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ORIGINAL ARTICLE

The Change of Bioactive Compounds in some Superior Genotypes of Orange during Maturation Stages Compared to the Commercial Orange (*Citrus sinensis*) cv. Mars

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Abstract
This research was carried out to investigate the changes in bioactive compounds within four orange-superior genotypes (G3, G4, G5 and G6) cultivated in the north of Iran. These genotypes were harvested at three different maturation stages, with a 30-day interval, and their bioactive compound profiles were compared to those of the commercially grown orange cv. Mars. The study revealed notable changes in various bioactive compounds in response to delayed harvest times. Specifically, as the harvest was delayed, significant increases were observed in vitamin C content, total phenol content (TPC), antioxidant activity, reduced and non-reduced sugar content, total sugar content, hesperidin content, and superoxide dismutase (SOD) activity. Conversely, fruit carotenoid content and titratable acidity (TA) experienced a considerable reduction. Among the studied genotypes, G6 demonstrated particularly elevated levels of carotenoids (0.19 mg 100 g^{-1} FW), vitamin C (6.29 mg 100 g^{-1} FW), TPC (4.13 mg 100 g^{-1} FW), antioxidant activity (5.32%DPPHsc), reduced sugar (3.31 mg 100 g^{-1} FW), non-reduced sugar (8.04 mg 100 g^{-1} FW), and total sugar content (3.54 mg 100 g^{-1} FW) compared to the orange cv. Mars during the second harvest period. Overall, G6, as an orange-superior genotype, harvested on December 10th, presents a promising candidate for early selection in developing early maturing commercial orange cultivars.

Keywords Carotenoids · Fruit quality · Polyphenols · Phytochemicals · Vitamin C

Introduction

Consuming a nutritious diet has become a fundamental aspect of daily life. In this context, fruits and vegetables hold significant importance in maintaining a well-balanced diet, primarily due to their role in disease prevention, such as obesity, diabetes, and specific types of cancer. Orange fruits are rich in minerals, biological chemicals, and antioxidants, making them particularly beneficial (Ye 2018; Saini et al. 2022). Pharmacological research suggests that citrus fruits offer various advantages, including antioxidant, anti-inflammatory, antibacterial, anti-ischemic, and antidiabetic properties (Tang et al. 2021).

Quality control of citrus fruits is crucial, given their wide range of applications as natural health products. Characteristics such as color, taste, presence of seeds, shape, peel color, and modifications impact consumer perception significantly, thus influencing the economic value of these fruits. The nutritional value of citrus fruits is influenced by phytochemicals and the fruit's structure (Lado et al. 2018; Ladaniya 2022). Several factors regulate the chemical profile levels, including maturation stage, genotype variations, rootstock, production methods, and climatic variables. Notably, changes in organic acids, sugars, and phenolics during the maturation stages substantially impact

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taste and overall nutritional properties (Zhang et al. 2022a; Salvatore et al. 2022).

Further research is needed to better understand the quality of orange fruits, as the maturation stage has been shown to affect various physicochemical characteristics significantly. Recent findings by Zhang et al. (2022b) revealed that the contents of total soluble solids (TSS), total polyphenol content (TPC), total flavonoid content (TFC), sucrose, and hesperidin gradually increase during fruit maturation, with slight declines observed during the late maturity stage of Gannan navel oranges (*C. sinensis* L. Osbeck 'Newhall'). Conversely, titratable acid (TA), vitamin C, and limonin levels decrease as the fruit mature. The concentrations of fructose, glucose, and narirutin also vary during the harvest period. However, the antioxidant capacity remains relatively unaffected during this period, as indicated by three in vitro antioxidant experiments (Zhang et al. 2022b). Investigating how orange quality changes fruit during maturation provides valuable insights into determining the optimal harvest time to meet consumer preferences.

Based on these considerations, this study aims to investigate how the maturation stage influences the bioactive components of orange fruits and evaluate the fruit quality of four orange genotypes compared to the commercial orange cv. Mars throughout their maturation periods.

