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# Evaluation of fruit quality of 'Navelina', 'Lane Late' compared to 'Thomson' Novel oranges on Citrumelo and C35 rootstocks during cold storage

# Javad Fatahi Moghadam<sup>1,\*</sup>, Seyedeh Elham Seyedghasemi<sup>1</sup> and Negin Akhlaghi Amiri<sup>2</sup>

- 1, Postharvest Physiology and Technology Department, Citrus and Subtropical Fruits Research Center, Horticultural Science Research Institute, Agricultural Research Education and Extension Organization (AREEO), Ramsar, Iran
- 2, Horticulture Crops Research Department, Mazandaran Agricultural and Natural Resources Research and Education Center, Agricultural Research Education and Extension Organization (AREEO), Sari, Iran

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### \*Corresponding author:

Postharvest Physiology and Technology Department, Citrus and Subtropical Fruits Research Center, Horticultural Science Research Institute, Agricultural Research Education and Extension Organization (AREEO), Ramsar, Iran.

#### Email: j.fattahi@areeo.ac.ir

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#### ABSTRACT

Purpose: Citrus is one of the most important horticultural products in Iran. Fruit quality is usually influenced by variety, rootstock, and postharvest conditions. Therefore, this study was carried out to determine the fruit quality and storability of three orange cultivars on two rootstocks. Research method: Fruits of Navelina, Lane Late, and Thomson Novel on two rootstocks (Citrumelo and C35) were harvested when the TSS/TA ratio reached about 6.5-7. Fruit quality characteristics were evaluated at harvest and in cold storage (5°C, RH=85%) for 60 days. Findings: The fruits of all three cultivars on both rootstocks had arithmetic, geometrical, and homogenous diameter values close to the equivalent diameter value. The weight loss of 'Lane Late' was higher than the 'Thomson' and 'Navelina' fruits on both rootstocks during storage. 'Navelina' fruits on Citrumelo had reserved more juice percentage during storage. The TSS/TA index was lower in 'Lane Late' compared to the other cultivars on both rootstocks. A decrease in ascorbic acid was observed during storage. The peel greenness ( $a^*$ =-10.53) was the lowest at 'Lane Late' fruit grafted on C35. The best CCI index was observed in 'Navelina' peel on both rootstocks. 'Lane Late' had the lowest antioxidant capacity on C35 (59.26%) and more antioxidant capacity (71.5%) on Citrumelo. Based on the sensory evaluation, 'Navelina' and 'Thomson' scored more points regarding the appearance and aroma of the C35 rootstock. Research limitations: No limitations were found. Originality/Value: The 'Navelina' has good color, good storage quality, and is early ripening. 'Lane Late' is a late ripening variety that is slow to de-green and is not suitable for cultivation in the north of Iran.



## **INTRODUCTION**

Citrus is one of the most important horticultural products in Iran. In 2023, the cultivated area was more than 280 thousand hectares, which produced nearly 6 million tons of citrus fruits. Mazandaran province is the first citrus producer, followed by the south of Kerman and Fars provinces. Different varieties of oranges and tangerines are produced in the north and south of Iran, but sour lemons/limes and grapefruits are only distributed in the southern regions of Iran (Anonymous, 2023).

Postharvest quality management of citrus fruit begins immediately after harvest from the orchard, so orchard practices must be monitored carefully to produce high-quality fruit. In addition, there is a need to develop postharvest technologies that provide conditions to delay aging, maintain fruit quality, increase shelf life, and minimize respiration (Motamedi et al., 2018).

More reports suggest that rootstock affects tree growth, yield, and fruit quality. However, the results may be contrary regarding the effect of rootstock on fruit quality, such as size, juice content, and total soluble solids (TSS) (Shafieizargar et al., 2012).

The effects of rootstocks (orange, Volcomer lime, Flying Dragon, Citrumelo, and Citrange C-35) were investigated on the quality parameters of Persian lime fruit. The results showed that the use of rootstock did not affect the amount of fruit juice, pH, titratable acid (TA), organic acids (oxalic, malic, and ascorbic), and sugar (fructose, glucose, and sucrose). The fruits of trees grafted on orange and Volcomer lemon showed the highest pectin yield, while Volcomer lemon and Flying Dragon showed the highest citric acid. The type of rootstock influenced the aromatic profile of Persian lime. The fruits of trees grafted on Flying Dragon showed a dark green color, the highest TSS, and a lower weight loss percentage (Raddatz-Mota et al., 2019).

Another important quality parameter in citrus fruits is peel thickness, especially for fresh consumption. In general, fruit with thick peel contains less juice percentage and is susceptible to physiological disorders such as fruit splitting and creasing (Juan & Jiezhong, 2017). On the other hand, several authors have reported that rootstock changes the peel thickness in citrus fruits (Castle et al., 2011; Legua et al., 2011). It has been found that different rootstocks affect fruit ripening, postharvest quality, and fruit appearance, including size, color, shape, texture, and form. Also, citrus rootstock has a significant effect on the internal characteristics of the fruit, such as total soluble solids, acidity, vitamin C, antioxidant capacity, aroma, taste, and juice percentage (Benjamin et al., 2013).

One of the main problems with citrus fruit after harvesting is weight loss during storage. Fruit weight loss increases due to post-harvest transpiration. Environmental conditions with low relative humidity and high temperatures increase transpiration and water loss, which accelerates aging and reduces fruit quality (Yadav et al., 2013).

