

## Phytochemical Properties and Volatile Composition Profile of Nine Early Maturing Mandarins Cultivated in South-East Spain

F. Garcia-Sanchez<sup>1\*</sup>, S. Simon-Grao<sup>1</sup>, V. Gimeno<sup>2</sup>, L. Galvez-Sola<sup>3</sup>, V. Lidon<sup>3</sup>, I. Simon<sup>3</sup>, F. Hernandez<sup>3</sup>, J. J. Martinez-Nicolas<sup>3</sup>, and A. A. Carbonell-Barrachina<sup>3</sup>

### ABSTRACT

Many new varieties of mandarins have not been characterized from the nutritional and organoleptic point of view. It is important to know this information in order to select the cultivars of the highest quality. We characterized the physicochemical properties of 9 commercial early-maturing mandarins from south-east Spain: Four "Traditional Clementines" (Clemenules, Orogrande, Arrufatina, Oronules), 4 "New Clementines" (Loretina, Mioro, Clemenpons and Clemenrubí or Prim-23) and one "Satsuma" (Iwasaki). 'Oronules', 'Clemenules' and 'Iwasaki' were the varieties that had the highest fresh weight (>120 g). The "Mioro" variety had the highest acidity (12.50 g L<sup>-1</sup>), and the juices from "Loretina" and "Mioro" showed the highest values of total soluble solids: 12.77 and 12.57 (°Brix), respectively. "Loretina" and "Oronules" showed the most elevated values of total phenolic compounds, with 78.75 and 75.56 mg L<sup>-1</sup> respectively. The main volatile compound was the monoterpene limonene. Following limonene in concentration was  $\beta$ -myrcene (25  $\mu$ g L<sup>-1</sup>). "Clemenrubí" was the best variety for fresh consumption among the 9 examined, due to its high content of total phenols and ascorbic acid. Limonene was the main aroma of the mandarin juice, and the "Mioro" cultivar showed a different profile from the rest of cultivars studied according to the principal component analysis performed.

**Keywords:** Citrus, Clementines, Physicochemical properties, Satsumas.

### INTRODUCTION

In recent years, global production of mandarins has been about 27 million metric tons. The Spanish production is about 1.87 million metric tons, this amount being equivalent to 6.90% of the global production. This indicates that Spain is the second highest mandarin producer in the world, and it's a leading exporter. Spanish mandarins are mainly exported to Western European countries, especially Germany,

France and the United Kingdom (FAO, 2014). The main exporting competitor countries are China, Morocco and Turkey. These countries are attempting to overtake Spain, bringing to the market new varieties of early mandarins to compete with the Spanish clementines, mainly from the "clemenules" variety. Therefore, the need of introducing new cultivars has emerged in Spain. The organoleptic and nutritional characteristics of the new products should allow them to compete in the international

<sup>1</sup> CEBAS-CSIC, University Campus of Espinardo, Espinardo, Murcia. E-30100, Spain.

\* Corresponding author: e-mail: fgs@cebas.csic.es

<sup>2</sup> Department of Agronomy, ISA University, Av. Antonio Guzmán Fdez. km 5 1/2, PO Box 166 La Herradura, Santiago, Dominican Republic.

<sup>3</sup> Polytechnic School of Orihuela (EPSO), Miguel Hernández University, Km 3.2 Carretera Beniel, Orihuela – 03312, (Alicante), Spain.



market and overcome the state of saturation regarding traditional fresh production.

In Spain, Clementines (*Citrus clementina* Hort. Ex Tan) cover about 71.23% of the surface dedicated to mandarin production, whereas Satsumas varieties (*Citrus unshiu*) only cover 6.33% of the mandarin growing area (MAGRAMA, 2014), in contrast to China, where more than 40% of the surface of mandarin crops area is farmed with Satsumas varieties. Also, within the wide range of clementines, the “Clemenules” is the most produced cultivar in the world (FAO, 2014). At present, nurseries that are dedicated to the commercialization of citrus seedlings offer a broad range of both mandarins and orange trees cultivars. Therefore, choosing the cultivar to grow is a difficult and important decision to be taken by the farmer. Normally, the market strategies, such as price, date of harvest, organic farming products, organoleptic characteristics and nutritional aspects that give an added value are the main factors on which the farmers can base their choice for a specific cultivar. The characteristics not only depend on the cultivar, but also on other factors such as climate or the soil type where the plant might grow, which are crucial for obtaining fruit with some added values. Therefore, it is necessary to analysis the agronomic performance of each variety in the conditions of the area where the crop will be grown (Yildiz, *et al.*, 2014).

Moreover, apart from the traditional requirements demanded by the consumers (good size, appearance and color, easy to peel, seedless, high juice quantity and quality, flavorful with pleasant aroma) there are other nutritional requirements, such as, high vitamin content, good mineral concentration etc. Owing to this fact, it is important to characterize all these requirements with the best available analytical methods and as complete as possible, to launch all those varieties onto the market as a product with an extra added value and on the other hand, knowing the suitable cultivars for fresh consumption or for the food industry (Legua *et al.*, 2014).

We performed a study for the identification of the physicochemical properties and volatile compounds of nine early maturing commercial mandarins in south-east Spain. The results obtained from the study of fruit quality parameters during the two consecutive seasons (2011 and 2012), included three different groups of mandarin cultivars: “Traditional Clementines”, “New Clementines” and “Satsumas”.

