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Morphological and Biochemical Characterization of Late-Season Varieties of Mandarin Growing in Spain under Homogeneous Growing Conditions

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Abstract: Mandarins are one of the most important citrus fruits in the world in terms of tons produced. The late-season varieties of mandarin have a great economic value due to their high production in a season with few mandarin varieties. The objective is to carry out a preliminary study of characterization and comparison of the morphological and biochemical properties of the late varieties 'Afourer', 'Tango', and 'Orri'. The characterization consisted of physicochemical parameters related to the quality of the fruits, highlighting the total antioxidant activity using ABTS and DPPH, the organic acids and sugars using HPLC and the metabolomics of the juice by ^{1}H-NMR. Afourer' mandarins were heavier and larger (120.75 g, 67.60 mm) than the other two varieties studied. Mandarins of the 'Orri' variety showed a different organic acid profile compared to the other varieties studied, and a higher amount of sugars (13.49 g/100 mL). 'Tango' variety mandarins grown on the Forner-Alcaide rootstock stood out for having a larger weight (113.52 g), a more intense color, and a greater amount of phenolic compounds (966.85 mg AGE/L Forner) than the fruits grown on *Citrus macrophylla*. The metabolomics analysis showed that these mandarin varieties had mainly non-essential amino acids.

Keywords: mandarin; late-season; Citrus rootstock; morphology; HPLC; metabolomics

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1. Introduction

Mandarins are one of the most important citrus fruits in the world in terms of tons produced. Asia has historically been the largest producer of this fruit, with 71.33% of the total world production, in 2019 China produced 52.62% of the world's mandarins [1]. Other large producers of mandarins in the world are Turkey (3.74%), Morocco (3.67%) or and Egypt (2.93 %) [1]. The largest exporter of mandarins in the world is Spain, which represents 26.77% of the traded tons of this citrus [1]. In Spain, mandarins account for almost 20% of the total citrus produced. Its high production and export make this fruit a key economic element for this sector [2].

The genus *Citrus* has a large number of varieties, cultivars, and hybrids that differ greatly in terms of their morphological and genetic traits, as well as in terms of their growth patterns and yield production [3]. Mandarins, *Citrus reticulata*, therefore have a wide number of varieties, among which the late-season varieties stand out. Some outstanding examples of late mandarin varieties are 'Afourer', also known as 'Nadorcott', was bred in 1988 in Morocco and its origin could be the hybridization between 'Murcott' variety and an unknown pollinator parent ('Murcott' is a tangor hybrid of tangerine and orange, *Citrus reticulata* Blanco × *Citrus sinensis* (L.) Osbeck) [4]. 'Orri' is a another late-season mandarin originated in Israel from irradiated bud wood of the cultivar 'Orah' ['Orah' is hybrid of 'Temple' (*Citrus temple* hort. ex Y. Tanaka) × 'Dancy' (*Citrus tangerina* hort. ex Tanaka)] [5]. 'Tango' mandarin is a hybrid citrus late-season fruit that was developed at the University of California, Riverside from an irradiated bud of the diploid mandarin hybrid 'Murcott' [6].

Mandarins, according to their ripening, can be classified into early, mid and late-season varieties. Late-season varieties are of great economic interest, for being collected in a winter period in which productions are limited and for their higher productivity. This late-season harvest provides the product with a high value, so it is of great interest to know the characteristics and agronomic-commercial advantages that each one of them presents. So that citrus growers can choose those varieties or variety/rootstock combinations that best suit their objectives.

The quality of mandarins and their appeal to consumers are determined by a combination of morphological and biochemial properties. From a morphological perspective, consumers value attributes such as color, the ease of peeling, the presence or absence of seeds or the amount of juice. While the flavor and nutritional properties of the fruits will be given by biochemical parameters such as acidity, maturity index, antioxidant activity, sugar content or organic acid concentration [7–10].

Mandarins have a high nutritional value. The main biochemical components of these fruits are sugars, organic acids, carotenoids, polyphenols (flavonoids and phenolic acids), limonoids and vitamins, highlighting vitamin C of the latter [11]. Ascorbic acid (vitamin C) is an organic acid that plays an important role in the prevention of many diseases, thanks to its antioxidant, anti-inflammatory and anticancer capacity [12–14].

The biochemical and morphological properties of the fruits can be different depending on the rootstock on which it is grown [15]. Citrus growers commonly choose the *Citrus macrophylla* rootstock to grow citrus because of its fast and abundant production [16]. However, this rootstock is sensitive to CTV (citrus tristeza virus) and to the attack of nematodes, which is why some farmers decide to cultivate their trees on a rootstock resistant to these diseases, such as Forner-Alcaide nº5 [16].

The objectives of this preliminary study were twofold. Firstly, to characterize the morphological and biochemical attributes of the late mandarin varieties 'Afourer', 'Orri', and 'Tango' under homogeneous growing conditions. This analysis aimed to compare their key characteristics and provide valuable information regarding the preferred varieties for cultivation based on fruit quality. Secondly, the study aimed to compare the main characteristics of 'Tango' mandarins when cultivated using two different rootstocks: Citrus macrophylla and Forner Alcaide nº5. The mandarins included in this study were grown across multiple farms situated in the Southeast of the Iberian Peninsula.

