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ORIGINAL PAPER

Differentiation of juice of mandarin-like hybrids based on physicochemical characteristics, bioactive compounds, and antioxidant capacity

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Abstract

In this study, samples of mandarin-like hybrids (Clemenvilla, Nadorcott and Ortanique) from two harvesting seasons (2017−2018 and 2018−2019) were analyzed, to evaluate its differences in physicochemical characteristics and nutritional properties and establish the parameters that allow classify these citrus cultivars. Results showed that Clemenvilla juice had the highest concentration of total phenolic and ascorbic acid and are strongly correlated to its higher antioxidant capacity. Flavonoids were higher in Nadorcott samples. Large differences of total carotenoids were observed in juice analyzed. Varieties and harvesting seasons significantly influenced ($p < 0.05$) the physicochemical properties, bioactive compounds content and antioxidant capacity of samples. The pH, flavonoids, ascorbic acid, DPPH and TEAC values were determined as predictor parameters to classify the groups according to the varieties, concluding that Nadorcott samples were clearly different. The data presented in this research will currently provide information about the physicochemical evaluation of mandarin-like hybrid varieties and their potential as source of bioactive compounds and antioxidant capacity.

Keywords Mandarin juice · Hybrid varieties · Physicochemical characteristics · Bioactive compounds · Antioxidant capacity

Introduction

Mandarins (*Citrus reticulata* Blanco) are one of the most important citrus crops and represented the 26% of worldwide citrus production in 2019 [1]. After oranges production, mandarins are the second of major citrus fruits produced in the Mediterranean region [2]. The citrus producers have introduced mandarin-like cultivars (varieties of hybrids and tangor mandarins) to provide mandarins throughout the year. The knowledge of organoleptic and nutritional properties of these mandarin-like cultivars could contribute to increasing their consumption and exploitation.

The extraction of juice is one of the main activities (approximately 18%) of citrus fruits grown worldwide for industrial processes [3]. In the last two decades, the bioactive constituents (flavonoids, carotenoids, and others) from citrus products as mandarin juice have been investigated. Citrus carotenoids are mainly responsible for the color in citrus juice. The antioxidant capacity of mandarin juice is mainly attributed to ascorbic acid and polyphenols content [4]. In addition to influencing the quality of citrus products, citrus bioactive compounds are important in food industry for their nutraceutical effects and health-related benefits in the prevention of metabolic and cardiovascular diseases and some types of cancer [5–7].

Numerous studies describe the extraction of bioactive compounds from most knowledge mandarin varieties juice [8–10]. In contrast, just few studies have analyzed the physical and nutritional characteristics of some mandarin-like hybrids selected. Also, recent studies are focused on the management in agriculture and organoleptic quality of citrus hybrids production [11, 12]. The aim of the study is providing a correlation of juice quality and nutritional parameters with the analyzed mandarin varieties. In this line, physicochemical properties, bioactive compounds content

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and antioxidant capacity of three mandarin-like hybrids (Clemenvilla, Nadorcott and Ortanique) juice were evaluated. The discriminant functions analysis could be a useful method to determine the physicochemical and phytochemical properties as predictor parameters to classifying these citrus cultivars.

Materials and methods

Chemicals and reagents

Gallic acid monohydrate, sodium hydroxide (NaOH), potassium persulphate ($K_2S_2O_8$) and 2,6-dichlorophenolindophenol (2,6-DCPI) were purchased from Panreac (Barcelona, Spain). Metaphosphoric acid (HPO_3), L-ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid], Folin–Ciocâlteu reagent, catechin and 2,2`-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich (Steinheim, Germany). Ethanol absolute, acetic acid 96%, methanol 99.9% and hexane 95% (analytical grade) were purchased from J.T. Baker Chemical Co. (Deventer, The Netherlands). Sodium carbonate anhydrous (Na_2CO_3), sodium nitrite ($NaNO_2$) and acetone were purchased from VWR Chemicals (Leuven, Belgium) and aluminum chloride hexahydrate ($AlCl_3.6H_2O$) was purchased from Acofarma (Terrasa, Barcelona).