## MATERIALS AND METHODS

### Plant material and experimental plot

The nine cultivars of early mandarins studied in this experiment were collected from the citrus collection available from the CEBAS-CSIC (Centro de Edafología y Biología Aplicada del Sureste-Consejo Superior de Investigaciones Científicas) experimental farm ‘Tres caminos’ in Santomera (Murcia, Spain). All mandarin trees were 15 years-old, they were grafted onto Carrizo citrange rootstock and irrigated with a dripper system with water coming from the Tajo-Segura aqueduct. The fertilizers applied every year contained ammonium nitrate (33% N) 3 kg tree<sup>-1</sup>; monoammonium phosphate 0.5 kg tree<sup>-1</sup>, KNO<sub>3</sub> 1.5 kg tree<sup>-1</sup>, and iron chelate 30 g tree<sup>-1</sup>. The soil was clay loam with more than 40% CaCO<sub>3</sub>. The weather was characterized by a scant seasonal precipitation profile capped by a severe drought in summer. The reference evapotranspiration according to Penman-Monteith and the average annual rainfall in the Southeast of Spain is 1,210±60 mm and 305 mm, respectively.

The nine mandarin cultivars studied were classified into three different cultivar groups: (i) “Traditional Clementines” (Clemenules, Orogrande, Arrufatina and Oronules; (ii) “New Clementines” (Loretina, Mioro, Clemenpons y Clemenrubí), and (iii) “Satsumas” (Iwasaqui). A completely

randomized experimental design was used, four trees at random locations were chosen from each cultivar, with 10 representative fruits collected from each tree. The collection data for each cultivar is reported in Table 1. Mandarin fruits were harvested from trees in the four quadrants to avoid fruit position effects.

### Physical Parameters of the Fruit

To assess the fruit's physical attributes, the following variables were measured: Fresh weight (g), width (mm), length (mm) and external Index of Color ( $IC_{ext}$ ). Fruits were cut along their equatorial zone and their juice was extracted. Thus, juice volume (mL), seed number and internal Index of Color ( $IC_{int}$ ) were measured. Fruit color was assessed using a tri-stimulus color spectrophotometer Minolta C-300 Chroma Meter (Minolta Corp., Osaka, Japan) coupled to a Minolta DP-301 data processor. Color Index (CI) was calculated as  $CI = a^* 10^3 (L^* b^*)^{-1}$  (Jiménez-Cuesta *et al.*, 1981), where  $L^*$  indicates Lightness,  $a^*$  and  $b^*$  are the chromaticity coordinates.

### Chemical Parameters of the Fruit

The juice was filtered and centrifuged by 10 minutes at 1,000 rpm to separate the pulp. The following chemical variables were then measured: pH, Titratable Acidity (TA, g citric  $L^{-1}$ ), Total Soluble Solids (TSS, °Brix), Maturity Index ( $MI = TSS \times 10 TA^{-1}$ ), composition and concentration of sugars (% sugar per 100 g fresh juice) and organic acids (% organic acids per 100 g fresh juice), mineral nutrients concentration (macronutrients and micronutrients, mg  $L^{-1}$ ), Total Antioxidant Activity (H-TAA and L-TAA), and total phenolic compounds (mg  $L^{-1}$ ).

Titrate Acidity (TA) was determined by acid-base potentiometry (0.1N NaOH up to 8.1), being expressed as g citric  $L^{-1}$ . Total Soluble Solids (TSS) were measured with a

**Table 1.** Physical characteristics of nine cultivars of early maturing mandarin in Spain.<sup>a</sup>

Cultivars	Collection data	Fresh weight (g)	Fruit width (mm)	Fruit length (mm)	Volume juice (mL fruit <sup>-1</sup> )	n° seeds	$IC_{ext}$	$IC_{int}$
Clemenules	07-11-2011	120.92±12.4 a	66.08±2.9 a	54.72±1.9 ab	46.25±6.2 ab	8.50±5.3 c	5.29±0.8 bc	14.50±1.2 a
Orogrande	25-10-2011	100.21±12.5 bcd	61.34±2.4 bc	51.82±3.7 bc	40.50±5.0 abc	13.50±8.4 c	6.68±3.3 bc	6.91±2.3 d
Arrufatina	11-10-2011	107.38±23.7 abc	59.79±4.0 cd	57.34±6.2 a	50.00±13.0 a	65.50±20.4 a	-2.09±1.1 d	10.45±1.6 bc
Oronules	26-10-2011	125.94±5.3 a	64.24±0.9 ab	59.01±2.2 a	35.75±12.0 bcd	4.25±3.8 c	-6.12±0.8 e	-5.84±1.1 e
Loretina	04-11-2011	83.80±7.2 de	55.86±1.2 e	53.54±3.6 ab	23.50±3.6 d	67.00±27.3 a	11.26±0.5 a	13.34±1.4 ab
Mioto	04-11-2011	70.08±1.9 e	51.75±0.5 f	47.67±1.0 c	29.50±1.0 cd	33.00±5.3 b	5.99±0.3 bc	14.72±0.5 a
Clemenpons	25-10-2011	94.22±14.4 cd	57.47±3.3 de	49.65±1.7 bc	43.88±6.2 ab	3.00±1.8 c	9.36±6.2 ab	7.71±3.2 cd
Clemenrubí	23-09-2011	108.16±10.0 abc	57.96±1.3 cde	58.79±3.7 a	48.67±6.1 ab	0.00±0.0 c	6.11±2.31 bc	11.63±1.7 ab
Iwasaki	01-10-2011	120.20±14.3 ab	64.30±1.9 ab	54.50±2.3 ab	50.00±11.9 a	0.00±0.0 c	2.81±1.78 c	14.36±0.8 a
ANOVA		***	***	***	***	***	***	***

<sup>a</sup> Inside each column, the means followed by the same statistical letter do not differ significantly at the 5%.  $CI_{ext}$  and  $CI_{int}$  represent the external and internal Colour Index, respectively. Each value is the mean of four samples. \*\*\* Indicates significant differences at  $P < 0.001$ .



refractometer model DCR-200. Maturity Index (MI) was calculated as the relation between TSS and TA.