Peel color is one of the most important quality indicators in citrus fruits, because it directly affects the appearance and visual quality. In addition, the color improvement in the edible part of the fruit indicates the presence of pigments with antioxidant properties such as carotenoids, anthocyanins, and flavonoids (Schifferstein et al., 2019; Vadiveloo et al., 2019).

Flavedo (peel) color in most citrus fruits, especially in lemons and limes, is one of the main characteristics that defines quality and traditional potential (Zhang & Zhou, 2019). The difference in citrus peel color can be due to the effect of the rootstock on the accumulation of pigments, which has also been referred to in various studies. Owari tangerine (*Citrus unshiu* Marc.) grafted on two rootstocks of Cleopatra mandarin and Troyer Citrange. It was found that fruits on Cleopatra rootstock have higher levels of  $\beta$ -cryptoxanthin and violaxanthin (in flavedo and albedo tissue) than were grafted on Troyer citrange (Cano & Bermejo, 2011).



It has been reported that rootstock can change the TSS content in citrus fruits. On the other hand, high TSS values in citrus juice have been reported to be associated with better fruit quality (Ladaniya & Mahalle, 2011). Titratable acidity in fruit juice is mainly due to organic acids (citric, malic, tartaric, ascorbic, oxalic, benzoic, etc.), which are mostly stored in the vacuoles of plant cells. Also, citric acid at 85 to 95 percent is the main organic acid in citrus fruits (Zhou et al., 2018). It has been reported that fruit Titratable acidity begins to decrease when organic acids enter in the citric acid cycle during fruit ripening (Paliyath et al., 2009).

The extent of shelf life and reduction of fruit loss of Salustiana orange (*Citrus sinensis* (L.) Osbeck) grafted on five rootstocks (Citrumelo, C35, Rangpur lime, Cleopatra mandarin, and Sankey) were investigated during postharvest. It was found that fruits on Citrange C13 and Cleopatra mandarin rootstocks had more flavor according to sensory characteristics, while Citrumelo showed a better overall appearance (de Carvalho et al., 2020).

The effect of rootstock on fruit quality has been widely considered by researchers. However, most evaluations have focused on a specific cultivar grafted onto different rootstocks, studying yield and vegetative indices. For this reason, to understand the effect of rootstock on fruit quality, a lot of information is needed, such as metabolic compounds, physicochemical properties, and the occurrence of physiological disorders, especially at harvest and postharvest, which can be used for scion/rootstock selection (Morales Alfaro et al., 2023).

Physio-chemical, quality and nutritive compounds in fruit are usually influenced by variety, rootstock, and postharvest conditions. It is important to attend to the physical and biochemical characteristics of the fruit, especially in newly introduced cultivars that may be unknown to the consumers on different rootstocks.

## **MATERIALS AND METHODS**

This study was conducted with eight-year-old citrus trees as a factorial experiment in a completely randomized block design. Three novel orange cultivars, including: 'Thomson Novel' (control), 'Navelina' and 'Lane Late', on two rootstocks of Swingle Citrumelo and Citrange C-35 (Fig. 1 to Fig. 6) with 4 replications and two trees in each replication with planting distances of  $3\times5$  meters, located at the Ghaemshahr Horticultural Research Station (Long. 52:52:53, Lat. 36:28:33). This Station has loamy soil with 11% total lime and pH 7.8. Horticultural operations such as the use of chemical fertilizers, pruning, irrigation, spraying, etc. were performed equally for all test trees.

In late November 2018 and 2019, fruits were harvested when the TSS/TA ratio of each variety reached an average of 6.5-7 from different parts of the tree (30 fruits for each replicate) and transferred to the Citrus and Subtropical Fruits Research Institute (Ramsar). After evaluating the fruit quality at harvest, they were transferred to cold storage (5°C and 85% RH). The physicochemical characteristics and bioactive compounds of fruits were measured by sampling on the 30th and 60th days of storage.





# Fig. 5. 'Lane Late' fruit grafted on C35

Fig. 6. 'Lane Late' fruit grafted on Citrumelo

## Physical characteristics

# Length, diameter, thickness, and related traits

A digital caliper (Digit-CAl, Swiss) with an accuracy of 0.1 mm was used to measure fruit length (L) (the distance from the stem end to the blossom end of the fruit), width (W), and thickness (T) in millimeters. Then, the values of arithmetic mean diameter (Da), geometric mean diameter (Dg), homogenous diameter (Dh), equivalent diameter (Deq), aspect ratio (%Ra), surface area (S), fruit sphericity (Ø) were calculated using equations (1) to (7) (Abdullah et al., 2012).

$$D_a = \frac{(L+W+T)}{3} \tag{1}$$

$$D_{g} = (LWT)^{1/3} \tag{2}$$

$$D_{h} = \frac{3}{\left(\frac{1}{\text{Length}}\right) + \left(\frac{1}{\text{Thickness}}\right) + \left(\frac{1}{\text{Wide}}\right)}$$
(3)



$$D_{eq} = [L \times \frac{(W+T)^2}{4}]^{1/3}$$
 (4)

$$\%R_{a} = \left(\frac{W}{L}\right) \times 100 \tag{5}$$

$$S = \pi D_g^2 \tag{6}$$

$$\emptyset = \frac{(LWT)^{1/3}}{L} \tag{7}$$

# True volume (Vt), apparent volume (Va), and error of two volumes

The true volume value was measured using the water displacement principle in m<sup>3</sup>. The apparent volume (Va) and the error value of apparent/true volumes (ev) were determined using equations (8) to (9), respectively (Razavi & Bahram Parvar, 2007).

$$V_{a} = \frac{\pi}{6} LWT \tag{8}$$

$$\%e_{v} = \frac{V_{a} - V_{t}}{V_{t}} \times 100 \tag{9}$$

# True density

The true density was determined using equation (10), which  $M_a$  is the mass and  $V_t$  is the true volume of the fruit (Razavi and Bahram Parvar, 2007).

$$\rho_t = M_a / V_t \tag{10}$$

# Fruit weight and Juice percentage

The fruit juice was extracted using a manual juicer and the juice percentage was calculated by measuring the ratio of juice weight to fruit weight.