Plant materials

Fruits of three mandarin-like hybrid varieties (Fig. 1): Clemenvilla (Nova) [*Citrus clementina* Hort *x* (*Citrus paradisi* Macf. *x Citrus tangerina* Hort)], Ortanique [*Citrus reticulata* Blanco *x Citrus sinensis* (L.) Osbeck] and Nadorcott (Afourer) ((*Citrus reticulata Blanco x Citrus sinensis* (L.) Osbeck) *x Citrus reticulata*) were harvested during the 2017–2018 and 2018–2019 harvesting seasons. These varieties were grown in a traditional citrus production orchard under standard agronomical and growing conditions located in the Valencia province (Spain). 35 lots of Clemenvilla, 22 of Nadorcott and 31 of Ortanique were obtained (Table 1). At least 15–20 fruits of each lot were harvested from different adult trees at commercial maturity stage. Chronologically, Clemenvilla mandarins were harvested since November 15th until February 1st. Ortanique fruits were harvested since January 1st until April 1st. Nadorcott samples were harvested during February 25th until March 25th. Samples were selected based on the external color and size uniformity by variety and were delivered immediately to the laboratory to analyze the fresh juice. After and adequate clean of fruits with tap water, the juice was obtained by cutting the selected fruits in half and hand-squeezing with a kitchen juicer, then was centrifuged (5 min, 4000*g*, room temperature) (EPPERDORF centrifuge 5810R, Germany) and filtered through Whatman no. 1 filter (Whatman International Ltd., UK). Three aliquots of juice of each lot were analyzed in triplicate. The juice/weight mean ratios (v/w) were 48, 44

Table 1 Samples according to varieties and harvesting seasons

Harvesting season	Variety		
	Clemenvilla	Nadorcott	Ortanique
2017–2018	10	10	13
2018–2019	25	12	18
Total	35	22	31

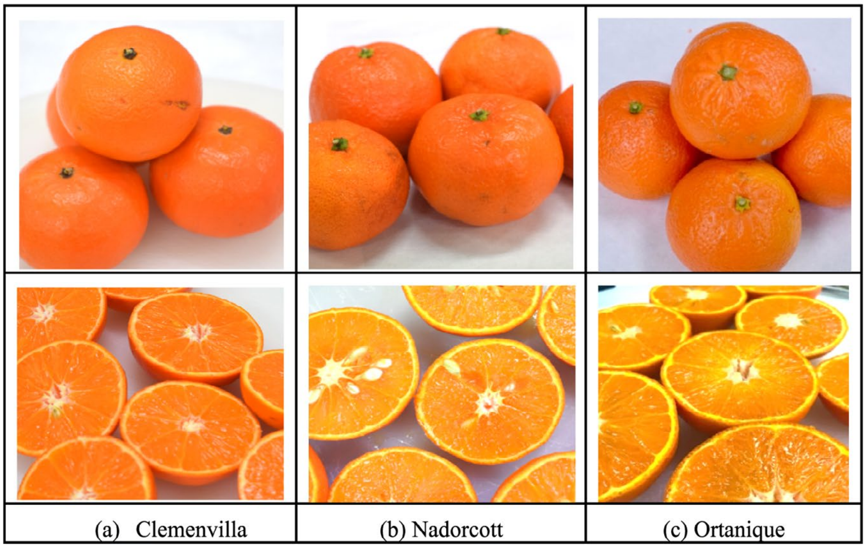


Fig. 1 Mandarin-like hybrid varieties included in the study. (**a**) Clemenvilla, (**b**) Nadorcott, (**c**) Ortanique

and 36 mL/100 g for Clemenvilla, Nadorcott and Ortanique, respectively.

Physicochemical properties (conductivity, total soluble solids, pH, color)

Conductivity was measured with a HANNA HI 5321 (Woonsocket RI, U.S.A.) conducti-meter and expressed in mS/cm. Total soluble solids were measured as Brix degree (°Brix) using an Atago MASTER-T digital refractometer (Atago CO Ltd., Tokyo, Japan) with automatic temperature compensation in 20 °C. The pH was determined using a Sension TM + MM340 pH-meter (HACH-LANGE, S.L.U., Barcelona, Spain). The color was determined with a Hunter Labscan II spectrophotometric colorimeter (Hunter Associates Laboratory Inc., Reston, VA., U.S.A.), controlled by a computer that calculates color ordinates from the reflectance spectrum. Results were expressed in accordance with the Commission International d`Eclairage LAB (CIELAB) system with three consecutive values: L^* (lightness [0 = black, 100 = white]), a^* ($-a^*$ = greenness, $+a^*$ = redness) and b^* ($-b^*$ = blueness, $+b^*$ = yellowness).

Bioactive compound content analysis

The total phenolic content (TPC) was determined according to the Singleton and Rossi [13] reported method. Absorbance was measured at 765 nm and the results were expressed as mg of gallic acid equivalent (GAE)/100 mL. The total flavonoid content (TF) of juice was carried out according to the method optimized by Alberti et al. [14]. The measurement at 510 nm was compared to a calibration curve of catechin standard. TF was expressed as mg of catechin equivalents (CE)/100 mL. The quantification of ascorbic acid (AA) was performed applying the AOAC volumetric technique [15] and results were expressed as mg AA/100 mL. The extraction of total carotenoids (TC) of juice was performed in accordance to the method of Bunio-wska et al. [16]. TC was calculated using an extinction coefficient of β-carotene ($E^{1\%} = 2505$). Results were expressed as μg β-carotene/100 mL.