Total Antioxidant Activity (TAA) was determined for each sample: 5 mL of mandarin juice was homogenized in 5 mL of 50 mM phosphate buffer pH 7.8 and 3 mL of ethyl acetate, and centrifuged at 10,000 rpm for 15 minutes at 4°C. The upper fraction was used for TAA due to Lipophilic compounds (L-TAA) and the lower fraction for TAA due to Hydrophilic compounds (H-TAA). TAA was determined in each extract (quadruplicate) using the enzymatic system composed of the chromophore 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), the Horseradish Peroxidase Enzyme (HRP) and its oxidant substrate (hydrogen peroxide), in which ABTS<sup>+</sup> radicals are generated and monitored at 730 nm. A calibration curve was calculated with Trolox ((R)-(+)-6-hydroxy-2, 5, 7, 8-tetramethyl-croman-2-carboxylic acid).

Total phenolic compounds were determined by diluting the juice sample 1:10 with methanol (80%). The sample was shaken for 30 minutes and centrifuged at about 6,000 rpm for 10 minutes. 500 µL of the previous extraction was poured into glass tubes, 500 µL of the previous extraction was added, as well as 2.5 mL of Folin-Ciocalteu reagent (10%) and 2 mL of Na<sub>2</sub>CO<sub>3</sub> (75 g L<sup>-1</sup>) and the mixture was shaken (in triplicate). The straight pattern was prepared using Gallic acid (0.1 g L<sup>-1</sup>). The glass tubes were introduced in a bath heated to 50°C for 5 minutes and the absorbance was measured using a spectrophotometer (Hitachi U-2000), at 760 nm.

Extraction and characterization of organic acids and sugars were carried out with 15 mL of juice, which was centrifuged at 10,000×g for 15 minutes. The supernatant was filtered through a cellulose nitrate membrane filter (0.45µm pore size). The aqueous extracts were analyzed using an HPLC (Hewlett-Packard series 1100) containing a Supelcogel C-610H column

(300×7.8 mm id), connected in series to a Supelcogel<sup>TM</sup> carbohydrate pre-column (50×4.6 mm) and with a stationary phase of sulfonated polystyrene divinilbenzene. The isocratic separation of sugars and organic acids was performed at 30°C, using a mobile phase of 0.1% phosphoric acid adjusted to a flow rate of 0.5 mL min<sup>-1</sup>. The quantification of sugars was carried out with a Refractive Index Detector (RID) and the organic acids were quantified using a Ultra-Violet (UV) Diode Array Detector (DAD) at a wavelength of 210 nm. The characterization and quantification of the sugars and organic acids in the samples were made by comparing the retention times and the areas under the peaks with standards. Sugar and organic acid standards were supplied by Supelco analysis (Bellefonte, PA, USA).

Mineral concentration was determined using 15 mL of juice centrifuged previously at a 9,500 rpm for 15 minutes. The supernatant was retrieved and passed through a 0.45 µm filter. Determination of Ca, K, Mg, P, S, Na, Cu and Zn was carried out using Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES, Iris Intrepid II, Thermo Electron Corporation, Franklin, USA).

#### Extraction procedure of volatile aroma compounds

The steps that were carried out were as follows: (1) Extraction of the sample. The filtered juice samples were diluted 1:5 with ultrapure water. 10 µL of the internal standard 3-hexanol were added at a concentration of 10,000 ppm. (2) Absorption of volatile compounds by fiber. The sample was put with a fiber (50/30 µm divinylbenzene/carboxen) in a water bath at 50°C for 1 hour. (3) Gas chromatography–mass spectrometry (GC/MS) analysis. The isolation, identification and quantification of volatile compounds was performed on a gas chromatograph Shimadzu GC-17A (Shimadzu Corp., Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector

GC-MS QP-5050A. The GC/MS system was equipped with a TRACSIL Meta.X5 column, 95% dimethyl-polysiloxane and 5% diphenyl-polysiloxane (Teknokroma S. Coop. C. LTD, Barcelona, Spain; 30 m×0.25 mm, 0.25 µm of film thickness).

Analyses were carried out using helium as a carrier gas at a column flow of 0.6 mL min<sup>-1</sup> in a split ratio of 1:5 and the following program was adopted: (a) 80°C for 0 minute; (b) rate of 3.0°C min<sup>-1</sup> from 80 to 210°C and held for 1 minute; (c) rate of 25°C min<sup>-1</sup> from 210 to 300°C and held for 3 minutes. The temperatures of the injector and detector were 230 and 300°C, respectively.

Most of the compounds (27 out of 29) were identified by using three different analytical methods: (1) Retention indexes; (2) GC-MS retention times [authentic standards of all compounds reported were used for identification purposes; differences between experimental and literature (NIST, 2015) retention indexes were always below 10 units], and (3) Mass spectra (authentic chemicals and NIST05 spectral library collection). The volatile studies were conducted in triplicate. The concentration of each compound was expressed as µg L<sup>-1</sup> after the use of the internal standard (3-hexanol).

### Statistical Analysis

The data were analyzed using the SPSS 22.0 program for Windows (SPSS Science, Chicago, IL, USA). The differences between cultivars ( $P < 0.05$ ) in the different parameters studied were evaluated by Analysis Of Variance (ANOVA) followed by Duncan's test for comparison of means. Principal Component Analysis (PCA) and Cluster Analysis (CA) were also performed. Cluster analysis was applied to the data for hierarchical associations, employing Ward's method for agglomeration and the squared Euclidean distance as a dissimilarity measure. The variables used in the multivariate analysis were those indicated in Table 5 (volatile chemicals).

## RESULTS AND DISCUSSION

### Physical Fruits Parameters

The 'Oronules', 'Clemenules' and 'Iwasaki' mandarins had the highest fresh weight, weighing more than 120 g on average. In contrast, the "Mioro" cultivar mandarins showed lower weight, not even reaching a value of 75 g (Table 1).

