5% and 1% probability levels, respectively. 1. Fruit weight, 2. total soluble solids, 3. DPPH, 4. APX, 5. SOD, 6. POX, 7. PPO, 8. PAL, 9. total phenol content, 10. total flavonoids content.



Discussion

Initial weight loss was greater in non-stressed samples than in stressed samples. However, after 60 and 90 days of storage under MAP conditions, no significant differences were detected between the two groups. This indicates that MAP effectively reduces decay during storage by regulating fruit respiration, making it a suitable method for preserving water-stressed fruits for up to 90 days. The reduced gas exchange and water loss under MAP contribute to minimizing overall weight loss.

These results align with previous studies. Baswal et al. (2020) reported similar findings for mandarins stored under MAP at 5–7 °C, while Ibrahim and Gad (2015) observed minimal weight loss in oranges stored under both passive and active modified atmospheres. The high humidity within polyethylene packaging significantly delayed juice loss and slowed ripening due to altered gas composition.

Although oranges are classified as non-climacteric fruits, their TSS content progressively increases during storage. Both TSS and titratable acidity are key indicators of citrus fruit taste and quality, with a higher TSS-to-titratable acidity ratio reflecting greater sweetness (Lado et al., 2018). Comparable trends in TSS changes across storage periods have been documented for 'Thompson Navel' oranges by Nasiri et al. (2019).

No significant differences were observed in DPPH antioxidant capacity between drought-stressed and non-stressed treatments in either cultivar. This may be explained by the adaptive mechanisms plants employ in response to water stress. In water-limited environments, where water availability is the main constraint, yield depends on efficient water absorption supported by root system adaptations (Bodner et al., 2015). A strong correlation between osmotic regulation capacity and drought tolerance has also been reported (Silva et al., 2023).

Drought stress significantly influenced the activity of antioxidant enzymes such as APX, SOD, and PAL in both cultivars. However, its effect on POX and PPO was significant only in 'Valencia', whereas 'Thompson Navel' showed no significant differences in POX and PPO activities compared with non-stressed conditions. Similar increases in SOD, APX, and catalase activity under drought stress have been reported in citrus (Dos Santos et al., 2019).

Environmental stresses impair photosynthesis by limiting CO_2 fixation, reducing $NADP^+$ production,

and disrupting the electron transport chain. These disruptions promote the accumulation of reactive oxygen species (ROS), including superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen (Dmitrieva et al., 2020). Excessive ROS can cause oxidative damage through lipid peroxidation as well as protein and nucleic acid degradation. To counteract this, plants activate antioxidant defense mechanisms, often characterized by enhanced activity of enzymes such as SOD, APX, and POX (Hasanuzzaman et al., 2020).

Beyond their damaging effects, ROS also function as signaling molecules, initiating defense responses and enzyme activation. Among these, SOD plays a pivotal role by catalyzing the conversion of superoxide radicals into hydrogen peroxide and oxygen, thereby serving as a primary scavenger of ROS (Delfani et al., 2021). Elevated SOD activity under drought stress not only reduces oxidative damage but also promotes superoxide-mediated signaling pathways and upregulates SOD gene expression. The resulting hydrogen peroxide is further neutralized by POX enzymes (Gupta et al., 2018; Hasanuzzaman et al., 2020). Although relatively stable, H_2O_2 can diffuse across cell membranes and react with superoxide to form highly reactive hydroxyl radicals, making its detoxification crucial for cellular integrity. Unlike many other antioxidant enzymes, peroxidases exhibit broad substrate specificity, enabling them to neutralize a wide range of oxidative stressors. Consistent with the present findings, previous studies have documented increased catalase, POX, and SOD activity in 'Thompson Navel' oranges exposed to drought stress (Delfini et al., 2021). Similarly, Habibi et al. (2022) reported elevated APX, SOD, PAL, PPO, and POX activities in citrus fruits under water-deficit conditions.