Materials and Methods

Plant Materials

The present study was conducted in 2021 and 2022 at the Kotra Research Station, belonging to Citrus and Subtropical Fruits Research Center, Iran. The experiment evaluated the performance of four orange genotypes: G3, G4, G5, and G6, and also orange cv. Mars (as control). These 18-year-old genotypes were collected two decades ago from different regions of the north of Iran and were cultivated in this research station with 4 × 3 m intervals under loamy-sandy soil conditions. The experimental design consisted of three replications, each comprising five trees. The research station is in a subtropical region with an average annual air temperature of 21 °C and an annual rainfall of 1200 mm. The experimental trees, which were 18 years old, were planted in loam soil with a pH of 6.9. The spacing between the trees was 4 m between rows and 3 m within rows. Orchard practices such irrigation, pest control and mineral fertilization were performed based on the commonly methods.

The fruits from all genotypes were harvested at three different maturity stages, spaced at 30-day intervals, starting from 11-November 2021 and continuing until 9-January 2022. The fruits were collected from various sections of the tree canopy and immediately transported to the laboratory for further analysis. Within each replication, only fruits exhibiting uniform size, color, and shape and without physical damage or disease were selected for subsequent assessments.

Several biochemical traits of the fruits were determined at the harvest time. For this purpose, 20 fruits were randomly sampled from each replication. The selected fruits were rapidly cut, combined into a pooled sample, frozen in liquid nitrogen, and stored at –80 °C until they were ready for further analysis.

Measurements

Using a digital titrometer, well-mixed juice titrated with 0.1 M NaOH was used to quantify titratable acidity (TA), which was represented as percent citric acid.

Using the technique described by Lichtenthaler (1987), carotenoid content was calculated. 80% acetone was pre-chilled to 4 °C and used to extract homogenized samples (2 g) while the sample was ground in the dark. CaCO3 (0.5 g) was added to the mixture to neutralize the organic acids while it was being crushed, and the mixture was then centrifuged at 10,000 g for five minutes at 4 °C. Carotenoids were once again extracted as described above after the supernatant was removed and 5 ml of cooled acetone was added to the waste. Using a UV-Vis spectrophotometer, the absorbance of the two supernatants was measured at 645, 663, and 470 nm. The blank was made of chilled 80% acetone.

The vitamin C content was determined by titration of 15 mL filtrated juice with 2,6-dichlorophenol indophenols (DCIP) containing $NaHCO_3$ and expressed as mg $100\,g^{-1}$ fresh weight (FW).

TPC was quantified using the Folin-Ciocalteu method, initially described by Singleton et al. (1999). The measurement was performed at a wavelength of 765 nm using a UV/Vis spectrophotometer. Gallic acid was used as a standard for obtaining the calibration curve. Data were expressed as milligrams of gallic acid equivalent (mg GAE) per 100 g of fruit FW.

With minor changes from Brand-Williams et al. (1995), the 2, 2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging technique was used to assess the antioxidant activity. In a nutshell, vortexing combined 2 mL of a 0.15 mM DPPH solution in methanol with 1 mL of methanolic extract, leaving the mixture to remain at room temperature in the dark. Using a UV/Vis spectrophotometer, the samples' absorbance was measured at 517 nm after 30 min. The amount of the fall in absorbance compared to the control, which corresponds to the amount of DPPH that was scavenged, was used to indicate the antioxidant activity.

Reducing and non-reducing sugar was estimated by the Nelson-Somogy method. Furthermore, the Anthrone method estimated the total sugar content.

Hesperidin was determined using high-performance liquid chromatography (HPLC, Agilent 1260 Infinity II) as described by Silva et al. (2014). Two milliliters of extraction solvent (methanol/acetic acid, 85:15, v/v) was added to 1 g of the fruit frozen powder and then kept in the refrigerator overnight. The aqueous part of the samples was centrifuged for 10 min at 8944 g. The supernatant of centrifuged samples was filtered through a disposable 0.45-mm syringe filter. Fifty microliters of the filtered sample were injected into the HPLC. The column was eluted with water as eluent A and methanol as eluent B. The column was run with gradient elution at 30 °C with 1 ml/min flow rate (0–10 min 80–60% A, 10–20 min 60–45% A, 20–25 min 45–20% A, 25–30 min 20–0% A). Hesperidin standard was purchased from Sigma Chemical Company. It was noted that hesperidin content was determined in first and third harvest times.