The 'Iwasaki', 'Arrufatina' and 'Clemenrubí' cultivars had the most amount of juice, 50 mL per fruit approximately (Table 1). In all cases, it was found that the volumes of juice were about 40% of the total weight of the fruit, except for the 'Loretina' cultivar, which had a value of less than 30% (data not shown). According to the European regulation (EU) No. 1234/2007 for the fruit sectors of the European Union member states, the percentage of juice in mandarins for fresh consumption has to be higher than 40%.

As for the undesirable characteristics for consumers, such as the number of seeds, it was found that 'Arrufatina', 'Loretina' and 'Mioro' cultivars had the highest number, with an average value of approximately 55 seeds per fruit. According to the European legislation for citrus commercialization, these varieties have to be labelled "with seeds" because the number of seeds is higher than 10 (DOUE, 2011); the 'Orogrande' cultivar fruits, although they did not present such a large number (14 seeds), have to be labelled similarly. As for 'Clemenules', 'Oronules' and 'Clemenpons' cultivars, the number of seeds ranged from 1 to 10, which means that they did not require the specific labeling (Table 1). Then, the only varieties that can be labelled as "without seeds" would be 'Clemenrubí' and 'Iwasaki'.

The color of the peel ( $IC_{ext}$ ) is considered to be one of the most important external factors that determines the quality in citrus fruit, and directly impacts consumer choice when the moment comes for deciding between buying one fruit or another (Olmo, *et al.*, 2000; Legua, *et al.*, 2011; Legua *et*



al., 2013). The cultivars with the lowest values were ‘Oronules’ (-6.12) and ‘Arrufatina’ (-2.09), both of which were harvested in early-mid October (Table 1). The other varieties showed colorations related to a degree of maturity ranging from 2.28 for ‘Iwasaki’ to 11.26 for ‘Loretina’.

Regarding the internal color (IC<sub>int</sub>) of fruits, it was observed that the ‘Oronules’ variety showed a negative value for this parameter, as the juice obtained from this cultivar was yellowish and was therefore unappetizing for consumers.

### Chemical parameters of the fruit

#### TA, TSS, and MI

As for the acidity, “Oronules” and “Clemepons” cultivars showed the lowest values: 7.34 and 8.33 g L<sup>-1</sup> respectively. In contrast, the “Mioro” cultivar had the highest acidity (12.50 g L<sup>-1</sup>), as shown in Table 2. With regards to the TSS content, significant differences between cultivars were observed. The juices from “Loretina” and “Mioro” showed the highest values: 12.77 and 12.57 °Brix respectively (Table 2). On the other hand, the cultivars that had the lowest TSS values were “Clemepons”, “Oronules” and “Iwasaki” (in decreasing order). The *MI* is a relevant parameter to be considered in citrus fruits when their purpose is fresh consumption (Altaf and Khan, 2009, Legua *et al.*, 2011). The cultivars that showed the highest *MI* values were “Loretina” and “Oronules” (14.00 and 13.43 respectively), compared to the “Clemenrubí” cultivar, which had the lowest value (9.25). If the fruits are intended for the juice industry, one of the most substantial parameters would be the sugar content per fruit. Considering this matter, the notable cultivars were “Clemenules” and “Clemenrubí” due to the fact that these were the ones that exceeded the amount of 5 grams of sugar per fruit.

**Table 2.** Internal quality parameters of Total Soluble Solids (TSS, °Brix), total sugar per fruit, Titratable Acidity (TA, g citric L<sup>-1</sup>), Maturity Index (MI), Lyophilic and Lipophilic antioxidant activity index (H-TAA and L-TAA), and total phenolic compounds (mg L<sup>-1</sup>) of the fruit juices of nine cultivars of early maturing mandarin cultivated in Spain.<sup>a</sup>

Cultivar	TA	TSS	g sugar fruit <sup>-1</sup>	MI	H-TAA (mg per 100 g)	L-TAA (mg per 100 g)	Totals phenolic (mg L <sup>-1</sup> )
Clemenules	10.33±0.3 b	11.40±0.8 bc	5.28±0.9 a	11.03±0.8 bcd	7.91±0.4 cd	1.75±0.9 a	49.89±4.6 c
Orogrande	9.50±1.1 bc	11.05±0.1 bc	4.47±0.6 ab	11.73±1.3 bc	17.91±2.3 ab	1.82±1.2 a	53.72±7.8 c
Arrufatina	11.57±1.1 a	11.57±1.3 b	5.67±0.8 a	10.00±0.7 de	12.93±8.4 bcd	1.30±0.8 a	64.78±6.2 b
Oronules	7.34±0.5 e	9.82±0.8 d	3.53±1.3 bc	13.43±1.6 a	7.57±1.2 cd	1.81±0.9 a	75.56±6.3 a
Loretina	9.16±0.7 cd	12.77±0.2 a	3.00±0.5 c	14.00±1.0 a	18.9±3.1 ab	1.85±0.9 a	78.75±8.7 a
Mioro	12.50±0.5 a	12.57±0.5 a	3.70±0.1 bc	10.07±0.6 de	14.16±6.8 b	2.77±1.3 a	47.53±5.2 c
Clemenpons	8.33±0.5 de	10.47±0.2 cd	4.57±0.5 ab	12.59±0.7 ab	7.36±0.1 cd	1.77±1.0 a	47.47±1.4 c
Clemenrubí	12.38±0.5 a	11.45±0.2 bc	5.60±0.5 a	9.25±0.3 e	21.52±5.4 a	1.21±0.3 a	63.35±3.8 b
Iwasaki	8.06±0.7 e	8.75±0.5 d	4.38±1.1 ab	10.91±1.2 cd	6.01±0.5 d	1.24±0.1 a	45.05±2.0 c
ANOVA	***	***	***	***	***	ns	***

<sup>a</sup> Inside each column, the means followed by the same statistical letter do not differ significantly at the 5%. Each value is the mean of four samples. \*\*\* Indicates significant differences at  $P < 0.001$ . <sup>ns</sup> Indicates non-significant at 5%.