The observed increase in antioxidant enzyme activities and the accumulation of phenolic and flavonoid compounds in response to chitosan and melatonin treatments, particularly under drought stress, are consistent with the findings of Saini et al. (2022), who reported a direct link between enhanced antioxidant potential and higher concentrations of bioactive compounds such as flavonoids and phenols. Our results, showing elevated antioxidant enzyme activities (DPPH, APX, SOD, POX, PPO, and PAL) alongside increased total phenolic and flavonoid content in treated fruits, support this relationship.

The fluctuations in antioxidant activity observed over the 90-day storage period likely reflect the dynamic changes in bioactive compound concentrations and ongoing metabolic processes, including respiration. A general decline in antioxidant levels was observed under both traditional and MAP storage, with the most pronounced reduction occurring in water-stressed samples stored conventionally for 90 days. This sharp decrease may result from the combined effects of pre-harvest water deficit, which depletes metabolic reserves, and the absence of modified atmosphere conditions to slow deterioration during extended storage.

Interestingly, MAP storage appeared to mitigate the adverse effects of pre-harvest drought stress on post-harvest antioxidant content. Reductions in antioxidant levels under MAP were not significantly different between stressed and non-stressed groups, suggesting that the controlled gaseous environment slowed respiration and degradation of bioactive compounds, thereby preserving antioxidant capacity. Notably, oranges subjected to drought stress and stored in MAP exhibited the highest total phenol content.

Total phenol levels increased over time under both storage conditions, with MAP consistently promoting greater phenolic compound accumulation than traditional storage. This may be attributed to differences in enzyme activity and phenylpropanoid pathway regulation (Song et al., 2025). Given the critical role of phenolic compounds in plant defense against oxidative stress and pathogens (Rao and Zheng, 2025), their higher accumulation in stress-treated samples is expected. While all antioxidant enzymes contributed to polyphenol accumulation during storage, further research is needed to clarify the mechanisms of polyphenol synthesis under drought conditions and MAP storage. It is hypothesized that the regulatory pathways governing phenolic biosynthesis differ between MAP and traditional storage methods. Similar trends have been reported for oranges during storage by Shu et al. (2025).

Environmental changes during storage influence flavonoid accumulation (Guo et al., 2022). Cold storage, in particular, has been shown to alter the phenolic composition of citrus fruits, often resulting in elevated flavonoid concentrations (Baswal et al., 2020). Since shelf life and quality are critical for tropical fruit imports stored under cold conditions (Saberi et al., 2018), strategies that mitigate quality loss are of great importance.

Pre-harvest foliar applications of chitosan and melatonin significantly improved fruit quality and alleviated the negative effects of drought stress, especially when applied in combination. These findings are consistent with those of Jafari and Shahsavar (2021), who demonstrated that melatonin application under drought conditions enhanced both quantitative and qualitative traits in citrus. Similarly, our results showing melatonin's role in maintaining post-harvest quality—by reducing weight loss and preserving biochemical attributes—resonate with the work of Hayati et al. (2023), who reported that melatonin extended the postharvest life of *Physalis* fruit while enhancing nutritional quality. The

reduction in weight loss observed in our melatonin-treated oranges under drought stress parallels this protective effect.

Additional evidence from Liao et al. (2024) supports these observations, showing that melatonin combined with interstock application preserved 'Kiyomi tangor' fruit quality during cold storage by activating antioxidant responses. Our findings of increased antioxidant enzyme activity in melatonin-treated fruits, particularly when combined with chitosan, suggest that melatonin mediates its protective effects partly through the upregulation of antioxidant defense systems. By counteracting oxidative stress induced by drought and storage conditions, this activation contributes to maintaining fruit quality.