The capacity of superoxide dismutase (SOD) to prevent the photochemical reduction of nitro blue tetrazolium (NBT) was used to measure the activity of SOD. Briefly, 100 µl of enzyme extract was collected and put in a cuvette. The cuvette was filled with 1 ml of phosphate buffer (pH 5), 1 ml of distilled water, 300 µl of 22 µM methionine, and 100 µl of 20 µM NBT before being exposed to UV radiation for 15 min. The reaction mixture was then given 100 µl of 0.6 µM riboflavin (as a substrate). Finally, the reaction mixture's absorbance was repeatedly measured in a UV-Vis spectrophotometer at 0, 30, 60, and 90 s at 560 nm, and the mean results were used to calculate the SOD activity (Giannopolitis and Ries 1977).

Experimental Design and Statistical Analysis

Our study was done as a factorial experiment (three harvest times and five genotypes) according to a randomized block design (RBD) and with three replications, and analysed by SAS software. Duncan's multiple range test was used to assess the differences between means.

Results and Discussion

TA

The results showed that TA content significantly affected by the individual effects of harvest time and genotype (Table 1). TA content significantly decreased from 1.65 to 1.36% at the third harvest time (Table 1). At all harvest times, G6 had the lowest TA content as compared to other genotypes (Table 2).

Table 1 Changes of some biochemical composition of some orange-same genotypes and orange cv. Mars in response to different harvest time

	TA (%)	Carotenoid (mg 100 g^{-1} FW)	Vitamin C (mg 100 g^{-1} FW)	Total phenol (mg 100 g^{-1} FW)	Antioxidant activity (%DPPHsc)	Reduced sugar (mg 100 g^{-1} FW)	Non-reduced sugar (mg 100 g^{-1} FW)	Total sugar (mg 100 g^{-1} FW)	Hesperidin (µg 100 g^{-1} FW)	SOD (IU mg^{-1} FW)
Harvest Time (HT)	**	**	*	**	*	**	*	**	*	*
Genotypes (G)	**	*	**	**	*	*	**	*	**	**
HT × G	NS	**	**	*	**	*	**	**	**	*
Harvest Time										
1	*1.65a*	*3.28a*	*43.81b*	*92.81b*	*51.38b*	*288.43b*	*206.82b*	*378.52b*	*193.52cb*	*0.308cb*
2	*1.57a*	*3.07a*	*47.05a*	*98.46a*	*57.04a*	*299.08ab*	*294.09a*	*406.27a*	*202.99b*	*0.358b*
3	*1.36b*	*2.41b*	*47.39a*	*95.27ab*	*58.26a*	*311.16a*	*301.96a*	*419.02a*	*219.74a*	*0.391a*

**, * and NS indicate significant at $P \leq 0.01$, $P \leq 0.05$ and non-significant
Means within each column with different letters denote significant differences
The values are the means ($n = 3$)

Table 2 Changes in some biochemical composition of four orange-same genotypes as compared to orange cv. Mars in response to different harvest time

Genotypes	TA (%)	Carotenoid (mg 100 g^{-1} FW)	Vitamin C (mg 100 g^{-1} FW)	Total phenol (mg 100 g^{-1} FW)	Antioxidant activity (%DPPHsc)	Reduced sugar (mg 100 g^{-1} FW)	Non-reduced sugar (mg 100 g^{-1} FW)	Total sugar (mg 100 g^{-1} FW)
First harvest time								
G3	1.66c	2.97d	43.72bc	93.31a	51.27c	288.51b	205.61a	352.63d
G4	1.84a	3.23c	41.21cd	91.62a	46.86d	273.54c	204.79a	388.44c
G5	1.72b	2.81d	39.60d	92.08a	47.74d	279.34c	207.42a	351.52d
G6	1.47e	3.89a	49.62a	94.59a	56.17a	294.61ab	208.21a	391.37b
Mars	1.57d	3.51b	44.90b	92.45a	54.86b	306.15a	208.07a	408.64a
Second harvest time								
G3	*1.56c*	*2.56d*	*44.81bc*	*98.35bc*	*53.16cd*	*289.93b*	*271.62d*	*403.51c*
G4	*1.73a*	*2.74c*	*42.52c*	*97.29c*	*55.74c*	*275.22c*	*299.06b*	*392.24d*
G5	*1.67b*	*2.77c*	*45.67bc*	*94.41d*	*52.35d*	*292.61b*	*281.63c*	*401.65c*
G6	*1.40e*	*3.63a*	*54.73a*	*103.82a*	*65.04a*	*320.04a*	*316.49a*	*420.38a*
Mars	*1.49d*	*3.46b*	*47.52b*	*98.43b*	*58.91b*	*317.61a*	*301.65b*	*413.57b*
Third harvest time								
G3	1.37c	2.37b	44.96c	94.78c	53.78d	294.63c	286.71e	407.81c
G4	1.54a	1.84c	43.01c	90.62d	56.02c	310.02b	301.13c	418.34b
G5	1.46b	1.95c	45.79c	93.75c	53.31d	308.82b	293.65d	405.01c
G6	1.17e	2.96a	55.07a	101.04a	67.86a	320.75a	318.72a	442.51a
Mars	1.26d	2.94a	48.12b	96.16b	59.33b	321.58a	309.59b	421.43b