Total antioxidant capacity assessment

The DPPH assay was performed as the method described by Brand-Williams et al. [17]. The DPPH-colored radical was used measuring the initial and final reaction absorbance at 515 nm. TEAC assay was applied using the method reported by Zulueta et al. [18]. The ABTS radical was diluted until an absorbance of 0.70 ± 0.02 reached at 734 nm and 30 °C, and the initial and final reaction absorbance was measured. In both assays, the percentage of inhibition (% I) was calculated using the following formula (Eq. 1):

$$\%I = \left[(A0 - A1)/A0\right] \times 100 \tag{1}$$

where A0 is the absorbance of the control, A1 is the absorbance in the presence of sample. Results were expressed as mM Trolox equivalent (mM TE).

Statistical analysis

Three biological replicates were included according to each lot by variety, and data were reported as mean ± standard deviation (SD). The analysis of variance (ANOVA) was performed to verify the significant differences in the parameters studied in relation to the sample analyzed and factors included (variety and harvesting season). A multiple regression analysis was carried out to study the influence of the factors to the parameters (results are shown in the significant cases, $p < 0.05$). The Pearson's correlation coefficients were obtained using R software [19] and the R packe "corrplot" [20] was used to illustrate the correlations and their significances. Discriminant functions analysis was performed to estimate the variables that allow to classify the samples according to the mandarin varieties. ANOVA and discriminant functions were performed using Statgraphics® Centurion XVI (Statpoint Technologies Inc., USA).

Results and discussion

Conductivity, total soluble solids content, pH, and color

Physicochemical properties are important parameters to evaluate the citrus quality and results are showed in Table 2. Al-Juhaimi and Ghafoor [21] indicated that compared to other citrus species, mandarin juice showed best-quality characteristics according to the physicochemical parameters. Lado et al. mentioned that juice content (%) and total soluble solids (°Brix) are some of the maturity indexes to evaluate the fruit quality in European Union markets. Also, these parameters are determined by the accumulation of primary and secondary metabolites during the ripening process on the tree [18]. Conductivity values were stronger influenced by variety ($p < 0.05$) being the Nadorcott juice, the samples with highest values (3.47 ± 0.47 and 3.37 ± 0.41 mS/cm from the first and second harvesting seasons, respectively). These differences may be due to the presence of ions from chemical components in the samples. A similar conductivity range (1.89–4.57 mS/cm) was obtained by Dragull et al. [22] in Satsuma (*C. unshiu* Marcovitch) juice. No significant differences were observed in °Brix of samples, determining a range from 12.8 to 13.5 in juice from first

Table 2 Physicochemical characteristics of mandarin juice by variety and harvesting season

Parameters (mean ± SD^A)	2017–2018^B			2018–2019^B		
	Clemenvilla	Nadorcott	Ortanique	Clemenvilla	Nadorcott	Ortanique
Conductivity (mS/cm)	3.10 ± 0.53^{a1}	3.47 ± 0.47^{a1}	3.33 ± 0.50^{a1}	3.05 ± 0.61^{d1}	3.37 ± 0.41^{e1}	3.31 ± 0.42^{e1}
ºBrix	13.4 ± 1.20^{a1}	13.5 ± 1.20^{a1}	13.2 ± 1.30^{a1}	12.8 ± 2.00^{d1}	13.3 ± 1.80^{d1}	13.4 ± 0.90^{d1}
pH	3.64 ± 0.28^{a1}	4.40 ± 0.25^{b1}	3.28 ± 0.1^{4c1}	4.14 ± 0.39^{d2}	4.21 ± 0.25^{d2}	3.71 ± 0.18^{e2}
L^*	4.27 ± 0.39^{a1}	3.55 ± 0.46^{b1}	4.22 ± 0.26^{a1}	4.56 ± 0.52^{d2}	3.63 ± 0.36^{e1}	4.04 ± 0.39^{f2}
a^*	6.05 ± 0.30^{a1}	5.94 ± 0.39^{a1}	5.94 ± 0.18^{a1}	6.32 ± 0.30^{d2}	6.17 ± 0.40^{e2}	6.04 ± 0.33^{e1}
b^*	7.10 ± 0.57^{a1}	6.02 ± 0.76^{b1}	7.12 ± 0.43^{a1}	7.46 ± 0.70^{d2}	6.15 ± 0.59^{e1}	6.82 ± 0.65^{f2}