## **Technology index**

This index was obtained using the following equation (11) (Castricini et al., 2017).

TI= fruit juice (%) 
$$\times$$
 TSS/ 100 (11)

## **Peel thickness**

The thickness of the peel was measured in millimeters with an accuracy of 0.01 mm using a digital caliper (Swiss Digit-Cal).

**Number of seeds:** With a crosswise cut in the diameter of the fruit then, the seeds are removed and counted.

# Fruit peel color

The peel color was determined by measuring  $L^*$ ,  $a^*$ , and  $b^*$ , hue angle, and chroma values on the central point of fruit using a chromometer (CR400-Minolta, Japan). Then, the color index of citrus fruits was calculated with equation (12) (Jimenez-Cuesta et al., 1983).

$$CCI = 1000 \ a^*/L^*.b^* \tag{12}$$



# Total soluble solids, Titratable acidity

The level of TSS was measured using a handheld refractometer (Atago ATC-20E, Japan) with a range of 0-20%. To measure TA, a titration method with 0.1 N sodium hydroxide was used (AOAC, 2002). After measuring TSS and TA, maturity index was calculated with following equation (13).

Maturity index = 
$$TSS/TA$$
 (13)

## Vitamin C

Titration method with 2,6-dichlorophenol-indophenol solution was used to measure vitamin C. So, 5 mL of 3% metaphosphoric acid was added to one gram of fruit juice to extract vitamin C. Then it was titrated with 2,6-dichlorophenol-indophenol color solution until the appearance of a light pink color that remained stable for 15 seconds. The amount of vitamin C was calculated in terms of mg per 100 grams of fresh weight (Ladaniya, 2010).

## Peel and pulp extraction procedure

One gram of tissue was extracted using methanol solvent (1:2 ratios). The extracts were stored in a freezer at -20°C for biochemical analysis.

# **Total phenolic content**

The amount of total phenol was measured spectrophotometrically by <u>Folin -Ciocalteu</u> reagent according to the method of Meyers et al. (2003) with a slight change in terms of mg of gallic acid per gram of sample.

# **Antioxidant capacity**

The antioxidant capacity of fruit was measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method (Brand-Williams et al., 1995). DPPH radical scavenging was calculated from the following formula (14):

$$DPPH_{Sc} (\%) = 100 (1 - As) / Ac)$$
 (14)

In this equation, Ac is DPPH absorption and As is DPPH absorption plus sample.

## **Organoleptic test**

For the organoleptic test at the end of the storage period, 9 evaluators were randomly selected from the total number of male and female employees with different age and job categories, and a numerical index was applied to the appearance characteristics of the peel and pulp, aroma, taste, sweetness, sourness, bitterness, and the overall acceptability of the fruit. 1 to 10 and assigned as 1=poor, 5=satisfactory, and 10=excellent (Fellers, 1985; Cano-Lamarid et al., 2018).

## **Statistical analysis**

Physicochemical data were analyzed with MSTAT–C statistical software (Michigan State University, version 1/42, USA). Treatments were arranged in a complete randomized block design (Compound analysis), and Tukey's test (p< 0.5) was used to reveal any differences for each orange variety.



## **RESULTS AND DISCUSSION**

The fruits of all three cultivars on both rootstocks had arithmetic, geometrical and homogenous diameter values close to the equivalent diameter value, indicating that the fruit had balanced dimensions (Table 1). It was found that the length and width of 'Thomson' and 'Lane Late' fruits were larger than Navelina on both rootstocks. For this reason, some dependent traits such as surface area, thickness, and volume were higher in 'Thomson' and 'Lane Late' than in 'Navelina' fruits. 'Navelina' fruit on C35 rootstock had a higher aspect coefficient (93.11) and sphericity (0.95), which showed that the fruits were slightly curved. The fruit of the Thomson variety, with an error volume of 13.33, showed a firmer texture than the fruit of the 'Navelina' orange, which in this respect was at the least (3.69).

Table 1. Physical characteristics of Thomson, Navelina and Lane late fruits on two rootstocks.

Variety	Rootstock	Arithmetic mean	Geometric mean	Homogenous	Equivalent
variety	ROOISIOCK	diameter (cm)	diameter (cm)	diameter (cm)	diameter (cm)
'Thomson'	Citrumelo	$7.85 \pm 0.26$ *	$7.84 \pm 0.26$	$7.84 \pm 0.26$	$7.84 \pm 0.26$
'Navelina'		$7.48 \pm 0.08$	$7.47 \pm 0.08$	$7.47 \pm 0.08$	$7.47 \pm 0.08$
'Lane Late'		$7.88 \pm 0.42$	$7.88 \pm 0.42$	$7.87 \pm 0.42$	$7.88 \pm 0.42$
'Thomson'	C-35	$7.94 \pm 0.05$	$7.94 \pm 0.06$	$7.93 \pm 0.05$	$7.94 \pm 0.06$
'Navelina'		$7.34 \pm 0.49$	$7.34 \pm 0.49$	$7.33 \pm 0.49$	$7.34 \pm 0.49$
'Lane Late'		$7.89 \pm 0.16$	$7.89 \pm 0.16$	$7.89 \pm 0.16$	$7.89 \pm 0.16$

<sup>\*</sup>Mean ± Sd

Table 1. (Continued).