2. Materials and Methods

2.1. Plant Material

For this study, mandarin trees were selected from two different fields located in Southeast Spain, both having similar edaphoclimatic conditions. The three mandarin varieties ('Afourer', 'Orri', and 'Tango'), grafted on the Citrus macrophylla rootstock, were cultivated in a field situated in Torre-Pacheco (Murcia, Spain; 37°46′11.2″ N 1°01′07.4″ W). The agrometeorological conditions recorded in 2021 for this area were an average temperature of 17.97 °C, average humidity of 69.76%, wind speed of 1.14 m/s, rainfall of 249.40 mm, and evapotranspiration of 1172 mm [17]. The trees, which were 14 years old, ranged in height from 3 to 4 m and were planted within a frame measuring 5.5 × 4 m.

In contrast, the 'Tango' variety grafted on the Forner-Alcaide nº5 rootstock (*C. reshni* × *P. trifoliata*) was cultivated in a farm located in the municipality of Torremendo (Alicante, Spain; 37°58′47.8″ N 0°50′24.5″ W). In 2021, the average temperature in this area was 18.61 °C, average humidity was 68.71%, wind speed was 4.09 m/s, rainfall was 441.72 mm, and evapotranspiration was 1158.36 mm [18]. The trees in this farm were 11 years old and had a similar crop frame of 5.5 × 4 m but varied in height between 2.5 and 3 m.

For all the trees, traditional crop management practices were carried out for commercial production, including the following: water application was done through a drip irrigation system with an average consumption ranging between 5500 and 6000 m^3/ha/year. Fertilization was performed with approximately 180–200 units of NPK per year, 100 units of P_2O_5 per year, and 50 units of K_2O per year. Annual pruning was conducted for all the trees. Phytosanitary treatments and addressing nutrient deficiencies were applied as needed, varying accordingly. At the time of fruit harvest, all the trees exhibited good development and phytosanitary conditions.

All the studied mandarin varieties were manually harvested when they reached their state of commercial maturity, as defined by DOUE-L-2021-81443 [19]. This state is determined based on several criteria: the juice content (>33%), the ratio of sugars to acids (7.5:1), the coloration (typical coloration of the variety on at least one-third of the fruit's surface), and the caliber (>45 mm). These criteria ensure that the mandarins meet the required quality standards for commercial purposes.

Fifty fruits were carefully collected from each variety/rootstock combination, sourced from five different trees. The selection of fruits was done randomly, ensuring an unbiased representation. To maintain consistency, the fruits were collected at the same height and from all sides of the trees. Subsequently, the mandarin fruits were transported to the laboratory and stored at a controlled temperature of 4 °C until the analysis and process. The fruits were analyzed on the same collected day.

2.2. Biometric and Physical Parameters

Fruit weight (g), equatorial diameter (mm), and polar diameter (mm) were the physical parameters measured of the fruit samples. The fruits were cut along the equatorial region and the peel thickness, number of carpels, and number of seeds were measured. For juice extraction, an electric juicer (Mpz22, Braun, Spain) was employed, and the volume of juice and the weight of the peel were accurately recorded. The weight of the juice was determined by calculating the difference between the fruit weight and the peel weight. Juice yield was calculated and expressed as the percentage of juice volume per fruit weight.

To evaluate the peel color, measurements were taken at three equidistant points from the equatorial region of the fruit using a colorimeter (CM-700d portable spectrophotometer, Konica Minolta, Osaka, Japan). The colorimeter used a view angle of 10º and standard illuminant D65, conforming to the standards set by the Commission Internationale de l'Eclairage (CIE) [20]. The values obtained included L*(brightness; where black = 0, white = 100), a*(↓a*: green, ↑a*: red), b*(↓b*: blue, ↑b*: yellow), h[h = arctang (b*/a*), where red = 0, yellow = 90, teal = 180, and blue = 270], and the C* [color intensity or saturation, C* = (a*2 + b*2)/2]. In addition, the Jimenez Cuesta citrus color index was calculated using the formula (CI) [CI = (1000*a)/(L*b)] [21]. The color evaluation of the juice was conducted separately. Measurements were performed in triplicate using a colorimetry cuvette, ensuring consistent and reliable results. The color evaluation followed the standardized guidelines established by the Commission Internationale de l'Eclairage (CIE).

2.3. Chemical Parameters

The titratable acidity (TA) expressed in g of citric acid/L and the pH were measured with an automatic acidity titrator (877 Titrino plus, Metrohm, Herisau, Switzerland) with 0.1 N NaOH until reaching a pH of 8.1, using 5 mL of mandarin juice diluted in 50 mL of distilled water. With the same device, the pH was also measured. Total soluble solids (TSS) were determined using a handheld refractometer (N1 model, Atago, Tokyo, Japan) and the results were expressed in ºBrix at 20 °C. The Maturity Index was calculated as the SST/TA ratio.

2.4. Antioxidant Activity

Total phenols were quantified following the methodology described by Singleton et al. [22]. The Folin-Ciocalteu reagent was utilized, and samples were analyzed in triplicate at a wavelength of 760 nm using a spectrophotometer (Helios γ, Thermo Spectronic, Cambridge, UK). The concentration of phenols was determined by constructing a calibration curve using gallic acid as the reference compound. The results were expressed as milligrams of gallic acid equivalent per liter of juice (mg GAE/L).

Total antioxidant activity was assessed according to the protocol outlined by Martínez-Nicolas et al. [23]. The ABTS+ (Azinobis [3-ethylbenzothiazolin-6-sulfonic] radical) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical methods were employed. Measurements were conducted in triplicate at 760 nm using a spectrophotometer (Helios γ, Thermo Spectronic, Cambridge, UK). The antioxidant activity results were expressed as milligrams of Trolox equivalent per milliliter of juice (mg Trolox/mL).