[A] *SD* standard deviation

[B] In the same row, different superscripts indicate that there are statistically significant differences ($p < 0.05$) between the values in the 2017–2018 (a–c) and 2018–2019 (d–f) seasons, by each variety (1–2)

and second harvesting seasons. This is interesting since both samples correspond a different harvesting season; however, these differences were not significant. A lower value in fresh Ortanique juice (12.67°Brix) was observed by Betoret et al. [23]. However, our results are in range of °Brix values reported in previous research for other mandarin cultivars [10, 24, 25]. The pH values ranged between 3.28 and 4.40 and the multifactorial analysis showed a significant difference ($p < 0.05$) in pH values of the juice analyzed. Similar results were obtained by Betoret et al. [23] who observed a pH of 3.23 in fresh Ortanique juice, while Simon-Grao [26] obtained a pH of 3.70 in the same variety. In Satsuma, Robinson and Fremont mandarin juice, similar pH values (3.5, 3.6 and 3.4, respectively), was reported by Kelebek and Selli [27]. Higher range of pH (3.82–4.62) was obtained by Legua et al. [10] in 14 rootstocks of Clemenules mandarin juice. Hunlun et al. [28] reported higher pH values (3.37–3.73) in Clementine and Satsuma juice and Li et al. [25] showed a range of 3.58–4.06 in Satsuma juice. These differences could be due to the genetic and chemical composition of different mandarin juice.

The color of citrus juice is one of the parameters considered for the commercial acceptance of the product in relation to its quality being habitual the practice of adding mandarin juice to enhance the color of commercial orange juice. Chemical composition of juice from different varieties can affect the color characteristics ($p < 0.05$). According to the L^* data, Nadorcott mandarin juice could be consider the darkest samples. Ortanique mandarins showed a higher green–red coordinate (a^*) compared to the results obtained from Beltrán et al. [29] in the same variety (− 1.53). Lower values of a^* were observed by Li et al. [25] in Satsuma mandarin juice (from − 0.23 to 2.0). The Ortanique (7.12 ± 0.43) and Clemenvilla (7.46 ± 0.70) juice showed the highest positive values for the b^* parameter (yellow axis) in first and second harvesting season, respectively.

Total polyphenols, flavonoids, ascorbic acid, and carotenoids content

Bioactive compounds content, responsible of functional characteristics of mandarin juice are reported in Table 3. Mandarins are a good source of phenolic compounds and their concentration influence on taste characteristics and organoleptic quality [27]. The main values of TPC in Clemenvilla were significantly ($p < 0.05$) higher (127 ± 21.2 mg GAE/100 mL), compared to Nadorcott and Ortanique juice (98.5 ± 20.8 and 116 ± 17.1 mg GAE/100 mL, respectively). Our results were in concordance to Xu et al. [4] who reported 77.5–155.5 mg GAE/100 mL in different mandarin juice from China. Al-Juhaimi and Ghafoor [21] determined 91.2 mg GAE/100 mL in juice of kinnow (*Citrus nobilis* × C. deliciosa) mandarins from Saudi origin, while Sicari et al. [30] obtained 92.0 mg GAE/100 mL in Italian mandarin juice. Higher concentrations of TPC were determined by Roussos et al. [31] who reported 132 and 135 mg GAE/100 mL in Clementine (*Citrus clementina* SRA63) juice from organic and integrated cultivation system, respectively. And Pyo et al. [32] determined 211 mg GAE/100 mL in flesh *Citrus unshiu* juice. Lower concentrations were obtained by some authors in different mandarin varieties [10, 22, 32] and Simon-Grao obtained 38.8 mg GAE/100 mL in Ortanique juice [26]. These differences suggest that phenolic compound's concentration is dependent on genes, geographical origin, environmental and cultural practices.

Concerning the flavonoids content, the highest concentrations were obtained in Nadorcott juice with a significantly difference ($p < 0.05$) compared to Clemenvilla and Ortanique. Lower flavonoids concentrations were obtained (8.50 mg CE/100 mL) in Tunisian Elarbi mandarin juice [33]. Hunlun et al. [28] suggested that the genetics of citrus species affects its polyphenol and flavonoids contents in juice, and is possible that these differences can be also, by the geographical origin. On the other hand, the determination of total flavonoids content of some studies with

Table 3 Bioactive compounds content antioxidant capacity of mandarin juice by variety and harvesting season