Variety	Rootstock	Weight (g)	Length (cm)	Wide (cm)	Thickness (cm)
'Thomson'	Citrumelo	$269.90 \pm 28.61$	$7.50 \pm 0.25$	$8.08 \pm 0.27$	$7.96 \pm 0.29$
'Navelina'		$217.23 \pm 1.98$	$7.35 \pm 0.24$	$7.60 \pm 0.05$	$7.48 \pm 0.09$
'Lane Late'		$256.80 \pm 43.93$	$7.85 \pm 0.16$	$7.95 \pm 0.55$	$7.84 \pm 0.54$
'Thomson'	C-35	$207.43 \pm 40.43$	$7.73 \pm 0.51$	$7.20 \pm 0.45$	$7.10 \pm 0.50$
'Navelina'		$215.10 \pm 16.39$	$7.41 \pm 0.05$	$7.58 \pm 0.21$	$7.47 \pm 0.27$
'Lane Late'		$259.67 \pm 16.88$	$7.75 \pm 0.12$	$8.09 \pm 0.13$	$7.84 \pm 0.23$

Table 1. (Continued).

Variety	Rootstock	Fruit sphericity	Aspect ratio (%Ra)	Surface area (m²)
'Thomson'	Citrumelo	$1.05 \pm 0.01$	$107.81 \pm 1.94$	$193.45 \pm 12.58$
'Navelina'		$1.02 \pm 0.02$	$103.48 \pm 3.53$	$175.46 \pm 3.74$
'Lane Late'		$1.00 \pm 0.03$	$101.08 \pm 4.89$	$195.93 \pm 20.63$
'Thomson'	C-35	$1.05 \pm 0.01$	$108.77 \pm 0.68$	$197.89 \pm 2.72$
'Navelina'		$0.95 \pm 0.01$	$93.11 \pm 1.26$	$170.42 \pm 22.26$
'Lane Late'		$1.02 \pm 0.01$	$104.37 \pm 0.94$	$195.68 \pm 7.78$

## Table 1. (Continued).

Variety	Rootstock	True volume (cm <sup>3</sup> )	Density	Apparent volume (cm <sup>3</sup> )	Apparent/true volumes (ev)
'Thomson'	Citrumelo	$288.50 \pm 35.57$	$0.94 \pm 0.02$	$253.89 \pm 24.28$	$13.33 \pm 6.45$
'Navelina'		$233.90 \pm 2.92$	$0.93 \pm 0.01$	$218.67 \pm 6.99$	$7.10 \pm 2.10$
'Lane Late'		$276.80 \pm 45.72$	$0.93 \pm 0.01$	$260.09 \pm 40.77$	$6.09 \pm 1.48$
'Thomson'	C-35	$224.00 \pm 43.33$	$0.93 \pm 0.01$	$211.91 \pm 41.14$	$5.67 \pm 1.01$
'Navelina'		$228.40 \pm 18.45$	$0.94 \pm 0.01$	$219.96 \pm 15.06$	$3.69 \pm 1.72$
'Lane Late'		$273.67 \pm 18.49$	$0.95 \pm 0.01$	$257.76 \pm 15.60$	$6.10 \pm 1.36$



It was reported that the rootstock did not affect the length/equatorial diameter ratio. Salustiana orange has a typical rectangular shape. Accordingly, this form was more noticeable in the fruits produced in grafted trees of Rangpur lime, Cleopatra tangerine, and Sankey tangerine, which reached indices equal to one (de Carvalho et al., 2020). Fruit size and weight parameters were similar to those reported by Roussos (2011) for fruit produced in Salustiana orange trees grafted onto 'Sour' orange in Greece, where fruit weights ranged from 170 to 197 g and diameter fruit was rated between 70.6 and 74.4 mm for trees grown under different farming systems.

# Fruit weight loss

The average weight loss was similar in the two study years. The rate of weight loss of 'Lane Late' was higher than 'Thomson' and 'Navelina' fruits on both rootstocks during storage (Table 2). The thinner peel was observed in the fruit of 'Lane Late'. It has been reported that thick peel can prompt the fruit to maintain the juice content and the weight of the fruit during storage (Morales Alfaro et al., 2023). It was reported that the fruit on C13 showed a higher percentage of weight loss than other rootstocks after 20 days in cold storage. This may be acceptable because C13 Citrange produces larger fruits, as large fruits have a high surface/volume ratio and lose water more and faster than small fruits (de Carvalho et al., 2020). In this experiment, the results related to the weight loss of the 'Thomson' variety were consistent with this research.

# Fruit peel thickness

The average peel thickness did not change significantly over two the years. Only the peel thickness of the 'Lane Late' fruit decreased during storage compared to at harvest on both rootstocks (Table 2). But, the peel thickness of 'Navelina' and 'Thomson' cultivars on both rootstocks did not change during storage. Usually, the thickness of the fruit peel decreases during storage. The decrease in peel thickness of stored fruits can be explained by increased dehydration caused by changes in humidity and storage temperature (Yadav et al., 2013). This parameter is critical in the fresh consumption and citrus juice industry because citrus with thinner peel correlates with a higher juice percentage (Raddatz-Mota et al., 2019). Recently, the effect of rootstock on the metabolism of endogenous hormones that regulate peel thickness has been investigated (Rong et al., 2019). The fruits of Tangur grafted on Junos citrus rootstock had higher peel thickness than citrus trifoliata, which was approved with the highest levels of IAA, GA3, and ZT hormones in Junos citrus rootstock. These hormones are directly related to increased peel thickness (Morales Alfaro et al., 2023).