2.5. Organic Acids and Sugars

Organic acids and sugars in the mandarin juice were quantified as described by Legua et al. [10], briefly: filtered mandarin juice was centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was filtered through a cellulose nitrate membrane filter (0.45 µm). Afterwards, it was analysed by High-Performance Liquid Chromatography (HPLC, Hewlett Packard 1100 series, Wilmington, DE, USA), using a Supelcocolumn column [Supelcogel C-610H column (30 cm × 7.8 mm ID), Supelco] and a Supelguard precolumn [C610H (5 cm × 4.6 mm), Supelco]. A 0.1% phosphoric acid mobile phase was used at a flow rate of 0.5 mL/min. Organic acids were detected by an ultraviolet diode detector at 210 nm, for sugars a refractive index detector was used. The quantification of organic acids and sugars was performed by comparing the retention times and the area of the peaks with a standard curve of pure acids and sugars. The results of acids and sugars were expressed in g/100 mL. All the chemical reagents and standards used in the HPLC analysis were appropriate and sourced from Sigma Aldrich. These high-quality materials ensure accuracy and reliability in the analytical process.

2.6. Juice Metabolomics

The metabolomics of mandarin juice was measured by nuclear magnetic resonance (1H-NMR) following the methodology described by Melgarejo et al. [24].

2.7. Statistical Analysis

For the statistical analysis, the Statgraphics -Centurion 18 (Chicago, IL, USA) program was used. An analysis of variance was performed using an ANOVA of one factor for the locations of Abanilla and Campo de Cartagena separately. For the separation of means, Tukey's HSD test was used with a confidence level of 95%.

3. Results and Discussion

3.1. Biometric and Physical Parameters

At first sight and externally, these three varieties differ slightly in color tones or size (Figure 1). When the physical characterization was carried out in the laboratory, differences were observed in some parameters such as the weight of the fruits (Table 1). The 'Afourer' variety exhibited significantly higher weight (120.75 g) compared to the other two, with a noticeable difference of up to 20%. The weight of the 'Afourer' variety was much higher than that described by Tarancon et al. for this same variety, even our results are close to the heaviest varieties of mandarin hybrids described in their study [25]. While the equatorial diameter of the 'Afourer' variety was significantly larger, no significant differences were observed in the polar and equatorial diameters among the three varieties studied.

The number of carpels per fruit showed no significant variation among the three varieties, averaging around 9 carpels per fruit. Moreover, most of the fruits in all three varieties were found to be seedless, which is considered a desirable trait by consumers. The thickness of the peel did not differ significantly among the three varieties, with values ranging between 3.25 and 4.13 mm. However, the 'Afourer' variety exhibited a higher peel weight (51.91 g), up to 18% heavier than the peels of 'Orri' and 'Tango' varieties.

In terms of juice characteristics, both weight and volume were higher in the 'Afourer' and 'Orri' varieties compared to 'Tango'. However, the juice yield, calculated as a percentage of juice volume to fruit weight, was higher in the 'Orri' variety (53.47%) than in the other two varieties. In contrast to other studies of mandarin hybrids, our results showed that there can be significant differences in juice yield between these varieties [25]. This difference in yield can be attributed to the fact that although 'Afourer' has a higher fruit weight than 'Orri', it contains a larger amount of pulp. Consequently, the 'Orri' variety, known for its juicy carpels, is likely to be preferred by consumers.

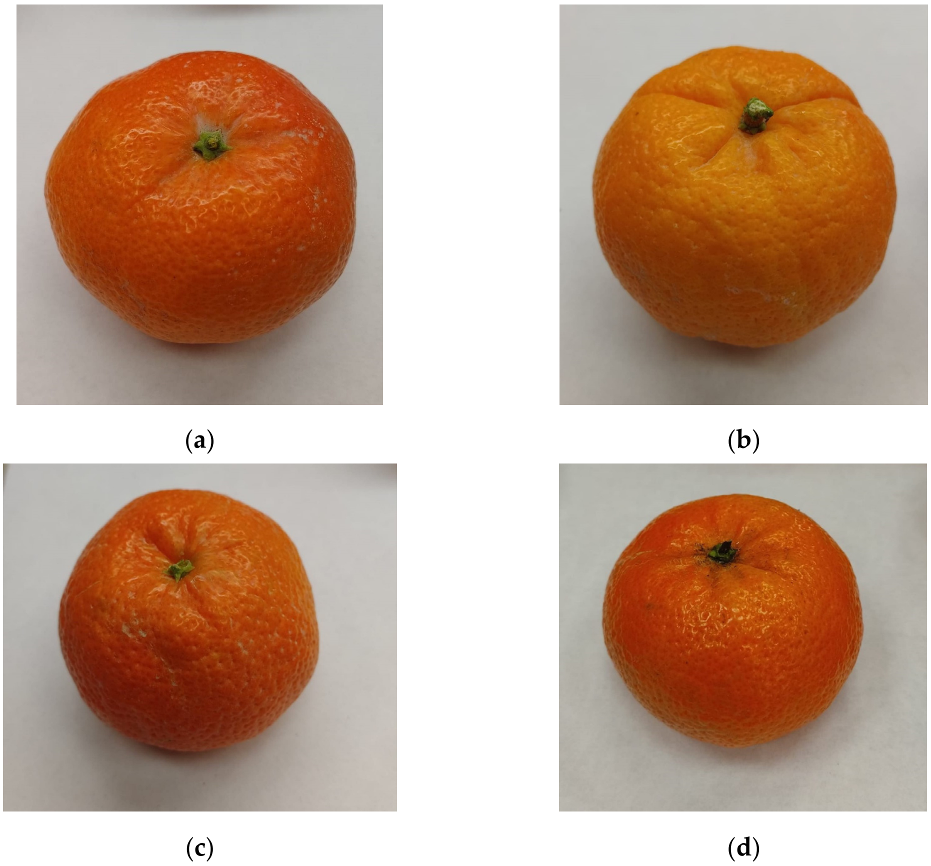

Figure 1. External photographs of the 3 mandarin varieties studied. (**a**) 'Afourer' variety; (**b**) 'Orri' variety; (**c**) 'Tango' variety cultivated on the *Citrus macrophylla* rootstock; (**d**) 'Tango' variety cultivated on the Forner-Alcaide nº5 rootstock.