Parameters (mean ± SD[A])	2017–2018[B]			2018–2019[B]		
	Clemenvilla	Nadorcott	Ortanique	Clemenvilla	Nadorcott	Ortanique
TPC[A] (mg GAE[A]/100 mL)	125 ± 13.0^{a1}	89.1 ± 16.5^{b1}	117 ± 15.5^{c1}	127 ± 22.6^{d1}	102 ± 21.4^{e2}	115 ± 17.8^{f1}
TF[A] (mg CE[A]/100 mL)	9.68 ± 1.19^{a1}	16.1 ± 3.49^{b1}	10.9 ± 1.87^{c1}	7.45 ± 1.46^{d1}	13.6 ± 3.50^{e1}	11.5 ± 2.07^{f2}
AA[A] (mg AA/100 mL)	49.1 ± 9.70^{a1}	11.9 ± 2.90^{b1}	31.9 ± 3.80^{c1}	39.2 ± 8.5^{d1}	13.2 ± 3.60^{e2}	22.3 ± 7.10^{f1}
TC[A] (μg/100 mL)	1461 ± 361^{a1}	1349 ± 423^{ab1}	1219 ± 400^{b1}	306 ± 49.2^{d2}	415 ± 225^{e2}	619 ± 327^{f2}
DPPH[A] (mM TE[A])	2.88 ± 1.25^{a1}	0.25 ± 0.14^{b1}	1.94 ± 0.39^{b1}	3.31 ± 0.63^{d2}	0.82 ± 0.34^{e2}	1.86 ± 0.36^{f1}
TEAC[A] (mM TE)	3.61 ± 0.62^{a1}	1.81 ± 0.50^{b1}	2.82 ± 0.55^{c1}	4.24 ± 0.87^{d2}	1.85 ± 0.32^{e1}	2.52 ± 0.35^{f2}

[A] *SD* standard deviation, *TPC* total phenolic compounds, *GAE* gallic acid equivalent, *TF* total flavonoids, *CE* catequin equivalent, *AA* ascorbic acid, *TC* total carotenoids, *DPPH* 2,2`-diphenyl-1-picrylhydrazyl scavenging assay, *TE* trolox equivalent, *TEAC* Trolox-equivalent antioxidant capacity

[B] In the same row, different superscripts indicate that there are statistically significant differences ($p < 0.05$) between the values in the 2017–2018 (a–c), 2018–2019 (d–f) seasons by each variety (1–2)

mandarin juice is expressed in different units: Sicari et al. [30] obtained a 11 mg rutin equivalent/100 mL of mandarin juice. While Roussos et al. [31] determined 1.6 and 1.2 mg caffeic acid equivalent/100 mL of TF in clementine juice from organic and integrated cultivation system, respectively. The standardization in the determination of total flavonoids content in fruit samples is necessary to eliminate this gap.

Mandarin juice is a rich source of AA, an important antioxidant and a significant indicator of mandarin juice quality [27]. The AA content in mandarin juice was significantly different ($p < 0.05$) between the varieties in both harvesting seasons. Clemenvilla juice had the highest concentration of AA ($p < 0.05$) in juice (40.8 ± 9.37 mg/100 mL). These results were in close agreement with the values obtained in juice of Clemenvilla mandarins (46.2–54.4 mg/100 mL) by Torregrosa [34]. Results suggest that mandarin juice from different origins and growing conditions is an excellent source of Vitamin C (10–60 mg/100 mL approximately) independently of its species. Also, Clemenvilla and Ortanique juice are a good option to enhance the Vitamin C concentration of commercial orange juice, that is 36 mg/100 mL reported by the European Fruit Juice Association [35].

Values of TC found in the present study slightly agreed with results found by Xu et al. [4] (292–1002 μg β-carotene/100 mL) in seven varieties of mandarins cultivated in China. Results of first harvesting season are in a range from 1219 to 1461 μg/100 mL, and are similar to Cheng et al. [36] results who reported 1220 μg β-carotene/100 mL of TC in *Citrus unshiu* juice. On the other hand, concentrations of TC in juice of second harvesting season (306–619 μg/100 mL) were like the range of cumulative concentrations obtained by Giuffrida et al. [37] in different mandarin varieties (279–808 μg/100 mL). A concentration of 420 μg β-carotene/100 mL was obtained in fresh Kinnow mandarin (*Citrus nobilis* × *C. deliciosa*) juice [38]. All of these differences are due to the citrus species analyzed; however, a study of the influence of the external factors is needed, to establish the discriminant grown conditions that enhance the carotenoids content in mandarin-like hybrid cultivars.

Antioxidant capacity

The DPPH and TEAC assays are the most employed methods to evaluate antioxidant capacity in fruits [39]. According to Table 3, DPPH and TEAC main values in Clemenvilla, Nadorcott and Ortanique juice have significantly differences ($p < 0.05$) in both harvesting seasons. As shown in Fig. 2, Clemenvilla juice exhibited the highest values of mM TE. These results are higher than the values obtained by Hunlun et al. [28] who reported 0.212 mM TE in Clementina and Satsuma mandarins. In this line, Pyo et al. [32] determined approximately 2 mM TE in juice of flesh Satsuma mandarins. In the case of TEAC assay, were obtained higher scavenging activity values. These results are slightly similar than Legua et al. [10] who obtained less than 0.2 mM TE in juice of 14 rootstocks for Clemenules mandarins. In addition, Betoret et al. [40] reported a 0.70 mM TE in Ortanique (*Citrus reticulata* × *Citrus sinensis* Osbeck) juice.