## Fruit juice percentage

The fruit juice percentage in the second-year study was higher (43.32%) than in the first-year study (32.46%). At harvest, the highest were in the 'Lane Late' and 'Thomson' cultivars on the Citrumelo rootstock (Table 2). 'Navelina' fruits on Citrumelo rootstock retained a higher juice percentage during storage. In general, Citrumelo rootstock had a greater role in juice production and preservation than C35 rootstock. Based on a research report, Valencia oranges on 12 rootstocks had juice contents between 56-61% with significant differences between rootstock (Castle et al., 2010). Conferring to these research results, Citrumelo Swingle produced the highest percentage of juice content, which was well distinguished from the Rangpur lime, Sanki tangerine, and C-13 Citrange rootstocks. The efficiency of Citrumelo Swingle in inducing higher juice content in citrus was also mentioned in previous reports. Despite significant differences, all rootstocks produced an acceptable amount of juice (35%) above the minimum fresh fruit market requirement (Raddatz-Mota et al., 2019).



Table 2. Chemical characteristics of Thomson, Navelina and Lane late fruits on two rootstocks during 60 days of storage.

Year	r Rootstock Variety Weight loss (%)		Weight loss (%)	Peel thickness (mm)	Juice percentage	Technological index	
2018			3.58	3.25	32.46 b	3.38 b	
2019			3.62	3.3	43.32 a	4.79 a	
Storage time							
0	Citrumelo	'Thomson' 'Navelina' 'Lane Late'	0.01 g 0.01 g 0.01 g	3.31 bc 4.36 a 3.42 bc	39.67 a-c 38.24 b-d 43.89 a	3.68 fg 4.37 b-e 4.12 b-f	
	C-35	'Thomson' 'Navelina' 'Lane Late'	0.01 g 0.01 g	3.79 a-c 4.02 ab 3.11 cd	37.79 b-d 36.96 b-d 38.01 b-d	3.91 d-g 4.29 b-f	
30	Citrumelo	'Thomson' 'Navelina' 'Lane Late'	0.01 g 5.8 b-d 5.09 c-f 7.62 a	3.34 bc 3.03 cd 2.39 de	37.18 b-d 41.04 ab 37.34 b-d	3.85 e-g 3.8 e-g 4.47 b-d 3.51 gh	
	C-35	'Thomson' 'Navelina' 'Lane Late'	5.69 b-e 4.77 d-f 6.41 b	3.65 a-c 3.83 a-c 2.41 de	34.48 cd 36.34 b-d 37.45 b-d	3.83 e-g 4.65 ab 4.29 b-f	
60	Citrumelo	'Thomson' 'Navelina' 'Lane Late'	4.66 ef 4.52 f 6.07 bc	3 cd 4 ab 2.07 e	38.11 b-d 43.32 a 33.38 d	3.97 c-g 5.13 a 3.05 h	
	C-35	'Thomson' 'Navelina' 'Lane Late'	4.79 d-f 4.16 f 5.18 c-f	3.5 a-c 3.4 bc 2.36 de	36.6 b-d 36.95 b-d 35.24 cd	4.23 b-f 4.56 bc 3.86 e-g	

<sup>\*</sup>Values in the same column for each variable and variety having different letters are significantly different (p=0.05) based on Tukey test.

Table 2. (Continued).

Year	Rootstock	Variety	TSS (%	6)	TA (9	%)	TSS/T	A	Vitami (mg/10	n C 0gFW)
2018			10.44	b*	1.51	a	7.43	b	35.18	a
2019			11.08	a	1.21	b	9.37	a	32.99	b
Storage time										
0	Citrumelo	'Thomson'	9.22	h	1.07	fg	8.59	a-d	35.31	b-d
		'Navelina'	11.4	bc	1.82	a	7.15	c-f	33.78	b-d
		'Lane Late'	9.36	gh	1.39	c-f	7.09	d-f	39.42	b
	C-35	'Thomson'	10.27	e-g	1.37	c-f	7.85	b-f	38.8	b
		'Navelina'	11.37	b-d	1.15	e-g	10	a	47.47	a
		'Lane Late'	10.08	f-h	1.49	b-d	7.02	d-f	33.65	b-d
30	Citrumelo	'Thomson'	10.35	d-g	1.22	d-g	8.75	a-d	30.53	cd
		'Navelina'	10.9	c-f	1.3	c-g	8.53	а-е	28.9	d
		'Lane Late'	9.38	gh	1.45	b-e	7.05	d-f	29.34	d
	C-35	'Thomson'	11.05	c-f	1.1	fg	10.19	a	29.07	d
		'Navelina'	12.8	a	1.29	c-g	10.18	a	36.6	bc
		'Lane Late'	11.3	с-е	1.7	ab	6.78	ef	28.37	d
60	Citrumelo	'Thomson'	10.33	d-g	1.05	g	10	a	34.49	b-d
		'Navelina'	11.85	a-c	1.39	c-f	8.89	a-c	34.79	b-d
		'Lane Late'	9.2	h	1.59	a-c	6.21	f	32.96	b-d
	C-35	'Thomson'	11.53	bc	1.26	d-g	9.4	ab	32.44	b-d
		'Navelina'	12.35	ab	1.22	d-g	10.28	a	33.42	b-d
		'Lane Late'	10.97	c-f	1.58	a-c	7.19	c-f	34.19	b-d

<sup>\*</sup>Values in the same column for each variable and variety having different letters are significantly different (p=0.05) based on Tukey test.