Table 1. Physical properties of the three varieties of mandarins, cultivated on the *Citrus macrophylla* (CM) rootstock. Values are expressed as mean ± SE (n = 50).

Parameters	'Afourer'	'Orri'	'Tango'
Fruit weight (g)	120.75 ± 3.53 a	110.35 ± 2.59 b	99.91 ± 2.28 c
Equatorial diameter (mm)	67.60 ± 0.77 a	63.59 ± 0.53 b	62.90 ± 0.54 b
Polar diameter (mm)	48.80 ± 0.49 a	48.61 ± 0.48 a	47.33 ± 0.42 a
Number of carpels	9.44 ± 0.15 a	9.52 ± 0.15 a	9.38 ± 0.13 a
Number of seeds	0.24 ± 0.09 a	0.24 ± 0.09 a	0.02 ± 0.02 a
Peel thickness (mm)	3.31 ± 0.09 a	4.13 ± 0.72 a	3.25 ± 0.08 a
Peel weight (g)	51.91 ± 2.04 a	43.95 ± 1.38 b	44.78 ± 1.46 b
Juice weight (g)	68.84 ± 1.78 a	66.40 ± 1.47 a	55.13 ± 1.15 b
Juice volume (mL)	59.10 ± 2.09 a	58.72 ± 1.55 a	47.44 ± 1.51 b
Juice yield (%)	49.05 ± 1.07 b	53.47 ± 1.03 a	47.41 ± 1.04 b

The different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$).

Table 2 presents the results of the physical characterization of 'Tango' mandarins grown on two different rootstocks, Forner-Alcaide nº5 (FA) and *Citrus macrophylla* (CM). The average weight of mandarins cultivated on FA rootstock was 113.52 g, which was approximately 13 g heavier than those grown on CM rootstock. While there were no significant differences in the polar diameter between the two rootstocks, the equatorial diameter of mandarins cultivated on FA rootstock was found to be statistically greater than those grown on CM. The number of carpels per fruit did not differ significantly between the two rootstocks, with an average of 9 carpels per fruit. Additionally, neither rootstock exhibited seeds in the 'Tango' mandarins.

Table 2. Physical properties of 'Tango' variety cultivated on the Forner-Alcaide nº5 rootstock (FA) compared to the *Citrus macrophylla* rootstock (CM). Values are expressed as mean ± SE (n = 50).

Parameters	CM	FA
Fruit weight (g)	99.91 ± 2.28 b	113.52 ± 3.07 a
Equatorial diameter (mm)	62.90 ± 0.54 b	66.46 ± 0.67 a
Polar diameter (mm)	47.33 ± 0.42 a	47.71 ± 0.49 a
Number of carpels	9.38 ± 0.13 a	9.40 ± 0.12 a
Number of seeds	0.02 ± 0.02 a	0 a
Peel thickness (mm)	3.25 ± 0.08 a	3.13 ± 0.08 a
Peel weight (g)	44.78 ± 1.46 b	49.42 ± 1.75 a
Juice weight (g)	55.13 ± 1.15 b	64.10 ± 1.73 a
Juice volume (mL)	47.44 ± 1.51 b	55.98 ± 1.64 a
Juice yield (%)	47.41 ± 1.04 a	49.43 ± 0.74 a

The different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$).

When it comes to juice characteristics, both the weight and volume of juice obtained from mandarins grown on FA rootstock (64.10 g, 55.98 mL) were higher compared to those grown on CM rootstock (55.13 g, 47.44 mL). However, no significant differences were observed in the juice yield between the two rootstocks.

Color is an important parameter in the quality of the fruits for consumers. In Table 3 we can see the results of peel color and juice in the three varieties studied. According to sensory analyzes carried out by Gámbaro et al. to consumers, the greater brightness and orange hue in peel is better valued by consumers [26]. There were significant differences in all peel color parameters among the three varieties. The peel of the 'Orri' variety was brighter (L*) than the other two varieties. According to the CI, the 'Afourer' variety showed the most orange color (14.32), followed by the 'Tango' variety (13.41) and the 'Orri' variety with less tonality (12.03). According to the CI, all the fruits displayed a vibrant orange color, which is characteristic of this citrus. Regarding the color of the juice, we see from the CI that the varieties of 'Afourer' (9.18) and 'Tango' (9.55) had a more orange color than the variety 'Orri' (7.50).

Table 3. CIE parameters and color index (CI) of the peel and juice of the three varieties of mandarins, cultivated on the *Citrus macrophylla* (CM) rootstock. Parameters: L* (brightness; where black = 0, white = 100), a* (↓a*: green, ↑a*: red), b* (↓b*: blue, ↑b*: yellow), h (where red = 0, yellow = 90, teal = 180, and blue = 270), C* (color intensity or saturation) and CI (Color Index). Values are expressed as mean ± SE (n = 50 peel samples, n = 5 juice samples).