### Total Antioxidant Activity and Total Phenolic Compounds

With regards to the TAA of the studied juices, it was found that the “Clemenrubí” cultivar showed the highest value in the Lydrophilic fraction (H-TAA) with 21.52 mg per 100 g (Table 2). No significant differences were observed in the TAA from the Lipophilic fraction (L-TAA).

“Loretina” and “Oronules” showed the most elevated values of total phenolic compounds, with 78.75 and 75.56 mg L<sup>-1</sup> respectively. The phenolic compounds (secondary metabolites of plants) have attracted recent interest owing to their antioxidant properties and possible salubrious actions in human health (Martínez-Valverde *et al.*, 2000; Robbins, 2003). Therefore, the juice from these fruits would present an added value over their nutritional properties versus the other cultivars.

### Organic Acids and Sugars

Organic acids have a considerable influence over both the taste and the nutritional properties of the fruits (Mahmood *et al.*, 2012). In the analyzed cultivars, the concentration decreased in this order: Phytic > citric > malic > succinic > ascorbic (Table 3). Furthermore, the differences in the juice acidity of the different cultivars were mainly due to the concentration of citric acid. A significant linear correlation between the titratable acidity and this acid was observed ( $r^2 = 0.705$ ,  $P < 0.001$ ) (*data not shown*).

Usually, ascorbic acid (vitamin C) in citrus fruits is a parameter that provides a significant added value to human diets given its high antioxidant capacity. In this study, the cultivars that had the highest concentration of ascorbic acids were those that belonged to the group of “new” clementines, with an average concentration of 0.053%, except for the “Clemepons”,

which along with “Iwasaki” had the lowest values (< 0.02%).

It is known that sugars are the main components of the citrus juices, with sucrose being the most common (Kelebek *et al.*, 2009; Legua *et al.*, 2014) and also significantly correlated with the TSS (Simón-Grao *et al.*, 2014). The same was observed in this study of nine mandarin cultivars ( $r^2 = 0.767$ ,  $P < 0.001$ ; *data not shown*). “Clemenules”, “Loretina” and “Mioro” showed the highest values, with an average of 10.9%. In contrast, the cultivar “Iwasaki” had the lowest value (5.85%). The concentrations of glucose and fructose varied between 2.5 and 1.5 g per 100 mL of juice (Table 3).

### Mineral Content

Minerals are as important as vitamins in the human diet in order to keep the body healthy (Koo, 1985). In this experiment, the distribution of the concentration of nutrients follows in decreasing order: K > P > Mg > Ca > S > Na > Zn > Cu. Therefore, it can be said that mandarins are a great source of K, P and Mg. Within the studied cultivars, the highest content of K was found in “Loretina” and “Clemenules”, with 1,678.5 and 1,668.0 mg L<sup>-1</sup> of K, respectively (Table 4), followed by “Clemepons” (1,569.8 mg L<sup>-1</sup>). All other cultivars had similar values, except for “Iwasaki”, which showed the lowest value (1,166.2 mg L<sup>-1</sup>).

As for the P content, it could be seen that the “Clemenules” and “Oronules”, with 155.6 and 128.1 mg L<sup>-1</sup> respectively, were those with the highest content for this macronutrient. In contrast, the cultivars that had the lowest values, with an average of 59.2 mg L<sup>-1</sup>, were “Clemepons”, “Iwasaki” and “Orogrande” (Table 4).

Regarding the concentration of Mg, “Iwasaki” was the cultivar with the lowest content (61.0 mg L<sup>-1</sup>), while “Clemenules” had the highest value (89.3 mg L<sup>-1</sup>), followed by “Loretina”, “Mioro” and “Oronules” that showed an average value of



**Table 3.** Profile of the organic acids and sugar compounds in the fruit juices of nine cultivars of early maturing mandarin cultivated in Spain.<sup>a</sup>

Cultivars	% (g acid per 100 g fruit juice)				
	Phytic	Citric	Malic	Ascorbic	Succinic
Clemenules	4.48±0.2 a	1.02±0.05 cd	0.36±0.02 ab	0.03±0.01 bc	0.10±0.01 e
Orogrande	3.09±0.2 b	1.12±0.05 bc	0.32±0.03 b	0.02±0.00 cd	0.17±0.04 bcd
Arrufatina	3.12±0.2 b	1.26±0.10 ab	0.30±0.03 b	0.04±0.01 b	0.13±0.06 cde
Oronules	2.39±0.4 c	0.80±0.02 e	0.34±0.01 ab	0.03±0.01 d	0.20±0.04 de
Loretina	2.36±0.2 cd	1.08±0.19 c	0.41±0.10 a	0.05±0.01 a	0.14±0.06 cde
Mioro	2.99±0.1 b	1.37±0.03 a	0.33±0.01 b	0.05±0.00 a	0.09±0.01 e
Clemenpons	2.87±0.5 b	1.03±0.05 cd	0.36±0.01 ab	0.02±0.00 d	0.12±0.01 de
Clemenrubí	1.95±0.3 d	1.25±0.16 ab	0.31±0.03 b	0.06±0.01 a	0.20±0.04 bc
Iwasaki	2.24±0.2 cd	0.91±0.03 de	0.22±0.01 a	0.01±0.00 e	0.300±0.04 a
ANOVA	***	***	***	***	***