*Means within each column with different letters denote significant differences
The values are the means ($n = 3$)
Slicing was performed based on harvesting times

As orange fruit matures and ripens, the organic acid content, this contributes to TA, decreases due to their utilization in respiration and conversion to sugars. This leads to a reduction in TA as the fruit remains on the tree for a longer period after reaching maturity. Additionally, the accumulation of total soluble solids (TSS), primarily sugars, increases with delayed harvest time. The increase in TSS and decrease in TA results in a higher TSS/TA ratio, which is an important indicator of fruit quality and sweetness perception (Rodríguez-Concepcion et al. 2018; Ma et al. 2018; Tadeo et al. 2020).

Carotenoid

As shown in Table 1, it was found that all evaluated traits except TA content were significantly affected by the individual and combined effects of harvest time and genotype. The delay in fruit harvest demonstrated a discernible impact on carotenoid content. A significant decrease from 3.28 to 2.41 mg 100 g^{-1} FW was observed at the third harvest time (Table 1). Comparing the genotypes, it was found that G4 exhibited the highest carotenoid content at the first and second harvest times with 3.89 and 3.69 mg 100 g^{-1} FW, respectively. However, at the third harvest time, both G6 and cv. Mars showed the highest fruit carotenoid content, with 2.96 and 2.94 mg 100 g^{-1} FW values, respectively (Table 2).

The alteration in fruit pigmentation during maturation, coupled with the transition from chloroplasts to chromoplasts, can be attributed to the down-regulation of carotenoid biosynthetic genes, leading to a decrease in carotenoid content. When the harvest of orange fruits is delayed, there is a reduction in carotenoid content, likely caused by a partial inhibition of lycopene β-cyclization in the carotenoid pathway. This inhibition results in the accumulation of carotenes upstream of lycopene and a diminished flow towards downstream xanthophylls and abscisic acid (ABA). Comparative transcriptome analysis has revealed a significant blockage in the carotenoid biosynthesis pathway in Navelate oranges and their mutant fruit Pinalate, leading to decreased levels of ABA (Romero et al. 2019; Cronje et al. 2022).

As fruits mature and undergo prolonged senescence, they generate higher levels of reactive oxygen species (ROS), which promote the oxidation and degradation of carotenoids. In overripe fruits, carotenoids may serve as antioxidants or quenchers of excess ROS generated during senescence, thereby leading to a decrease in carotenoid content (Rodríguez-Concepcion et al. 2018; Ma et al. 2018; Tadeo et al. 2020).

Vitamin C

During the first harvest, the fruit vitamin C content measured 43.81 mg 100 g^{-1} FW and significantly increased to 47.39 mg 100 g^{-1} FW at the third harvest time (Table 1). Among the studied genotypes, G6 consistently exhibited the highest fruit vitamin C content across all harvest times (Table 2).

Delaying the harvest allows the fruit to remain on the tree for an extended period, during which metabolic processes, including vitamin C synthesis, continue. As long as the oranges remain attached to the tree and the leaves remain green, photosynthesis supplies the necessary energy and nutrients to support vitamin C production (Boonyakiat et al. 2016; Fenech et al. 2019). However, delayed harvest exposes the fruit to prolonged environmental stressors, such as sunlight and temperature fluctuations, which can induce oxidative stress. In response, the fruit may increase the production of antioxidants, including vitamin C, to mitigate the harmful effects of ROS. Furthermore, delayed harvest can also convert other compounds, such as sugars and organic acids, into vitamin C. (Yang et al. 2011; Caruso et al. 2021; Zheng et al. 2022).