The combined chitosan–melatonin treatment further reduced weight loss and helped sustain TSS in both 'Valencia' and 'Thompson Navel' oranges under well-watered and drought-stressed conditions. This outcome can be attributed to the complementary protective mechanisms of the two compounds. Chitosan, as a natural biopolymer, likely formed a semi-permeable coating on the fruit surface, reducing transpiration and respiration rates and thereby minimizing weight loss—consistent with its role as a physical barrier (Massimo and Cerana, 2018). In addition, chitosan may have elicited plant defense responses, preserving cell integrity and reducing metabolic degradation during storage. Concurrently, melatonin's strong antioxidant activity, as described by Mansouri et al. (2021), likely scavenged ROS generated during stress and senescence, protecting cellular components and delaying quality decline.

The synergistic effects of chitosan and melatonin suggest that the physical barrier and defense elicitation properties of chitosan act in concert with melatonin's antioxidant and signaling functions to provide superior protection against post-harvest deterioration. This raises the possibility of cross-talk between their respective signaling pathways, a mechanism that warrants further investigation.

Conclusion

This two-year study demonstrates the significant and interactive effects of pre-harvest drought stress, foliar applications of chitosan and melatonin, and post-harvest storage conditions on the fruit quality and antioxidant properties of 'Valencia' and 'Thompson Navel' sweet oranges. Prolonged storage (up to 90 days) resulted in an inevitable decline in fruit weight and total soluble solids (TSS); however, MAP effectively mitigated weight loss compared with conventional storage. Pre-harvest drought stress exacerbated these declines, underscoring the sensitivity of citrus fruit to water scarcity.

The foliar application of chitosan (500 ppm) and melatonin (100 µM)—particularly in combination—significantly inhibited post-harvest weight loss, demonstrating a synergistic effect with clear potential for maintaining fruit marketability. Antioxidant enzyme activities generally increased during storage, but their expression was strongly influenced by both the storage environment and pre-harvest treatments. MAP tended to suppress DPPH, SOD, and POX while enhancing APX and PAL, indicating differential impacts on specific antioxidant pathways. Notably, pre-harvest drought stress as well as chitosan and melatonin application consistently enhanced overall antioxidant enzyme activity, reflecting an induced defense response.

Storage also promoted the accumulation of total phenolic content, which was further enhanced by chitosan and melatonin, especially under drought stress and conventional packaging. By contrast, MAP appeared to limit this accumulation. Flavonoid content displayed more complex patterns, varying according to pre-harvest treatment and storage duration, suggesting intricate biochemical regulation. Correlation analysis further clarified these interactions, revealing potential trade-offs between certain antioxidant enzymes and fruit weight, while highlighting positive associations between phenolic and flavonoid levels with antioxidant enzyme activities.

Acknowledgments

This article resulted from a PhD dissertation approved by the Vice-Chancellery for Research and Technology at Islamic Azad University of Quchan.

Author Contributions

TK, EAS, and EM designed the study and performed data analysis; TK and JFM conducted the experiments and collected the data; TK wrote the manuscript; EAS supervised the project. All authors have read and approved the final version of the manuscript.

Funding

This research received no external funding.

Conflict of Interest

The authors indicate no conflict of interest in this work.

References

Aghdam MS, Fard JR. 2017. Melatonin treatment attenuates postharvest decay and maintains nutritional quality of strawberry fruits (*Fragaria*× *anannasa* cv. Selva) by enhancing GABA shunt activity. Food Chemistry 221, 1650-1657. https://doi.org/10.1016/j.foodchem.2016.10.123

Ahluwalia O, Singh PC, Bhatia R. 2021. A review on

drought stress in plants: Implications, mitigation and the role of plant growth promoting rhizobacteria. Resources, Environment and Sustainability 5, 100032. https://doi.org/10.1016/j.resenv.2021.100032

Ahmad MA, Javed R, Adeel M, Rizwan M, Yang Y. 2020. PEG 6000-stimulated drought stress improves the attributes of in vitro growth, steviol glycosides production, and antioxidant activities in *Stevia rebaudiana* Bertoni. Plants 9, 1552. https://doi.org/10.3390/plants9111552