Since the antioxidant capacity is influenced by different variables and its synergistic effect, use of two or more methods to assess the antioxidant capacity in fruit samples is recommended [33]. DPPH assay is more sensitive to hydrophobic compounds while TEAC assay is more sensitive to hydrophilic antioxidants like ascorbic acid and polyphenols [39]. As is observed (Fig. 2), mandarin juice exhibited an elevated antioxidant capacity measured with TEAC compared to DPPH assay, in agreement with the study of Zhang et al. [41]. In this line, our results suggest that hydrophilic composition contributed to antioxidant capacity in higher amounts than hydrophobic ones in the juice analyzed. In contrast, Sicari et al. [30] determined higher

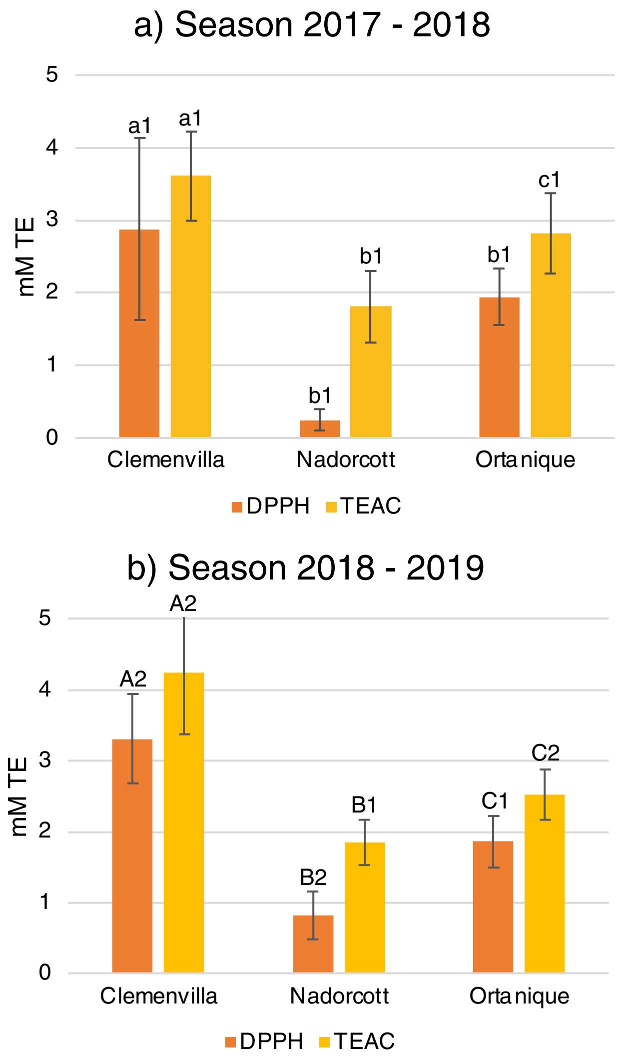


Fig. 2 Antioxidant capacity of mandarin juice by varieties and harvesting seasons. *TE* trolox equivalent, *DPPH* 2,2`-diphenyl-1-picrylhy-drazyl scavenging, *TEAC* Trolox-equivalent antioxidant capacity. In the same color, different superscripts (**a–c** and **A–C**) indicate that there are statistically significant differences ($p < 0.05$) between the values of each variety in the 2017–2018 and 2018–2019 seasons, respectively by each variety (1–2)

antioxidant values in mandarin (*Citrus reticulata*) juice analyzed by DPPH assay compared to TEAC assay. This can be explained because the ability to scavenge free radical was attributed to the their samples polyphenols composition (92 mg GAE/100 mL of juice) [30]. However, in our samples, we obtained higher polyphenols contents, (Table 3) and due to this, TEAC mM TE values were higher.

The multiple regression study showed that the antioxidant capacity was significantly influenced ($p < 0.05$) by the TPC, TF, AA and TC content, in agreement of previous studies [4, 23]. However, the lineal regression analysis showed that the only the AA explains more than 50% on antioxidant capacity of juice assessed by DPPH (70.32%) and TEAC (58.31%), in agreement to what was reported by Xu et al. [4]. Also, the correlation coefficients show the relation of bioactive compounds with the antioxidant capacity values (Table 4). Scatter charts evidence a lineal correlation between AA and DPPH and TEAC values, respectively, as is observed in Online Resource 1.

Zhang et al. [41] indicated that the TPC and TF were significantly correlated ($p < 0.01$) with the antioxidant capacity values obtained by DPPH assay in *Citrus reticulata* Blanco juice. Roussos et al. [31] mentioned in their study with Clementine hybrid juice that only a specific flavonoid (hesperidin) was strongly correlated ($p < 0.05$) to DPPH radical scavenge ability. In Fig. 3, is observed that the correlation between DPPH and TEAC values was significant ($p < 0.05$) according to previous results in vegetables and fruit juice [42]. This suggests that both methods (DPPH and TEAC assays) are suitable for the assessment of the antioxidant capacity of the mandarin juice.