TPC

The study's results revealed that at the first harvest time, the fruits' TPC measured 92.81 mg 100 g^{-1} FW. This value significantly increased to 98.46 mg 100 g^{-1} FW at the second harvest time (Table 1). No significant differences among the evaluated genotypes regarding fruit TPC at the first harvest time were observed. However, at the second and third harvest times, G6 exhibited the highest TPC values of 103.82 mg 100 g^{-1} FW and 101.04 mg 100 g^{-1} FW, respectively (Table 2).

The postponement of fruit harvest allows for extended fruit retention on the tree, enabling continuous metabolic processes, including the biosynthesis of phenolic compounds. The prolonged exposure of the fruit to environmental factors, such as sunlight, temperature fluctuations, and oxidative stress, induces the activation of defense mechanisms within the fruit, leading to an augmented production of phenolic compounds. This initial increase in total phenol content can be attributed to the fruit's adaptive response to environmental stressors aimed at mitigating oxidative damage (Li et al. 2019; Tadeo et al. 2020; Kołton et al. 2022).

Antioxidant Activity

In general, the fruit's antioxidant activity exhibited a significant increase from 51.38%DPPHsc to 58.26%DPPHsc at the third harvest time (Table 1). Across all harvest times, the highest antioxidant activity was consistently observed in G4 (56.17%DPPHsc, 65.04%DPPHsc, and 67.86%DPPHsc) (Table 2).

In line with our findings, Cardeosa et al. (2015) also observed increased citrus fruit antioxidant activity depending on the genotype during the harvest season.

As oranges progress toward their optimal harvest time, there is a concurrent increase in the accumulation of antioxidants, including phenolic compounds and flavonoids. The delay in fruit harvest allows for extended biosynthesis and enhanced accumulation of these antioxidants, resulting in elevated antioxidant activity. The prolonged fruit retention on the tree triggers the activation of defense mechanisms driven by environmental stressors such as sunlight, temperature fluctuations, and oxidative stress. These stressors stimulate the production of antioxidants as a protective response in the fruit (Kim et al. 2022; Corpas et al. 2023). The longer duration of these processes afforded by delayed harvest further contributes to heightened antioxidant activity.

Sugars

The data in Table 1 shows that the fruit's reduced sugar content measured 288.43 mg 100 g^{-1} FW at the first harvest time, then it rose to 311.16 mg 100 g^{-1} FW at the third harvest time. Across most harvest times, both G6 and cv. Mars displayed the highest fruit-reduced sugar content (Table 2).

Furthermore, the analysis revealed a significant increase in the content of non-reduced sugars, rising from 206.82 mg 100 g^{-1} FW at the first harvest time to to 301.96 mg 100 g^{-1} FW at the third harvest time (Table 1). No significant difference was observed among the evaluated genotypes regarding fruit non-reduced sugar content at the first harvest time. However, at the second and third harvest times, G6 and cv. Mars exhibited the highest levels of non-reduced sugars (Table 2).

Based on the findings in Table 1, the total sugar content of the fruit measured 378.52 mg 100 g^{-1} FW at the first harvest time. This value significantly increased to 406.27 mg 100 g^{-1} FW at the second harvest time and reached 419.02 mg 100 g^{-1} FW at the third harvest time. Among the evaluated genotypes, G6 consistently exhibited the highest total sugar content across all harvest times (Table 2).

As oranges progress toward maturity, they undergo physiological changes characterized by the accumulation of sugars. Reduced sugars (monosaccharides such as glucose and fructose) and non-reduced sugars (disaccharides such as sucrose) tend to increase during extended fruit maturation. The delay in fruit harvest allows for extended fruit retention on the tree, providing more time for sugar accumulation. During fruit maturation, starch reserves are converted into

sugars. The delayed harvest provides additional time for this conversion process, resulting in increased sugar content in the fruit (Lin et al. 2015; Wang et al. 2020, 2022).

Hesperidin

The results presented in Table 1 demonstrate a significant increase in fruit hesperidin content, rising from 193.52 µg 100 g^{-1} FW at the first harvest time to 219.74 µg 100 g^{-1} FW at the third harvest time. No significant differences were observed among the evaluated genotypes regarding fruit hesperidin content at the first harvest time. However, at the third harvest time, both G6 and cv. Mars exhibited the highest hesperidin content, with values of 238.71 and 236.55 µg 100 g^{-1} FW, respectively (Fig. 1).