# **Technological index**

The technological index had no significant changes in the two years of study. 'Navelina' fruit had a higher technological index on both rootstock at harvesting time and during storage (Table 2). The reason for the increase in this index in 'Navelina' could be due to the high amount of total soluble solids in 'Navelina' fruit compared to the other cultivars. Based on the technological index, this variety is also useful for the fruit juice industry in addition to fresh consumption.

# TSS, TA and mature index (TSS/TA)

The fruits of 'Navelina' had the highest and 'Lane Late' had the lowest TSS on both rootstocks at the harvesting time and during storage (Table 2). The average TSS in the second year (11.08%) was higher than in the first year (10.44%). The amount of TA was highest at harvest in the 'Navelina' variety on Citrumelo rootstock, but during storage from the 30th day of storage, the amount of TA in the 'Lane Late' fruit was higher than other varieties on both rootstocks.

The average TSS/TA ratio in the second year was higher (9.37) than the first-year of this experiment (7.43). Also, the fruit of 'Navelina' and 'Thomson' cultivars had the higher TSS/TA level at harvest and during storage. This index was lower in 'Lane Late', indicating that it is a late ripening cultivar compared to the other cultivars on both rootstocks.

Many studies have addressed the effect of rootstock on sugars and acids, which are important factors that strongly influence flavor characteristics of citrus fruits. Although the content of sugars and acids depends on the rootstock/scion interaction, some rootstocks have been observed to have similar effects on different cultivars. Similarly, when different citrus verities were grafted on sour orange, the TSS and TA levels increased and delayed commercial maturity (Morales Alfaro et al., 2023). Rivera et al. (2009) reported that Persian lime fruit grafted on Flying Dragon had a higher TSS percentage than other rootstocks. It has been reported that the use of Flying Dragon rootstock causes more water stress during fruit development, which may accelerate the accumulation of sugars (Forner-Giner et al., 2014).

Several authors have observed a similar effect on TA associated with citrus rootstock such as lemon, orange, and tangerine (Emmanouilidou & Kyriacou, 2017; Continella et al., 2018). The citric acid percentage was inversely related to sugar content, which increased during storage (de Carvalho et al., 2020). Similarly, in this study, the amount of sugar increased during storage.

The TSS/TA ratio is an index of citrus fruit maturity. Fruits with lower ratios ripen later than fruits with higher ratios (Castle et al., 2010). According to previous research, sweet orange fruit has good consumption quality when the TSS/TA range is between 8.8 and 15.4 (de Carvalho et al., 2020). At harvest, 'Thomson' cultivars on Citrumelo and 'Navelina' on C35 reached this index. In the conditions of northern Iran, the harvest index for orange fruit for fresh consumption is defined as a TSS/TA ratio between 6.5 and 7 (Fatahi Moghadam et al., 2017).

# Vitamin C

The highest amount of vitamin C at harvesting time belonged to the 'Navelina' followed by 'Thomson' fruit on the C35 rootstock (Table 2). Although a decrease in the amount of ascorbic acid was observed during storage, this variation was not significant compared to variety and rootstock kind.

According to Candir et al. (2013), vitamin C content shows a significant decrease during storage. This vitamin is degraded by the activity of the ascorbic acid oxidase enzyme and then hydrolyzed. Higher water loss after harvest leads to faster loss of vitamin C. Also, the



decrease in TA is closely related to the decrease in vitamin C (Candir et al., 2013). It has been found that increasing the concentration of vitamin C can prolong the storage life of fruit (Zacarias-Garcia et al., 2021).

# Fruit peel color indices

The  $L^*$  value in the 'Lane Late' fruit peel on both Citrumelo and C35 rootstocks (58.87 and 53.75 values, respectively) was lower at harvest compared to the other cultivars, but these differences were not significant during storage (Table 3). In a study, the  $L^*$  value decreased as the storage period progressed, which means a decrease in the brightness of the peel fruit (de Carvalho et al., 2020).

The amount of  $a^*$  value of the 'Lane Late' fruit peel at harvest was negative on both rootstocks. The degree of peel greenness was the highest at 'Lane Late' fruit grafted on C35, with a -10.53 value. The highest amount of  $a^*$  value belonged to 'Navelina' peel fruit at the time of harvesting. In total, the value of  $a^*$  increased during storage. Generally, this index has an inverse relationship with the *hue* angle. Because the 'Lane Late' peel was green on both rootstocks, the *hue* angle was the highest, which indicated that the fruit peel was not well colored. Similarly, a report found a decrease in *hue* angle on all used rootstock at the end of storage. This reduction in *hue* values can be explained by senescence development. The green color of the fruit vanishes due to the breakdown of chlorophyll and then the fruit turns green-yellow due to the presence of carotenoids during the senescence process (Alvarez-Armenta et al., 2008).