	Parameters	'Afourer'	'Orri'	'Tango'
Peel	L*	58.70 ± 0.26 b	62.65 ± 0.20 a	59.45 ± 0.24 b
	a*	37.89 ± 0.27 ab	38.18 ± 0.22 a	37.02 ± 0.36 b
	b*	45.45 ± 0.42 c	50.89 ± 0.28 a	46.73 ± 0.41 b
	C*	59.37 ± 0.34 b	63.69 ± 0.17 a	59.76 ± 0.39 b
	h	59.37 ± 0.34 a	53.09 ± 0.28 b	51.60 ± 0.37 c
	CI	14.32 ± 0.24 a	12.03 ± 0.16 c	13.41 ± 0.22 b
Juice	L*	40.77 ± 0.44 b	43.90 ± 0.13 a	41.11 ± 0.50 b
	a*	3.92 ± 0.33 a	4.70 ± 0.12 a	4.30 ± 0.38 a
	b*	10.42 ± 0.58 b	14.32 ± 0.46 a	10.90 ± 0.81 b
	C*	11.13 ± 0.66 a	15.07 ± 0.46 a	11.72 ± 0.89 a
	h	69.49 ± 0.52 b	71.79 ± 0.46 a	68.57 ± 0.32 b
	CI	9.18 ± 0.16 a	7.50 ± 0.21 b	9.55 ± 0.07 a

The different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$).

According to the color data, the external appearance of 'Tango' variety fruits cultivated on the CM rootstock (Table 4) showed higher brightness (L*) but a less orange color compared to those grown on the FA rootstock. In contrast, the fruits cultivated on the FA rootstock displayed a more intense orange color. Additionally, the juice obtained from the FA rootstock exhibited a richer, more vibrant orange hue.

Table 4. CIE parameters and color index (CI) of the peel and juice of 'Tango' variety cultivated on the Forner-Alcaide nº5 rootstock (FA) compared to the Citrus macrophylla rootstock (CM). Parameters: L* (brightness; where black = 0, white = 100), a* (↓a*: green, ↑a*: red), b* (↓b*: blue, ↑b*: yellow), h (where red = 0, yellow = 90, teal = 180, and blue = 270), C* (color intensity or saturation) and CI (Color Index). Values are expressed as mean ± SE (n = 50 peel, n = 5 juice).

	Parameters	CM	FA
Peel	L*	59.45 ± 0.24 a	58.05 ± 0.21 b
	a*	37.02 ± 0.36 b	38.95 ± 0.25 a
	b*	46.73 ± 0.41 a	43.35 ± 0.32 b
	C*	59.76 ± 0.39 a	58.36 ± 0.33 b
	h	51.60 ± 0.37 a	48.02 ± 0.24 b
	CI	13.41 ± 0.22 b	15.54 ± 0.18 a
Juice	L*	41.11 ± 0.50 b	42.84 ± 0.03 a
	a*	4.30 ± 0.38 b	5.77 ± 0.13 a
	b*	10.90 ± 0.81 b	13.31 ± 0.19 a
	C*	11.72 ± 0.89 b	14.51 ± 0.22 a
	h	68.57 ± 0.32 ab	14.51 ± 0.22 a
	CI	9.55 ± 0.07 b	10.12 ± 0.10 a

The different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$).

3.2. Chemical Parameters

Analysis of the chemical parameters among the three varieties studied are shown in Table 5. The pH was lower in the variety 'Afourer' (3.69) and higher in 'Orri' (4.24), the results obtained in 'Tango' variety were intermediate between the other two varieties. The titratable acidity was up to 2 g of citric acid/L higher in 'Afourer' and 'Orri' varieties than in 'Tango' variety. Soluble solids are linked to the sweetness of mandarin because 80% of these are sugars [27]. The total soluble solids were higher in the fruits of 'Orri' (13.72 ºBrix), followed by those of 'Afourer' (12.36 ºBrix) and in lower quantity in those of 'Tango' (10.80 ºBrix). The total soluble solids of 'Orri' variety was similar to that observed in other commercial mandarin varieties, such as 'Fortuna' (13.8 ºBrix) [28]. No significant differences were seen in the maturity index among the three varieties studied. In all cases, the minimum MI of 6.5 was reached for the commercialization of late-season mandarins [29].

Table 5. Chemical properties of the three varieties of mandarins, cultivated on the *Citrus macrophylla* (CM) rootstock. Parameters: pH, acidity titratable (TA), total soluble solids (TSS), maturity index (MI). Values expressed as mean ± SE (n = 5).

Parameters	'Afourer'	'Orri'	'Tango'
pH	3.69 ± 0.09 b	4.24 ± 0.19 a	3.83 ± 0.03 ab
TA (g citric acid/L)	9.25 ± 0.55 a	9.51 ± 0.29 a	7.40 ± 0.38 b
TSS (ºBrix)	12.36 ± 0.25 b	13.72 ± 0.22 a	10.80 ± 0.31 c
MI	13.52 ± 0.68 a	14.47 ± 0.39 a	14.74 ± 0.87 a

The different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$).

About the chemical properties of 'Tango' variety mandarins cultivated on the FA rootstock (Table 6), the pH was 4.00, significantly higher than that observed in 'Tango' cultivated on CM. The titratable acidity was 9.30 ± 0.41 g of citric acid/L, being statistically higher than the acidity observed on CM. 11.72 ºBrix detected in the juice, these data were similar to those obtained by this same study in CM, although slightly lower than those shown by Morales et al. for this same variety [30]. The MI of the fruits in FA reached the minimum required for its commercialization [29].

Table 6. Chemical properties of 'Tango' variety cultivated on the Forner-Alcaide nº5 rootstock (FA) compared to the *Citrus macrophylla* rootstock (CM). Parameters: pH, acidity titratable as citric acid (TA), total soluble solids (TSS), maturity index (MI). Values expressed as mean ± SE (n = 5).