In the study of the variables evolution during the harvesting seasons (Online Resource 2), interactions were observed in all mandarin juice according to DPPH values and collection time (*t*) and, the model obtained in Nadorcott samples ($0.91–7.31 \cdot 10^{-4}$ ct) explains the 62.38% of DPPH values variability suggesting that was a useful method to assess the antioxidant capacity in Nadorcott juice according to the collection time.

Classification of mandarin-like hybrid juice according to physicochemical characteristics, bioactive compounds content and antioxidant capacity

To study the data and group them naturally in accordance with their characteristics, we performed a discriminant analysis to estimate the parameters that allow classifying the samples according to the mandarin-like hybrid varieties. Similar parameters and bioactive compounds were previously analyzed for some of the varieties used in this work [43].

Using a stepwise selection algorithm, five variables were predictors (pH, TF, AA, DPPH, TEAC) to classify the groups and their coefficients are show in Table 5. This allows us to determine how the independent variables are being used to discriminate between the groups (Clemenvilla,

Table 4 Correlation coefficients of bioactive compounds and antioxidant capacity

Variable	M^A	SD^A	DPPH^A	TEAC^A	TPC^A	TF^A	AA^A
DPPH	2.09	1.21					
TEAC	3.00	1.11	.86**^{B}				
			[.79, .90]^C				
TPC	115.38	22.38	.58**	.61**			
			[.42, .70]	[.46, .73]			
TF	10.88	3.57	– .68**	– .53**	– .24*		
			[– .78, – .55]	[– .67, – .36]	[– .43, – .03]		
AA	29.02	14.09	.84**	.77**	.50**	– .57**	
			[.77, .89]	[.66, .84]	[.32, .64]	[– .69, – .40]	
TC	762.41	536.05	– .29**	– .23*	– .15	.28**	.01
			[– .47, – .08]	[– .42, – .03]	[– .34, .07]	[.08, .46]	[– .20, .22]

[A] *M* mean, *SD* standard deviation, *DPPH* 2,2`-diphenyl-1-picrylhy-drazyl scavenging, *TEAC* Trolox-equivalent antioxidant capacity, *TPC* total phenolic compounds, *TF* total flavonoids, *AA* ascorbic acid, *TC* total carotenoids

[B] Statistically significant at $*p < 0.05$ and $**p < 0.01$, respectively

[C] Values in square brackets indicate the 95% confidence interval for each correlation

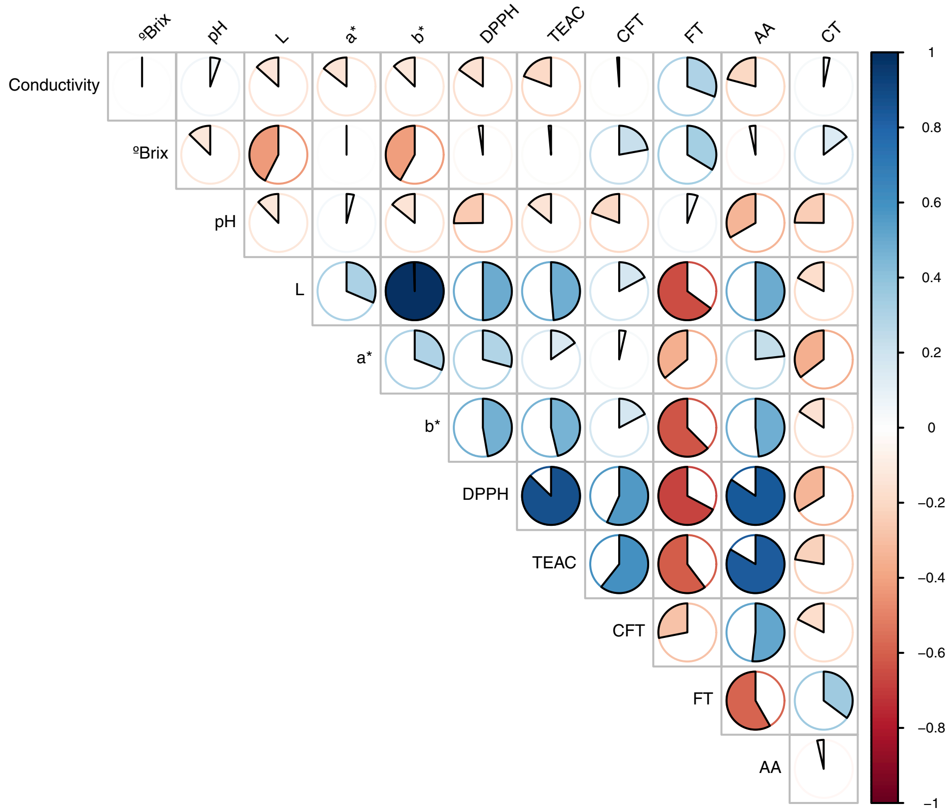


Fig. 3 Pie charts of statistical correlation between the physicochemical characteristics of mandarin juice. The pie charts and color gradients illustrate the strength of each of the correlations (e.g., DPPH and TEAC). *TPC* total phenolic compounds, *TF* total flavonoids, *AA* ascorbic acid, *TC* total carotenoids, *DPPH* 2,2`-diphenyl-1-picrylhydrazyl scavenging assay, *TEAC* Trolox-equivalent antioxidant capacity