Cultivars	% (g sugar per 100 g fruit juice)			
	Sucrose	Glucose	Fructose	
Clemenules	11.21±0.1 a	1.56±0.1 c	1.78±0.1 bcd	
Orogrande	9.49±0.3 b	1.55±0.05 c	1.27±0.1 d	
Arrufatina	9.40±0.8 b	1.89±0.3 b	2.17±0.3 ab	
Oronules	7.29±0.9 d	1.26±0.15 d	1.46±0.2 cd	
Loretina	10.63±0.6 a	1.82±0.15 b	2.17±0.1 ab	
Mioro	10.86±0.4 a	2.26±0.1 a	2.50±0.1 a	
Clemenpons	8.61±0.4 c	1.50±0.1 c	1.84 ±0.1 bcd	
Clemenrubí	9.07±0.5 bc	2.24±0.2 a	2.68±0.25 a	
Iwasaki	5.85±0.4 e	1.57±0.1 c	2.08±0.14 abc	
ANOVA	***	***	***	

<sup>a</sup> Inside each compound, the means followed by the same statistical letter do not differ significantly at the 5%. Each value is the mean of four samples. \*\*\* Indicates significant differences at  $P < 0.001$ .



approximately 83.3 mg L<sup>-1</sup> (Table 4). With regard to the Ca concentration, it was low, not exceeding 80 mg L<sup>-1</sup> for all the cases. Therefore, the studied mandarin fruits were quite poor in this macronutrient. It was an expected result, since it is known that mandarin fruits are not an important source of Ca (George and Doris, 1971). On the same topic, the “Loretina” cultivar had the highest concentration of Ca (71.2 mg L<sup>-1</sup>) and the lowest content was found in the “Clemepons” cultivar (37.81 mg L<sup>-1</sup>).

With respect to the micronutrients, the low concentration of Na in all cultivars (< 10.0 mg L<sup>-1</sup>) was noteworthy. This aspect can make the mandarin juices be recommended by nutritionists and is indicated for people with cardiovascular problems. The European Fruit Juice Association (AIJN) sets a maximum limit of 300 mg L<sup>-1</sup> of Na content in citrus juices and 10 mg L<sup>-1</sup> for beverages made from the juice (AIJN, 2014). “Mioro” and “Loretina” cultivars were those with the highest values (7.27 and 6.94 mg L<sup>-1</sup>, respectively), whereas the rest of the cultivars were below 5.5 mg L<sup>-1</sup> (Table 4). As for the S content, the cultivars with the highest concentration of this element were “Iwasaki” and “Clemenules”, with an average around 35.93 mg L<sup>-1</sup>, while the rest of the cultivars did not show significant differences between them. In relation to the Cu and Zn contents, it is recommended that the concentrations of these micronutrients in fruit juices never exceed 5 mg L<sup>-1</sup> (Goldman *et al.*, 1999). For the Cu content, no studied cultivar exceeded 0.25 mg L<sup>-1</sup>. In the case of the Zn concentration, all cultivars had less than 0.5 mg L<sup>-1</sup>, except for “Clemenules”, “Mioro” and “Loretina”, which had quite high values above 2.0 mg L<sup>-1</sup>.

### Volatile Aroma Compounds

Apart from the texture and color of the fruits, taste is an extremely important factor for the ready acceptance of edible products by consumers. Taste is determined by

organic acids, sugars and aromas. A pleasant and easily recognizable aroma is usually a guarantee for consumers, and a criterion by which they may accept the product. On the other hand, if the discerned aroma is not the one traditionally associated with the product, the consumer is likely to reject it. Nevertheless, flavor is one of the most sensitive and subjective characteristics, given its extreme complexity from the metabolic and biochemical point of view. During the analysis of aromas in our study, we identified 29 compounds (Figure 1), the majority of which belonged to the group of monoterpenes (compounds of 10 carbon atoms). Other compounds were also identified that did not belong to this group, for example, alcohols: 1-nonanol, decanal and hexanal, or the neryl acetate and *trans*-caryophyllene compounds. All these compounds had already been identified in previous studies of mandarin cultivars (Lota *et al.*, 2001; Dugo *et al.*, 2002; Merle *et al.*, 2004; Pérez-López and Carbonell-Barrachina, 2006). The main volatile compound (95% with respect to the total aromas detected) was the monoterpene limonene, as is usually observed in the majority of juices and essential oils from citric fruits (Goldman *et al.*, 1999). Following limonene as a function of concentration was  $\beta$ -myrcene, with a concentration of 25  $\mu$ g L<sup>-1</sup>. The rest of the detected volatile compounds had a concentration below 10  $\mu$ g L<sup>-1</sup>.

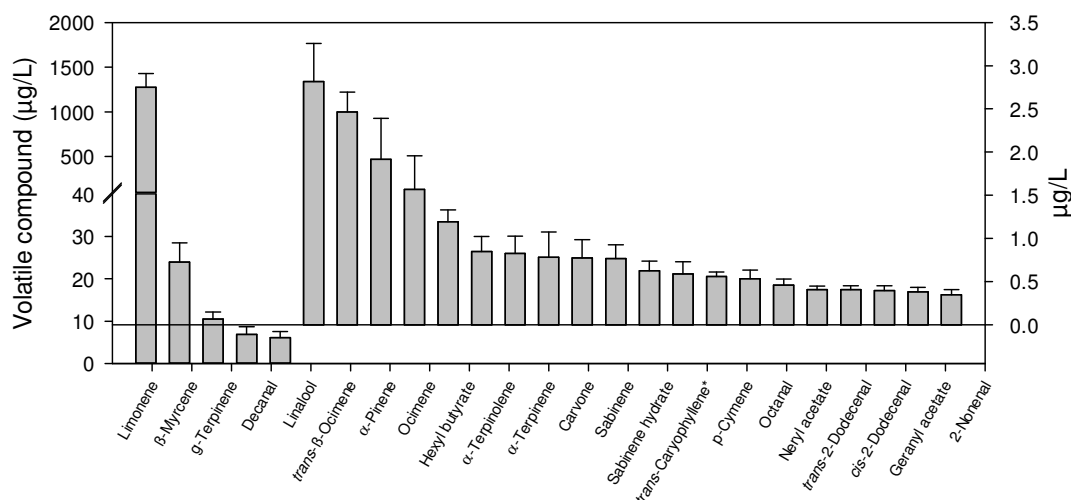
For a further understanding of the trends and relationships among the main volatile aroma compounds within sets of cultivar genotypes, a Principal Component Analysis (PCA) was used. The total variability was explained by 18 principal components (Table 5). The first four principal compounds explained 90.81% of the total variation, whereas nearly 84.52% of the variability was explained by the first three compounds. PC1 (59.11%) showed a high discriminating power and allowed for the differentiation of the “Mioro” cultivar from the rest, due to its high content of the following volatile compounds in its juice:



**Table 4.** Profile of mineral nutrients in the fruit juices of nine cultivars of early maturing mandarin cultivated in Spain.

Variety	Macronutrients (mg L <sup>-1</sup> )			
	Ca	K	Mg	P
Clemenules	43.72±7.7 cde	1668±105 ab	89.34±2.7 a	155.57±8.5 a
Orogrande	38.39±4.6 de	1340±107 ef	74.37±2.8 b	58.47±5.0 d
Arrufatina	58.06±5.3 b	1443±109 ce	82.08±5.5 a	103.88±23.9 c
Oronules	57.86±14.0 b	1470±77 ce	82.84±9.8 a	128.09±26.8 b
Loretina	71.22±11.5 a	1678±141 a	83.62±0.6 a	80.28±10.3 cd
Mioro	54.85±6.4 bcd	1429±86 ce	83.53±1.9 a	79.28±7.7 cd
Clemenpons	37.81±5.0 e	1570±99 abc	64.09±3.7 c	59.82±17.0 d
Clemenrubí	39.31±7.9 de	1483±239 bce	74.21±5.2 b	88.79±26.3 c
Iwasaki	50.92±4.0 bcd	1166±21 f	60.99±2.7 c	59.21±5.8 d
ANOVA	***	***	***	***
Variety	Micronutrients (mg L <sup>-1</sup> )			
	S	Na	Cu	Zn
Clemenules	35.60±1.46 a	5.13±0.41 bc	0.20±0.02 ab	3.38±0.8 a
Orogrande	28.19±1.98 b	3.40±0.08 cd	0.18±0.02 bcd	0.23±0.02 b
Arrufatina	29.72±1.52 b	4.76±0.85 c	0.22±0.04 a	0.25±0.01 b
Oronules	27.80±2.44 b	5.32±0.68 bc	0.21±0.03 ab	0.35±0.07 b
Loretina	30.87±2.62 b	6.94±1.59 ab	0.16±0.02 cd	2.07±1.5 ab
Mioro	28.68±2.61 b	7.27±2.59 a	0.19±0.02 abc	3.17±1.7 a
Clemenpons	28.42±6.02 b	4.96±0.82 c	0.14±0.01 d	0.19±0.02 b
Clemenrubí	29.53±4.52 b	3.76±1.47 cd	0.14±0.04 d	0.26±0.06 b
Iwasaki	36.26±1.94 a	2.66±0.28 d	0.22±0.01 a	0.26±0.01 b
ANOVA	***	***	***	**

<sup>a</sup> Inside each compound, the means followed by the same statistical letter do not differ significantly at the 5%. Each value is the mean of four samples. \*\* and \*\*\* Indicate significant differences at  $P < 0.01$  and  $P < 0.001$ , respectively.

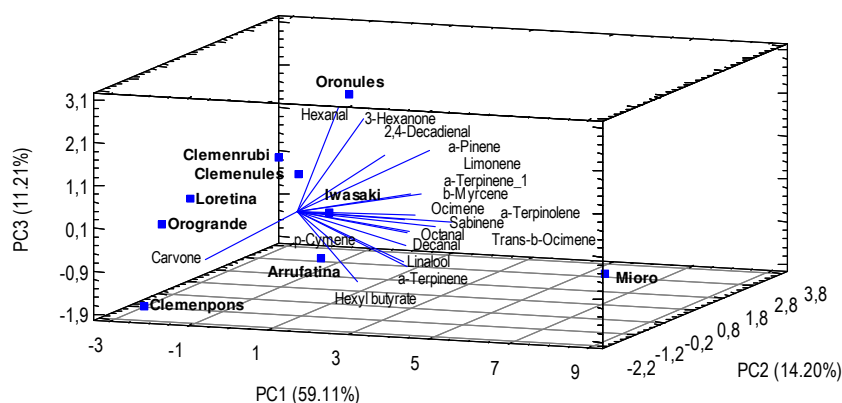


**Figure 1.** Profile of the volatile composition compounds in the fruit juice of 9 cultivars of early maturing mandarin in Spain. Each value is the mean ( $\pm$ SE) of 36 samples.

limonene,  $\beta$ -myrcene, decanal, linalool, *trans*- $\beta$ -ocimene, sabinene and octanal and  $\alpha$ -terpinene (Table 5, Figure 2). PC2 (14.20%) mainly explained the variables  $\gamma$ -terpinene, *p*-cymene and carvone (Table 5). This principal component allowed us to discriminate cultivars by their  $\gamma$ -terpinene, *p*-cymene and carvone contents; it was found that “Iwasaki” cultivar was the one that had the highest concentration of *p*-cymene and the lowest of carvone, whereas the “Clemepons” cultivar showed the highest carvone content and the

lowest concentration of  $\gamma$ -terpinene. PC3 (11.21%) provided information about the volatile components  $\alpha$ -pinene, hexanal, 2,4-decadienal and hexyl butyrate. The cultivars “Oronules” and “Iwasaki” could be discriminated against the other cultivars due to their high hexanal and 2,4-decadienal contents.