Parameters	CM	FA
pH	3.83 ± 0.03 b	4.00 ± 0.03 a
TA (g citric ac./L)	7.40 ± 0.38 b	9.30 ± 0.41 a
TSS (ºBrix)	10.80 ± 0.31 a	11.72 ± 0.34 a
MI	14.74 ± 0.87 a	12.66 ± 0.42 a

The different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$).

3.3. Antioxidant Activity

The quantification of total phenols in juice enables us to estimate the antioxidant activity of the fruits, because these compounds have the ability to neutralize free radicals by donating hydrogen atoms [31]. In Table 7 we observed a higher amount of total phenols in 'Orri' variety (1097.21 mg AGE/L), up to 40% more than those observed in 'Tango' variety (752.14 mg AGE/L), 'Afourer' variety (857.31 mg GAE/L) did not show significant differences with Orri or Tango. The values of 'Tango' and 'Afourer' were similar to those described by Rekha et al. for mandarins (*Citrus reticulata*) (800 µg GAE/mL) [32]. The results in total phenols of these three late-season varieties were lower than those reported for clementines in another study [33]. On the other hand, no significant differences were observed in the total antioxidant activity among the three varieties with any of the two methods.

Table 7. Total phenols and antioxidant activity of the three varieties of mandarins, cultivated on the *Citrus macrophylla* (CM) rootstock. Parameters: Total phenols (TP), and total antioxidant activity according to the ABTS and DPPH methods. Values are expressed as mean ± SE (n = 3).

Parameters	'Afourer'	'Orri'	'Tango'
TP (mg AGE/L)	857.31 ± 79.70 ab	1097.21 ± 97.90 a	752.14 ± 35.62 b
ABTS (mg Trolox/mL)	3.32 ± 0.40 a	2.35 ± 0.04 a	3.85 ± 0.48 a
DPPH (mg Trolox/mL)	4.79 ± 0.08 a	3.56 ± 0.49 a	4.14 ± 0.08 a

The different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$).

Regarding the fruits of 'Tango' variety cultivated on FA (Table 8), they had significantly more total phenols than those described for this same variety in CM, 28% more of these biomolecules. The AAT results in AF with the ABTS method were shown to be statistically inferior to those obtained in the fruits of 'Tango' cultivated on CM. However, no differences were observed between both rootstocks using the DPPH method.

Table 8. Total phenols and antioxidant activity of 'Tango' variety cultivated on the Forner-Alcaide nº5 rootstock (FA) compared to the *Citrus macrophylla* rootstock (CM). Parameters: Total phenols (TP), and total antioxidant activity according to the ABTS and DPPH methods. Values expressed as mean ± SE (n = 3).

Parameters	CM	FA
TP (mg AGE/L)	752.14 ± 35.62 b	966.85 ± 24.88 a
ABTS (mg Trolox/mL)	3.85 ± 0.48 a	2.61 ± 0.10 b
DPPH (mg Trolox/mL)	4.14 ± 0.08 a	4.23 ± 0.21 a

The different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$).

3.4. Organic Acids and Sugars Content

Table 9 shows the organic acids detected by HPLC. Citric, malic, ascorbic, succinic, and formic acids were detected in all the samples studied. When comparing the three mandarin varieties studied, a significant difference was observed in the organic acid profiles. Citric acid was the majority in 'Afourer' variety (1.02 g/100 mL), it was in large quantities in 'Tango' (0.79 g/100 mL), but it was in low quantities in 'Orri' (0.11 g/100mL). The results in 'Afourer' and 'Tango' are similar to those detected in tangerines [34]. Malic acid was the majority in 'Orri' variety (0.61 g/100 mL) and was also statistically higher than in 'Afourer' and 'Tango' varieties (0.28 g/100 mL, 0.27 g/100mL). The very high values of malic acid obtained in 'Orri' variety are like those we would find in apples [35]. 0.02 g/100 mL of ascorbic acid was detected in 'Afourer' and 'Tango' varieties, and 0.01 g/100 mL in 'Orri' variety. A significantly higher amount of succinic acid was observed in the varieties 'Afourer' and 'Tango' than in the variety 'Orri'. While the variety 'Orri' shows similar values to other late-season mandarins, the values of 'Afourer' and 'Tango' are substantially higher [3]. A significantly higher amount of formic acid was found in 'Orri' variety, somewhat lower in 'Afourer' fruits and a lower amount in 'Tango'. The total amount of organic acids show a lower amount of acids in 'Orri' variety (1.44 g/100 mL) compared to 'Afourer' and 'Tango' varieties (2.3 g/100 mL, 2.07 g/100mL). The organic acid profile of 'Orri' variety is remarkable as it does not resemble two other mandarin varieties. This variation could be due to an adaptation of this variety to dry climates, as indicated in a study on Orah mandarins, a variety phylogenetically close to 'Orri' variety, growing in dry climates would increase the presence of malic acid [36]. Such a high presence of malic acid in 'Orri' would cause differences in flavour concerning the other two varieties, although the characteristic flavour of citrus fruit would be maintained since this acid together with the citric acid gives the characteristic mandarin flavour [37].

Table 9. Organic acids and sugars (g/100 mL) of the three varieties of mandarins, cultivated on the *Citrus macrophylla* (CM) rootstock. Values are expressed as mean ± SE (n = 3).