The chroma and  $b^*$  indices in all cultivars and rootstocks were near and above 70, which showed that the yellow color saturation of the fruit was sufficient based on the citrus color index standard (Jimenez-Cuesta et al., 1983). Based on the CCI data, it was also found that only the green color of the 'Lane Late' fruit on both rootstocks did not change well due to the negative value of CCI. Emmanuelidou and Kyriacou (2017) observed a variation in the peel color of 'Lane Late' (*Citrus sinensis* L. Osbeck) and 'Delta' (*Citrus sinensis* L. Osbeck) varieties when grafted on five different rootstocks. Rootstocks influenced the maturity status and fruit ripening process (Morales Alfaro et al., 2023). In this study, the CCI index became positive during storage and the best coloring was observed in 'Navelina' peel on both rootstocks. In contrast, in a study on 'Navelina' orange (*Citrus sinensis* L. Osbeck) grafted on 14 rootstocks, fruits developed on 'Cleopatra' rootstock showed the lowest external color (Forner-Giner et al., 2003).

## **Antioxidant capacity**

In general, the antioxidant capacity of the fruit of all three cultivars on both rootstocks increased during storage compared to at harvest (Table 4). After 60 days of storage, the fruit of 'Lane Late' had the lowest antioxidant capacity on C35 rootstock (59.26%) and had the highest antioxidant capacity (71.5%) on Citrumelo rootstock.

The antioxidant capacity of Yashar mandarin on Citrange and Citrumelo was higher than three other rootstocks at harvest and during storage (Fatahi Moghadam et al., 2017). Antioxidant compounds in both lipophilic and hydrophilic types are affected by rootstock type (Fatahi Moghadam et al., 2019). Clemenols tangerines had the highest hydrophilic antioxidant content on the Forner-Alkaid 41, while it had the lowest hydrophilic antioxidants on the Forner-Alkaid 21 and 418. In terms of the lipophilic component, the grafted fruits on the Carrizo citrange rootstock had the highest antioxidant capacity (Legua et al., 2014).



**Table 3.** Peel color indices of Thomson, Navelina and Lane late fruits on two rootstocks during 60 days of storage.

Year	Rootstock	Variety	$L^*$		$a^*$		$b^*$	Chroma	hue	CCI
2018			64.45	a*	9.98		73.52	75.09	83.02	1.85
2019			62.60	b	9.57		73.56	74.76	83.30	1.83
Storag	e									
time										
0	Citrumelo	'Thomson'	64.23	a-d	1.77	hi	73.40 c-g	73.59 d-f	88.78 cd	0.30 fg
		'Navelina'	61.90	с-е	11.88	d-f	69.59 fg	70.83 ef	80.61 g-i	2.65 cd
		'Lane Late'	58.87	e	-4.80	j	62.94 h	63.58 g	95.18 b	-1.76 h
	C-35	'Thomson'	66.51	a-c	4.40	gh	71.89 e-g	72.48 d-f	86.92 d-f	0.78 e-g
		'Navelina'	63.05	b-e	13.39	c-f	72.52 d-g	74.21 d-f	79.81 g-j	2.89 b-d
		'Lane Late'	53.75	f	-10.53	k	55.46 i	56.70 h	101.20 a	-3.87 i
30	Citrumelo	'Thomson'	68.11	a	11.51	d-f	81.98 ab	82.88 ab	82.03 f-h	2.06 de
		'Navelina'	63.18	b-e	18.66	a-c	73.78 с-д	76.13 с-е	76.52 i-l	4.02 a-c
		'Lane Late'	62.78	b-e	4.25	gh	73.43 с-д	73.96 d-f	87.20 с-е	0.72 e-g
	C-35	'Thomson'	68.05	a	13.52	c-f	80.02 a-c	81.26 a-c	80.38 g-i	2.51 cd
		'Navelina'	63.92	a-d	20.26	ab	75.43 b-g	78.32 b-d	74.93 j-l	4.28 ab
		'Lane Late'	60.45	de	-2.27	ij	68.70 gh	68.90 fg	91.98 bc	-0.56 gh
60	Citrumelo	'Thomson'	67.04	ab	15.62	b-e	83.53 a	85.00 a	79.38 g-j	2.80 b-d
		'Navelina'	62.93	b-e	21.41	ab	73.58 с-д	76.74 b-e	73.77 kl	4.66 a
		'Lane Late'	63.55	a-d	9.28	e-g	76.16 b-f	76.89 b-e	83.19 e-h	1.89 de
	C-35	'Thomson'	67.06	ab	16.12	b-d	79.28 a-d	80.97 a-c	78.45 h-k	3.07 b-d
		'Navelina'	63.06	b-e	23.20	a	74.16 c-g	77.80 b-d	72.60 1	5.01 a
		'Lane Late'	65.03	a-d	8.31	fg	77.85 a-e	78.34 b-d	83.92 d-g	1.67 d-f
	C-35	'Navelina'	63.06	b-e	23.20		74.16 c-g	77.80 b-d	72.60 1	5.01 a

<sup>\*</sup>Values in the same column for each variable and variety having different letters are significantly different (p=0.05) based on Tukey test.

**Table 4.** Chemical characteristics of Thomson, Navelina and Lane late fruits on two rootstocks during 60 days of storage.