Ahmad S, Peng D, Liu Z. 2025. Controlling mechanisms of hydrogen peroxide signaling in plants. in, Hydrogen Peroxide (CRC Press). https://doi.org/10.1201/9781032647692

Ahmed AHH, Nesiem MRA-E, Allam HA, El-Wakil AF. 2016. Effect of pre-harvest chitosan foliar application on growth, yield and chemical composition of 'Washington navel' orange trees grown in two different regions. African Journal of Biochemistry Research 10, 59-69. https://doi.org/10.5897/AJBR2016.0908

Amiri ZS, Pakkish Z, Nasibi F. 2024. Selenium nanoparticle treatments alleviate chilling injury and preserve the quality of 'Valencia' orange fruit in cold storage. International Journal of Horticultural Science and Technology 11, 411-422. https://doi.org/10.22059/ijhst.2023.358947.641

Arzani K, Khoshghalb H, Malakouti M-J, Barzegar M. 2008. Postharvest fruit physicochemical changes and properties of Asian (*Pyrus serotina* Rehd.) and European (*Pyrus communis* L.) pear cultivars. Horticulture Environment and Biotechnology 49, 244-252.

Asami DK, Hong Y-J, Barrett DM, Mitchell AE. 2003. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. Journal of Agricultural and Food Chemistry 51, 1237-1241. https://doi.org/10.1021/jf020635c.

Baswal A, Dhaliwal H, Singh Z, Mahajan B. 2020. Influence of types of modified atmospheric packaging (MAP) films on cold-storage life and fruit quality of 'Kinnow' Mandarin (*Citrus nobilis* Lour X C. deliciosa Tenora). International Journal of Fruit Science 20, S1552-S1569. https://doi.org/10.1080/15538362.2020.1818163

Bodner G, Nakhforoosh A, Kaul H-P. 2015. Management of crop water under drought: a review. Agronomy for Sustainable Development 35, 401-442. https://doi.org/10.1007/s13593-015-0283-4

Delfani K, Asadi M, Golein B, Babakhani B, Razeghi Jadid R. 2021. Study of the physiological changes of 'Thompson Navel' orange in response to foliar application of jasmonic acid under drought stress conditions. Journal of Plant Process and Function 10, 71-90.

Dmitrieva VA, Tyutereva EV, Voitsekhovskaja OV. 2020. Singlet oxygen in plants: Generation, detection, and signaling roles. International Journal of Molecular Sciences 21, 3237. https://doi.org/10.3390/ijms21093237

Dos Santos IC, de Almeida AAF, Pirovani CP, Costa MGC, da Conceição AS, dos Santos Soares Filho W, Coelho Filho MA, Gesteira AS. 2019. Physiological, biochemical and molecular responses to drought conditions in field-grown grafted and ungrafted citrus plants. Environmental and Experimental Botany 162, 406-420. https://doi.org/10.1016/j.envexpbot.2019.03.018

Dradrach A, Iqbal M, Lewińska K, Jędroszka N, Rana MAK, Tanzeem-ul-Haq HS. 2022. Effects of soil application of chitosan and foliar melatonin on growth, photosynthesis, and heavy metals accumulation in wheat growing on wastewater polluted soil. Sustainability 14, 8293. https://doi.org/10.3390/su14148293

Dumanović J, Nepovimova E, Natić M, Kuča K, Jaćević V. 2021. The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. Frontiers in Plant Science 11, 552969. https://doi.org/10.3389/fpls.2020.552969

Faghih Nasiri M, Omidbaigi R, Fakhr Tabatabaie SM, Arzani K, Zare R. 2019. Comparison of the effect of physical and chemical treatments on decay control, qualitative characteristics and some flavonoids of 'Thomson-Navel' orange fruits in cold storage. Journal of Food Science and Technology (Iran) 16, 133-147.