	Parameters	'Afourer'	'Orri'	'Tango'
Organic acids	Citric acid	1.02 ± 0.03 a	0.11 ± 0.01 c	0.79 ± 0.02 b
	Malic acid	0.28 ± 0.02 b	0.61 ± 0.02 a	0.27 ± 0.01 b
	Ascorbic acid	0.02 a	0.01 b	0.02 a
	Succinic acid	0.76 ± 0.06 a	0.37 ± 0.04 b	0.87 ± 0.03 a
	Formic acid	0.22 ± 0.02 b	0.34 ± 0.01 a	0.12 c
	Total acids	2.3 ± 0.03 a	1.44 ± 0.07 c	2.07 ± 0.02 b
Sugars	Sucrose	5.58 ± 0.07 ab	5.99 ± 0.16 a	5.27 ± 0.03 b
	Fructose	2.95 ± 0.05 a	2.91 ± 0.03 a	2.78 ± 0.16 a
	Glucose	3.87 ± 0.06 b	4.59 ± 0.11 a	3.43 ± 0.09 c
	Total sugars	12.4 ± 0.11 b	13.49 ± 0.29 a	11.48 ± 0.27 b

The different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$).

As for 'Tango' variety cultivated on Forner-Alcaide nº5 (Table 10), we see that citric acid is the majority in these mandarins with 0.99 g/100 mL, unlike those analysed in this study on the CM rootstock. Succinic acid was also found in large quantities (0.81 g/100 mL). Malic, ascorbic and formic acids were detected in smaller quantities, in quantities similar to those obtained in 'Tango' on CM. The amount of organic acids was considerably higher than those described by Morales et al. (12.07–13.69 g/L) in this same variety and rootstock [30].

Table 9 also shows the results of the sugars detected by HPLC. In mandarins, the concentration ratio of sucrose, fructose and glucose is usually 2:1:1, a similar ratio was identified in all our analysed fruits [38]. The sugar contents among the three varieties studied showed that 'Orri' variety (13.49 g/100 mL) has a higher amount of sugars than in the other two varieties, with a significant difference of up to 18% between the values of 'Orri' and 'Tango'. The sucrose values reached 5.99 g/100 mL in 'Orri' variety, being statistically higher than those observed in 'Tango' (5.27 g/100 mL), with the concentrations of sucrose remaining in intermediate statistical values than the variety 'Afourer'. No significant differences were seen in the amounts of fructose among the three varieties, with values between 2.78 g/100 mL and 2.95 g/100 mL. As for glucose, 'Orri' mandarins again had the highest amount of this sugar (4.59 g/100 mL), followed by 'Afourer' variety and finding less of this sugar in 'Tango' variety (3.43 g/100 mL). The values of each of the sugars were similar to those that we could find in clementine varieties [33]. The glucose and fructose values were higher than those described by Sdiri et al. in other late-season mandarin varieties [7].

Table 10. Organic acids and sugars (g/100 mL) of 'Tango' variety cultivated on the Forner-Alcaide nº5 rootstock (FA) compared to the *Citrus macrophylla* rootstock (CM). Values are expressed as mean ± SE (n = 3).

	Parameters	CM	FA
Organic acids	Citric acid	0.79 ± 0.02 b	0.99 ± 0.02 a
	Malic acid	0.27 ± 0.01 a	0.25 ± 0.02 a
	Ascorbic acid	0.02 a	0.02 a
	Succinic acid	0.87 ± 0.03 a	0.81 ± 0.1 a
	Formic acid	0.12 a	0.13 ± 0.02 a
	Total acids	2.07 ± 0.02 a	2.21 ± 0.09 a
Sugars	Sucrose	5.27 ± 0.03 a	5.47 ± 0.09 a
	Fructose	2.78 ± 0.16 a	2.65 ± 0.06 a
	Glucose	3.43 ± 0.09 a	3.62 ± 0.06 a
	Total sugars	11.48 ± 0.27 a	11.74 ± 0.16 a

The different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$).

This study failed to identify any significant difference in the concentration of the three sugars between the fruits of 'Tango' variety cultivated on the FA and CM rootstocks (Table 10). The sweetness in the fruits of both rootstocks resulted identical. These results show a higher amount of sugars than those described by Morales et al. for this same variety [30].

3.5. *Juice Metabolomics*

Metabolomic analysis with 1H-NMR (Tables 11 and 12) showed the presence of the amino acids GABA, Alanine, Arginine, Asparagine, Aspartate, Glutamine, Leucine, Isoleucine, Proline, Tyrosine, Valine in all the samples analysed. The most abundant amino acid was aspartate, with values ranging from 21.75 mM ('Tango' on FA rootstock) to 108.2 mM ('Orri'). No significant differences were found in any amino acid among the three varieties studied, except for proline, which was found in higher amounts in the 'Orri' variety. Proline is closely linked to plant response to abiotic stress, so this higher concentration in 'Orri' variety suggests greater adaptability to stress [39]. As for the analysis of the 'Tango' variety grown on different rootstocks, there were no significant differences in most amino acids, except for GABA, Asparagine, Aspartic Acid and Tyrosine. The greater amount of these metabolites in the CM rootstock, which is known to be involved in the response to biotic and abiotic stress, this could indicate that this rootstock may have a higher resistance to certain types of stresses [40,41]. In general, citrus fruits have a low amount of amino acids and those present are usually non-essential such as alanine, arginine, asparagine, glutamine, aspartic acid, tyrosine or proline [42] presents in our analysis. Although we also found some essential amino acids in our samples such as leucine, isoleucine, or valine, these are found in the lowest concentrations.

Table 11. Amino acids and other metabolites (mM) of the three varieties of mandarins, cultivated on the *Citrus macrophylla* (CM) rootstock. Values are expressed as mean ± SE (n = 3).