Storage	Rootstock	Variety		dant capacity		henolics (mg
time	ROOTSTOCK	(%DPPHsc)		Gallic	acid.g <sup>-1</sup> FW)	
	Citrumelo	'Thomson'	54.22	c-e*	0.31	e
0		'Navelina'	50.70	de	0.35	e
		'Lane Late'	51.79	с-е	0.30	e
	C-35	'Thomson'	56.11	с-е	0.43	e
		'Navelina'	58.60	b-e	0.25	e
		'Lane Late'	52.92	с-е	0.26	e
30	Citrumelo	'Thomson'	55.99	с-е	0.94	cd
		'Navelina'	61.41	a-e	1.14	a-d
		'Lane Late'	49.65	e	0.72	de
	C-35	'Thomson'	61.51	а-е	1.23	a-c
		'Navelina'	70.21	ab	1.64	a
		'Lane Late'	63.93	a-c	1.25	a-c
60	Citrumelo	'Thomson'	63.44	a-c	1.17	a-d
		'Navelina'	69.08	ab	1.18	a-d
		'Lane Late'	71.50	a	1.20	a-d
	C-35	'Thomson'	63.82	a-c	1.28	а-с
		'Navelina'	62.75	a-d	1.45	ab
		'Lane Late'	59.26	b-e	0.96	b-d

<sup>\*</sup>Values in the same column for each variable and variety having different letters are significantly different (p=0.05) based on Tukey test.



# **Total phenolics**

Phenolic compounds showed an increasing trend from the time of harvest to the end of storage, as well as the antioxidant capacity (Table 4). The fruit of the 'Lane Late' cultivar on both rootstocks had lower phenol levels, especially at the end of storage, although there was no significant difference from the 30th day of storage with other cultivars.

The increasing trend in phenolic compounds during storage is due to high phenol biosynthesis in exposure to the low temperature stress in cold storage. In this way, the l-phenylalanine is converted into trans-cinnamic acid by the PAL enzyme, which is produced by hydroxylation or methylation of this substance and other hydro-cinnamic acids (Legua et al., 2014).

# **Organoleptic evaluation**

In terms of sensory characteristics such as appearance and aroma, the fruits of 'Navelina' and 'Thomson' on the C35 rootstock had higher scores than the fruits on the Citrumelo rootstock (Fig. 7 to Fig. 9). On the other hand, the 'Lane Late' on Citrumelo rootstock had the superior characteristics in terms of pulp appearance, aroma, and total acceptance of fruit.

Based on a report, by performing sensory evaluation at harvest, fruits grafted on C13 Citrange and 'Cleopatra' tangerine showed higher values for flavor, juiciness, and preference (taste attributes) because of the rating scale of the panel participants. Also, fruits on Citrumelo 'Swingle' were highlighted for visual (peel color and color uniformity) and physical (peel texture and fruit shape) characteristics. Fruits produced in grafted trees on C13 and Cleopatra tangerine were the most popular in taste characteristics at 20 and 40 days of storage (de Carvalho et al., 2020). Similarly, citrumelo 'Swingle' produces a better overall appearance fruit, based on visual and physical characteristics during storage (Cruz et al., 2019).

It has been confirmed that rootstock does not affect some sensory quality of the fruit. This fact shows that fruit characteristics such as overall appearance (size, shape, color, absence of defects, and decay), form, texture, and desirability (total soluble solid, acids, and volatile aromatic substances) are controlled by the level of scion: rootstock adaptation (Benjamin et al., 2013).

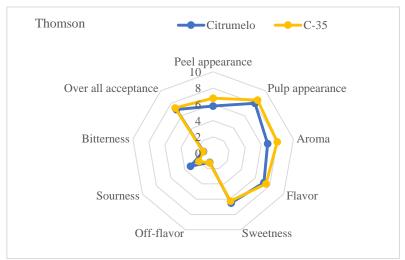
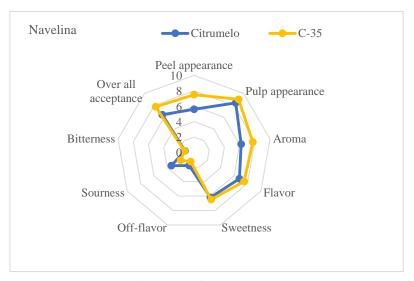


Fig. 7. Organoleptic evaluation results of Thomson fruits on two rootstocks at the end of cold storage (60 days).





**Fig. 8.** Organoleptic evaluation results of Navelina fruits on two rootstocks at the end of cold storage (60 days).

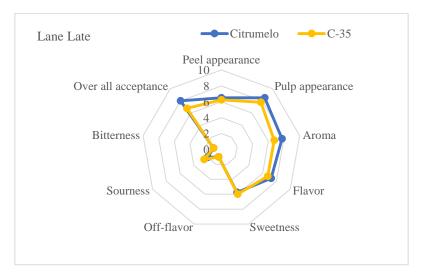


Fig. 9. Organoleptic evaluation results of Lane Late fruits on two rootstocks at the end of cold storage (60 days).

## **CONCLUSION**

Generally, the fruit dimensions (length, width, and thickness) of the new cultivars were smaller than the Thomson fruit on C-35 rootstock. Based on the TSS/TA ratio, it was the highest (early ripening) in Navelina and the lowest (late ripening) in Lane Late oranges at harvest. Citrumelo rootstock increased late ripening in Lane Late fruit and contrary, C-35 rootstock increased early ripening in Navelina fruit. According to the *a\** and CCI indices, the Lane Late fruits did not have suitable coloring at harvest, and Citrumelo rootstock delayed the color change of the fruit peel. In general, the organoleptic characteristics of Navelina as Thomson fruit on C-35 were better than Citrumelo. Overall, it seems that the combination of Navelina /C-35 is more suitable than Lane Late /Citrumelo. The 'Navelina' variety has good color, good storage quality, and is early ripening.



## **Conflict of interest**

There is no potential conflict of interest between the authors to declare.

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