Garcia-Caparros P, De Filippis L, Gul A, Hasanuzzaman M, Ozturk M, Altay V, Lao MT. 2021. Oxidative stress and antioxidant metabolism under adverse environmental conditions: a review. The Botanical Review 87, 421-466. https://doi.org/10.1007/s12229-020-09231-1

Giannopolitis C, Ries S. 1977. Superoxide dismutases: I. Occurrence in higher plants. Plant Physiology 59, 309–314. https://doi.org/10.1104/pp.59.2.309.

Gonçalves LP, Alves TF, Martins CP, de Sousa AO, dos Santos IC, Pirovani CP, Almeida A-AF, Filho MAC, Gesteira AS, Soares Filho WdS. 2016. Rootstock-induced physiological and biochemical mechanisms of drought tolerance in sweet orange. Acta Physiologiae Plantarum 38, 174. https://doi.org/10.1007/s11738-016-2198-3

Guo X, Liu Q, Du J, Guo Y, Hu X, Yu J, Bai J, Li X, Kou L. 2022. X-rays irradiation affects flavonoid synthesis and delays reddening of winter jujube (*Zizyphus jujuba* Mill. cv. Dalidongzao) during cold storage. Postharvest Biology and Technology 193, 112048. https://doi.org/10.1016/j.postharvbio.2022.112048

Gupta DK, Palma JM, Corpas FJ. 2018. Antioxidants and antioxidant enzymes in higher plants. Springer. https://doi.org/10.1007/978-3-319-75088-0

Habibi F, Guillén F, Serrano M, Valero D. 2022. Postharvest treatment with glycine betaine enhances chilling tolerance of blood orange fruit by increasing antioxidant defense systems and osmoregulation during cold storage. Scientia Horticulturae 305, 111352. https://doi.org/10.1016/j.scienta.2022.111352

Hammerschmidt R, Nuckles E, Kuć J. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiological Plant Pathology 20, 73-82. https://doi.org/10.1016/0048-4059(82)90025-X

Hasanuzzaman M, Bhuyan MB, Parvin K, Bhuiyan TF, Anee TI, Nahar K, Hossen MS, Zulfiqar F, Alam MM, Fujita M. 2020. Regulation of ROS metabolism in plants under environmental stress: A review of recent experimental evidence. International Journal of Molecular Sciences 21, 8695. https://doi.org/10.3390/ijms21228695

Hayati P, Hosseinifarahi M, Abdi G, Radi M, Taghipour L. 2023. Melatonin treatment improves nutritional value and antioxidant enzyme activity of *Physalis peruviana* fruit during storage. Journal of Food Measurement and Characterization 17(3), 2782-2791. https://doi.org/10.1007/s11694-023-01819-6

Hussain S, Rao MJ, Anjum MA, Ejaz S, Zakir I, Ali MA, Ahmad N, Ahmad S. 2019. Oxidative stress and antioxidant defense in plants under drought conditions. Plant Abiotic Stress Tolerance: Agronomic, Molecular and Biotechnological Approaches 207-219. https://doi.org/10.1007/978-3-030-06118-0_9

Ibrahim MM, Gad MM. 2015. The relationship between harvest date and storage life of Washington navel orange fruits. Middle East Journal of Applied Science 5, 1247-1256.