Delaying fruit harvest can result in an elevation of fruit hesperidin content. Hesperidin synthesis occurs throughout fruit development and maturation processes. By prolonging the time that the fruit remains on the tree, the biosynthesis and accumulation of hesperidin are afforded more time. This extended synthesis duration contributes to the increased hesperidin content observed in the fruit. The delay in harvest can also stimulate the activity of specific enzymes involved in hesperidin biosynthesis. The maturation processes experienced by the fruit can impact the expression and activity of these key enzymes responsible for hesperidin production. The prolonged duration on the tree facilitates heightened enzymatic activity, leading to augmented hesperidin levels in the fruit (Li et al. 2019; Kołton et al. 2022).

SOD Activity

The results of our study revealed a significant enhancement in SOD activity with the delay of fruit harvest, increasing from 0.308 to 0.391 IU mg^{-1} FW (Table 1). Among the genotypes evaluated, G3 exhibited the lowest SOD activity at the first harvest time, while no significant differences were observed among the other genotypes (Fig. 2). Notably, G6 displayed the highest SOD activity in the fruit at the third harvest time, reaching 0.429 IU mg^{-1} FW (Fig. 2).

By delaying the harvest of oranges, the fruit is subjected to extended periods of exposure to environmental stressors, including sunlight, temperature fluctuations, and oxidative stress. These stressors can induce oxidative damage in the fruit, generating ROS. In response to this oxidative challenge, the fruit activates its antioxidant defense system, which includes upregulation of SOD activity to mitigate the detrimental effects of ROS. During the maturation process, the fruit undergoes metabolic and physiological changes that can influence the activity of antioxidant enzymes such as SOD (Gmitter et al. 2020; Saini et al. 2022).

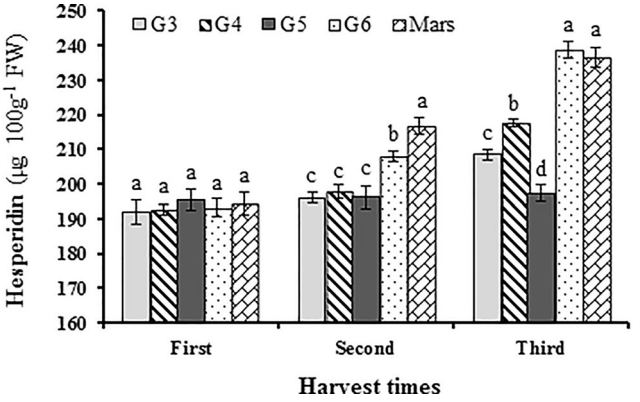


Fig. 1 Changes in hesperidin content of four orange-same genotypes as compared to orange cv. Mars in response to different harvest time. The values are the means ($n = 3$) ± standard error. Different letters indicate significant differences at $P < 0.01$. Slicing was performed based on harvesting times

Genetic variations significantly impact the intricate interactions and balances within biochemical pathways, thereby resulting in variations in the composition of biochemical compounds. The metabolism and transport of these compounds can differ among genotypes and cultivars due to variations in enzymatic activities, transport proteins, or compartmentalization within cells. Epigenetic modifications, such as DNA methylation and histone modifications, represent another layer of regulation that can control gene expression without altering the underlying genetic sequence. Epigenetic variations among genotypes can influence the activity of genes involved in synthesizing biochemical compounds, leading to variations in their content (Zhong et al. 2020; Legua et al. 2022). Furthermore, even within the same genotype or cultivar, phenotypic plasticity allows for variations in the expression of biochemical compounds in response to environmental cues. Genetic