Table 5 Coefficients of the classification function by variety

	Clemenvilla	Nadorcott	Ortanique
pH[A]	46.7	49.3	41.9
TF[A]	– 1.05	– 0.03	– 0.14
AA[A]	0.80	0.46	0.53
DPPH[A]	– 9.10	– 58.9	– 19.6
TEAC[A]	$-6.52\cdot10^{-3}$	– 0.06	– 0.04
Constant	– 166.33	– 196.64	– 141,85

[A] *TF* total flavonoids, *AA* ascorbic acid, *DPPH* 2,2`-diphenyl-1-picrylhy-drazyl scavenging, *TEAC* Trolox-equivalent antioxidant capacity

Nadorcott and Ortanique). The pH and AA were positively correlated with the mandarin varieties analyzed.

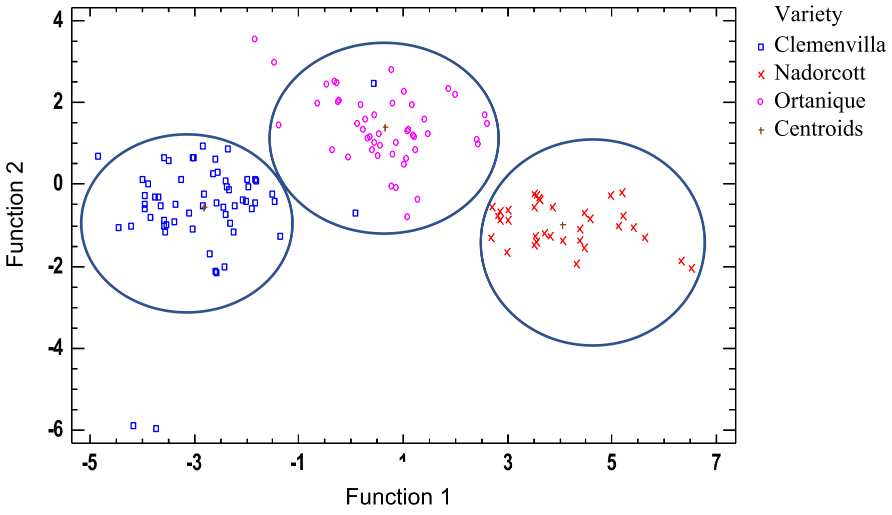
Applying these functions, we obtained a model with which it was possible to classify 98.59% of the samples correctly. The observed coefficients will allow us to differentiate the mandarins according to the varieties analyzed. The fitted model obtained for the discriminant functions was:

Function 1: 0.065*pH + 0.382*TF−0.402*AA−0.393 *DPPH−0.457*TEAC.

Function 2:—0.927*pH + 0.427*TF−0.342*AA−0.394 *DPPH−0.165*TEAC.

Figure 4 shows the ability of the two functions to differentiate the varieties studied. As is observed, Nadorcott

Fig. 4 Differentiation of mandarin-like hybrid varieties according to the discriminant functions



sample is clearly different, due to its high content of TF and lower concentration of AA and antioxidant capacity (DPPH and TEAC values) compared to Clemenvilla and Ortanique samples.

Conclusions

This research provides additional data concerning physicochemical characteristics of mandarin-like hybrid (Clemenvilla, Nadorcott and Ortanique) juice. Clemenvilla juice showed the highest content of TP and AA, and high scavenging activity in both assays employed. Nadorcott juice had the higher concentrations of TF and, in Ortanique juice from second harvesting seasons, the highest TC content was obtained. Variety and harvesting seasons significantly influenced ($p < 0.05$) on the bioactive compounds content and antioxidant capacity of juice. Also, the bioactive compounds were significantly ($p < 0.05$) correlated with the antioxidant capacity evaluated by DPPH and TEAC assays. The link of each assay with the hydrophobic and hydrophilic fraction will be very helpful in future research. In addition, pH, TF, AA, DPPH and TEAC were determined as the predictor parameters to classify the mandarins and group them according to the varieties. This information could be useful to citrus producers, processors, and consumers, for choosing cultivars of mandarin-like hybrids with high nutraceutical faculties with potential health benefits.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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