Jafari M, Shahsavar A. 2021. The effect of foliar application of melatonin on changes in secondary metabolite contents in two citrus species under drought stress conditions. Frontiers in Plant Science 12, 692735. https://doi.org/10.3389/fpls.2021.692735

Lado J, Gambetta G, Zacarias L. 2018. Key determinants of citrus fruit quality: Metabolites and main changes during maturation. Scientia Horticulturae 233, 238-248. https://doi.org/10.1016/j.scienta.2018.01.055

Liao L, Li J, Lan X, Li Y, Li Y, Huang Z, Jin Z, Yang Y, Wang X, Zhang M. 2024. Exogenous melatonin and interstock treatments confer chilling tolerance in citrus fruit during cold storage. Scientia Horticulturae 327, 112802. https://doi.org/10.1016/j.scienta.2023.112802

Lin D, Zhao Y. 2007. Innovations in the development and application of edible coatings for fresh and minimally processed fruits and vegetables. Comprehensive Reviews in Food Science and Food Safety 6, 60-75. https://doi.org/10.1016/j.foodcont.2022.109188

Mansouri S, Sarikhani H, Sayyari M, Solimani Aghdam M, Askari Sarcheshmeh MA. 2021. Effect of preharvest treatment of melatonin on ripening and postharvest qualitative characteristics of strawberry (*Fragaria* × *anannasa* cv. Queen Elisa). Journal of Plant Research (Iranian Journal of Biology) 34, 643-657.

Massimo M, Cerana R. 2018. Recent advances of chitosan application in plants. In: Polymers, 10, 118, https://doi.org/10.3390/polym10020118

Moradinezhad F, Dorostkar M. 2021. Effect of vacuum and modified atmosphere packaging on the quality attributes and sensory evaluation of fresh jujube fruit. International Journal of Fruit Science 21, 82-94. https://doi.org/10.1080/15538362.2020.1858470

Nakano Y, Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology 22, 867-880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Naserzadeh Y, Mahmoudi N. 2025. Management of Fruits to Increase Shelf Life. in, Plant Diseases and Their Management (Apple Academic Press).

Park GH, Lee SH, Kim HY, Jeong HS, Kim Ey, Yun YW, Nam SY, Lee BJ. 2011. Comparison in antioxidant effects of four citrus fruits. Journal of Food Hygiene and Safety 26, 355-360.

Paulauskienė A, Tarasevičienė Ž, Žebrauskienė A, Pranckietienė I. 2020. Effect of controlled atmosphere storage conditions on the chemical composition of super hardy kiwifruit. Agronomy 10, 822. https://doi.org/10.3390/agronomy10060822

Pizzocaro F, Torreggiani D, Gilardi G. 1993. Inhibition of apple polyphenoloxidase (PPO) by ascorbic acid, citric acid and sodium chloride.

Journal of Food Processing and Preservation 17, 21-30. https://doi.org/10.1111/j.1745-4549.1993.tb00223.x

Rao MJ, Zheng B. 2025. The role of polyphenols in abiotic stress tolerance and their antioxidant properties to scavenge reactive oxygen species and free radicals. Antioxidants 14, 74. https://doi.org/10.3390/antiox14010074

Roppolo P, Culmone A, Passafiume R, Pirrone A, Tinebra I, Gugino I, Monte M, Naselli V, Francesca N, Gaglio R. 2025. Effect of hot air-drying technique on the quality and stability of blood orange slices in modified atmosphere packaging. Horticulturae 11, 116. https://doi.org/10.3390/horticulturae11020116

Roussos P, Flessoura I, Petropoulos F, Massas I, Tsafouros A, Ntanos E, Denaxa N. 2019. Soil physicochemical properties, tree nutrient status, physical, organoleptic and phytochemical characteristics and antioxidant capacity of clementine mandarin (*Citrus clementine* cv. SRA63) juice under integrated and organic farming. Scientia Horticulturae 250, 414-420. https://doi.org/10.1016/j.scienta.2019.02.082

Saberi B, Golding JB, Chockchaisawasdee S, Scarlett CJ, Stathopoulos CE. 2018. Effect of biocomposite edible coatings based on pea starch and guar gum on nutritional quality of 'Valencia' orange during storage. Starch-Stärke 70, 1700299. https://doi.org/10.1002/star.201700299

Sadeghi S, Jabbarzadeh Z. 2025. Vase life and postharvest attributes of *Alstroemeria aurea* 'Orange Queen' as influenced by the application of sodium nitroprusside before and after harvest. International Journal of Horticultural Science and Technology 12, 421-434. https://doi.org/10.22059/ijhst.2024.366925.713

Saini S, Sagore B, Jain S, Maurya P. 2022. Advances in the exploitation of xenia and metaxenia in fruit and nut crops. Advances in Horticulture Crops and Their Challenges 50, 1-10.