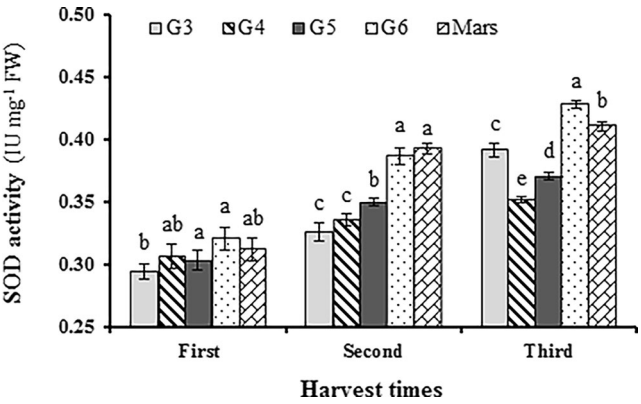
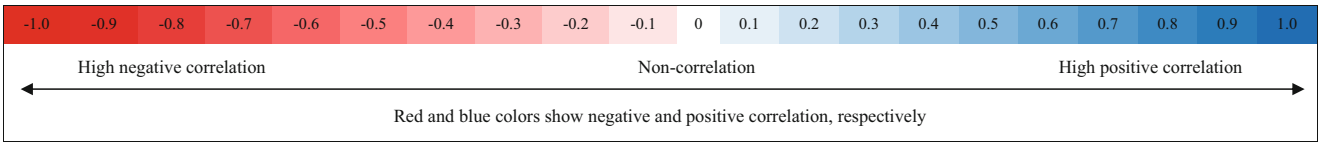


Fig. 2 Changes in superoxide dismutase (SOD) activity of four orange-same genotypes as compared to orange cv. Mars in response to different harvest time. The values are the means ($n = 3$) ± standard error. Different letters indicate significant differences at $P < 0.05$. Slicing was performed based on harvesting times

	TA	Carotenoid	Vitamin C	TPC	Antioxidant activity	Reduced sugar	Non-reduced sugar	Total sugar	Hesperidin
Carotenoid	0.63**								
Vitamin C	-0.52**	-0.38*							
TPC	-0.48*	-0.31	0.71**						
Antioxidant activity	-0.56**	-0.48*	0.65**	0.67**					
Reduced sugar	-0.32 ns	-0.29 ns	0.51**	0.49**	0.25 ns				
Non-reduced sugar	-0.39*	-0.36ns	0.63**	0.42*	0.31 ns	0.62**			
Total sugar	-0.37 ns	-0.30 ns	0.55**	0.38*	0.29 ns	0.84**	0.76**		
Hesperidin	-0.45*	-0.41*	0.69**	0.53**	0.72**	0.57**	0.49*	0.66**	
SOD	-0.59**	-0.52**	0.61**	0.62**	0.89**	0.33 ns	0.30 ns	0.37 ns	0.78**



**, * and ns indicate significant at $P \leq 0.01$, $P \leq 0.05$ and non-significant.

Fig. 3 Correlation coefficients between some bioactive compounds in some superior genotypes of orange during maturation stages. **, * and ns indicate significant at $P \leq 0.01$, $P \leq 0.05$ and non-significant

factors contribute to this plasticity and can result in variations in the composition of compounds even under similar environmental conditions. It is crucial to emphasize that while environmental conditions and cultivation practices can influence the overall composition of biochemical compounds in oranges, genetic variations play a fundamental role in determining these compounds' specific quantities and ratios. Consequently, different genotypes and cultivars can exhibit variations in the composition of biochemical compounds, even under identical environmental and cultivation conditions. The interplay between genetics and the environment contributes to the diverse array of biochemical profiles observed in citrus fruits (Wu et al. 2018; Ben Hsouna et al. 2023).

Correlations

The correlation coefficients between bioactive compounds mentioned that TA and carotenoid content had a positive correlation with each other but had a negative correlation with other traits (Fig. 3). A positive significant correlation was observed between vitamin C content and TPC, antioxidant activity, sugars, hesperidin and SOD activity. Furthermore, TPC showed a positive significant correlation with antioxidant activity, sugars, hesperidin and SOD activity. A positive significant correlation was found between antioxidant activity with hesperidin and SOD activity. Sugars had positive significant correlations with each other's and also with hesperidin. SOD and hesperidin mentioned a positive correlation (Fig. 3).

Conclusion

The study demonstrates that delayed harvest times lead to substantial increases in vitamin C content, total TPC, antioxidant activity, reduced and non-reduced sugar content, total sugar content, hesperidin content, and SOD activity. Conversely, fruit carotenoid and TA content decreases considerably with delayed harvesting. These findings indicate that G6, harvested on December 10th, presents a highly promising candidate for early selection in the development of early maturing commercial orange cultivars, offering enhanced nutritional and bioactive properties. The results provide valuable insights for breeding programs aimed at improving the quality and health benefits of commercial orange varieties.

Conflict of interest F.E. Shahrestani, P. Rahdari, J.F. Moghadam, B. Babakhani and M. Asadi declare that they have no competing interests.

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