	Parameters	**'Afourer'**	**'Orri'**	**'Tango'**
Amino acids	GABA	2.68 ± 0.6 a	3.43 ± 1.04 a	1.92 ± 0.08 a
	Alanine	2.36 ± 0.69 a	1.35 ± 0.41 a	1.37 ± 0.07 a
	Arginine	12.05 ± 1.93 a	19.02 ± 3.47 a	7.32 ± 0.13 a
	Asparagine	14.15 ± 6.25 a	15.51 ± 4.51 a	6.42 ± 0.05 a
	Aspartic Acid	36.73 ± 17.56 a	108.2 ± 18.8 a	36.61 ± 0.69 a
	Glutamine	2.03 ± 0.39 a	5.15 ± 1.18 a	1.81 ± 0.13 a
	Isoleucine	0.07 ± 0.02 a	0.08 ± 0.02 a	0.05 a
	Leucine	0.04 a	0.06 ± 0.02 a	0.04 a
	Proline	10.55 ± 0.83 ab	37.7 ± 8.66 a	5.97 ± 0.13 b
	Tyrosine	1.12 ± 0.2 a	2.4 ± 0.59 a	1.06 a
	Valine	0.22 ± 0.05 a	0.19 ± 0.04 a	0.16 a
Other metabolites	Choline	0.49 ± 0.17 a	0.65 ± 0.14 a	0.53 ± 0.12 a
	Ethanol	3.77 ± 1.09 a	1.62 ± 0.13 a	1.63 ± 0.42 a
	Trigonelline	0.14 ± 0.03 a	0.16 ± 0.05 a	0.09 ± 0.01 a

The different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$).

Table 12. Amino acids and other metabolites (mM) of 'Tango' variety cultivated on the Forner-Alcaide nº5 rootstock (FA) compared to the *Citrus macrophylla* rootstock (CM). Values are expressed as mean ± SE (n = 3).

	Parameters	**CM**	**FA**
Amino acids	GABA	1.92 ± 0.08 a	0.65 ± 0.13 b
	Alanine	1.37 ± 0.07 a	2.46 ± 0.52 a
	Arginine	7.32 ± 0.13 a	8.7 ± 0.32 a
	Asparagine	6.42 ± 0.05 b	7.14 ± 0.02 a
	Aspartic Acid	36.61 ± 0.69 a	21.75 ± 0.05 b
	Glutamine	1.81 ± 0.13 a	1.92 ± 0.23 a
	Isoleucine	0.05 a	0.05 a
	Leucine	0.04 a	0.06a
	Proline	5.97 ± 0.13 a	10.77 ± 1.35 a
	Tyrosine	1.06 a	0.89 ± 0.03 b
	Valine	0.16 a	0.17 a
Other metabolites	Choline	0.53 ± 0.12 a	0.36 ± 0.01 a
	Ethanol	1.63 ± 0.42 b	4.59 ± 0.37 a
	Trigonelline	0.09 ± 0.01 a	0.16 ± 0.02 a

The different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$).

Other metabolites detected (Tables 11 and 12) in the mandarin juice were choline, ethanol and trigonelline. No significant differences were found between the three varieties in any metabolite; however, we observed a higher amount of ethanol in 'Tango' variety grown on FA than on CM. Ethanol was the most abundant secondary metabolite, with concentrations between 1.08 mM and 5.9 mM. Ethanol is a secondary metabolite produced by anaerobic respiration, it is involved in fruit ripening and in the generation of volatile compounds that give the aromas [43]. In smaller amounts, we find choline, with values between 0.76 mM and 0.36 mM and trigonelline 0.28 mM and 0.09 mM. Choline (also known as vitamin B4) is essential for humans, this biomolecule is involved in the formation of cell membranes and is a precursor of the neurotransmitter acetylcholine [44]. Trigonelline is an alkaloid present in plants, commonly found in coffee in concentrations of around 1%, giving this fruit part of its characteristic aroma [45]. Trigonelline accumulates in plants under biotic and abiotic stress conditions, and together with choline it can protect the plant from fungal infections [46,47]. Trigonelline as a biomolecule has antioxidant and antidiabetic effects that could be of interest to the pharmaceutical industry.

4. Conclusions

Three late varieties of mandarin have been characterized and compared under homogeneous growing conditions. 'Afourer' variety stood out for being larger and weighing up to 20% more than the rest of the varieties. 'Afouer' also contains a large volume of juice and is more orange. Its main organic acid was citric. 'Orri' variety stood out for having a large amount of juice, higher brightness, and a sugar concentration 18% higher than in the other two sugar concentration varieties. However, its organic acid profile was different from that of the other varieties, with malic as the main acid. The total antioxidant activity was not significantly different between the three varieties.

'Tango' mandarins cultivated on two different rootstocks were analyzed, highlighting that the fruits cultivated on FA have a greater weight and size, as well as a more orange hue than those cultivated on the CM rootstock. The HPLC results showed that the organic acid profile was quite similar between both rootstocks, citric acid was found in high amounts in both rootstocks. The total antioxidant activity was similar, although up to 28% more phenols were observed in the FA rootstock. All this indicates that the fruits in both rootstocks will have a very similar organoleptic quality.

The metabolomics analysis revealed that all the analyzed mandarin samples contained predominantly non-essential amino acids such as aspartate, alanine, or proline. Other metabolites such as ethanol, choline or trigonelline were detected. Significant differences were observed in some amino acids such as GABA or aspartic acid in 'Tango' fruits grown on different rootstocks.

The results presented in this preliminary study are based on data collected from a single year. However, comprehensive analyses over multiple years will be conducted in the future to validate and reinforce the findings reported in this study.

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