Sajid M, Basit A, Ullah Z, Shah ST, Ullah I, Mohamed HI, Ullah I. 2020. Chitosan-based foliar application modulated the yield and biochemical attributes of peach (*Prunus persica* L.) cv. Early Grand. Bulletin of the National Research Centre 44, 1-11. https://doi.org/10.1186/s42269-020-00405-w

Shu X, Xie M, Zhang X, Wang N, Zhang W, Lin J, Yang J, Yang X, Li Y. 2025. Untargeted metabolomics comparison and nutrition evaluation of geographical indication 'Newhall Navel' oranges in China. Foods 14, 355. https://doi.org/10.3390/foods14030355

Silva SF, Miranda MT, Cunha CP, Domingues-Jr AP, Aricetti JA, Caldana C, Machado EC, Ribeiro RV. 2023. Metabolic profiling of drought tolerance: revealing how citrus rootstocks modulate plant metabolism under varying water availability. Environmental and Experimental Botany 206, 105169. https://doi.org/10.1016/j.envexpbot.2022.105169

Singh A, Rajput VD, Sharma R, Ghazaryan K, Minkina T. 2023. Salinity stress and nanoparticles: Insights into antioxidative enzymatic resistance, signaling, and defense mechanisms. Environmental Research 116585. https://doi.org/10.1016/j.envres.2023.116585

Song Z, Lin H, Liu Y, Zhang H, Wei B, Gu M. 2025. Integration of volatile and nonvolatile metabolite profiles, and PPO activity reveals the methyl jasmonate protective advantage and metabolite characteristics of *Houttuynia cordata* during storage. Food Chemistry 143307. https://doi.org/10.1016/j.foodchem.2025.143307

Tadayon MS, Safaiefarahani B, Sadeghi S. 2023. Evaluation of chitosan encapsulated salicylic acid, metal oxide, and endophytic *Bacillus subtilis* in amelioration of citrus decline. Physiological and Molecular Plant Pathology 127, 102104. https://doi.org/10.1016/j.pmpp.2023.102104

Tinebra I, Sortino G, Inglese P, Fretto S, Farina V. 2021. Effect of different modified atmosphere packaging on the quality of mulberry fruit (*Morus alba* L. cv Kokuso 21). International Journal of Food Science 2021. https://doi.org/10.1155/2021/8844502

Wang JW, Zheng LP, Wu JY, Tan RX. 2006. Involvement of nitric oxide in oxidative burst, phenylalanine ammonia-lyase activation and Taxol production induced by low-energy ultrasound in *Taxus yunnanensis* cell suspension cultures. Nitric Oxide 15, 351-358. https://doi.org/10.1016/j.niox.2006.04.261

Wang Z, Hao S, Xu H, Ji N, Guo Y, Asim M. 2025. Transcriptomics and metabolomics profiling revealed the adaptive mechanism of *Penicillium digitatum* under modified atmosphere packaging-simulated gas stress. Postharvest Biology and Technology 219, 113290. https://doi.org/10.1016/j.postharvbio.2024.113290

Zaher-Ara T, Boroomand N, Sadat-Hosseini M. 2016. Physiological and morphological response to drought stress in seedlings of ten citrus. Trees 30, 985-993. https://doi.org/10.1007/s00468-016-1372-y

Zhang J. 2007. Flavonoids in Grapefruit and commercial grapefruit juices: concentration, distribution, and potential health benefits. In: Proceedings of the Florida State Horticultural Society, 288-294.