According to the information shown in Figure 2, the relationship between the studied cultivars and the contents of volatile compounds of the obtained fruits as well as a first array in terms of those characteristics



**Figure 2.** Principal Component Analysis (PC1, PC2 and PC3) of volatile composition compounds with 3D-plot position of 9 cultivars of early maturing mandarin in Spain as given by first three PCs.

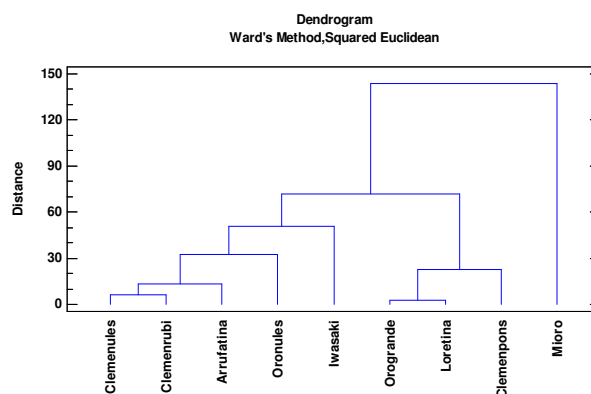
**Table 5.** Eigenvalues, proportion of variation and eigenvectors associated with three axes of the PCA in the main aromatic compounds in nine cultivars of mandarin.

Principal components (Axes)	1	2	3
Eigenvalues	10.64	2.55	2.02
Cumulated proportion of variation	59.11	73.31	84.52
Characters	Eigenvectors		
Limonene	0.293	-0.055	0.144
$\beta$ -Myrcene	0.304	-0.053	0.034
$\gamma$ -Terpinene	0.083	0.552	-0.094
Decanal	0.293	-0.090	-0.111
Linalool	0.276	-0.057	-0.205
trans- $\beta$ -Ocimene	0.280	0.130	-0.093
$\alpha$ -Pinene	0.238	0.204	0.269
Ocimene	0.264	-0.021	-0.003
$\alpha$ -Terpinolene	0.262	0.280	-0.123
3-Hexanone	0.032	0.323	0.365
Hexanal	0.127	-0.071	0.569
2,4-Decadienal	0.222	-0.037	0.327
Hexyl butyrate	0.230	-0.224	-0.258
Sabinene	0.297	-0.077	-0.043
Octanal	0.290	-0.071	-0.053
$\alpha$ -Terpinene	0.274	-0.030	-0.243
<i>p</i> -Cymene	-0.088	0.459	-0.333
Carvone	-0.067	-0.396	-0.118

could be established. This result was highlighted and made clear through cluster analysis, as shown by the dendrogram in Figure 3. The cultivars that were nearest to each other were “Loretina” and “Orogrande”, followed by “Clemenules” and “Clemenrubi”, whereas “Mioro” presented a larger distance from the other cultivars and consequently, was the one that showed a significantly different behavior.

## CONCLUSIONS

It can be concluded that the “Clemenrubi” fruits are the most suitable for fresh consumption. These fruits had a good relationship between the size (108 g, average fruit weight) and volume of juice (49 mL fruit<sup>-1</sup>).

**Figure 3.** Dendograms using Ward's methods based on squared Euclidean distance.

Moreover, these are seedless fruits and showed both external and internal color indexes within normal ranges for mandarins. By their chemical properties, it was agreed that they were pleasant-to-eat fruits, whose juice had a TSS content of 11.45, although they had an acidic flavor due to the high titratable acidity ( $12.38 \text{ g L}^{-1}$ ). This cultivar is also noted for the nutritional characteristics of the fruits, as they had a high concentration of both ascorbic acid and total phenols. The fruits of the “Clemenules” cultivar also had good physicochemical characteristics highlighting their high mineral content, especially K, Mg and P. For juice production, the cultivar “Arrufatina” could be the ideal one, owing to the high quantity of juice together with the high TSS concentration of its fruits, and also the nutritional characteristics that were similar to those of the other studied cultivars. As for the analytical profile of the volatiles, limonene was the main aroma found in the mandarin juices. The cultivar “Mioro” showed a different profile from the rest of the cultivars according to the principal multi-components analysis performed.

## ACKNOWLEDGEMENTS

Funding for this research came from the Ministry of Education and Science, government of Spain (Project Plan National AGL2007-65437-C04-02/AGR; AGL2011-24795) and FONDOCYT, Dominican Republic, Subvention No. 2013-2C1-106.

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### خواص فیتو شیمیایی و شماسه ترکیب فرار ۹ نارنگی زودرس کشت شده در جنوب شرقی اسپانیا

ف. گارسیا-سانچس، س. سایمون-گراو، و. جیمینو، ل. گالوز-سولا، و. لیدون، ی.  
سایمون، ف. هرناوندز، ج. ج. مارتینز-نیکولاس، و. ا. ا. کاربونل - باراچینا

### چکیده

بسیاری از گونه های نارنگی از نظر تغذیه ای و حسی طبقه بندی نشده اند. برای انتخاب ارگانیزم کشت با کیفیت بالا، دانستن این نکته بسیار مهم است. ما خواص فیزیکی شیمیایی ۹ نارنگی تجاری در حال بلوغ از جنوب شرقی آسیا را طبقه بندی کرده ایم: ۴ کلمنتین سنتی (Clemenules, Orogrande, Arrufatina, Oronules)، ۴ کلمنتین جدید (Iwasaqui, Loretina, Mioro, Clemenpons and Clemenrubí or Prim-23) و یک ساتسوما (Iwasaqui). 'Clemenules'، 'Oronules' و 'Iwasaki' گونه هایی بودند که بیشترین وزن را وقتی که تازه بودند داشتند ( $>120\text{ g}$ ). بالاترین حالت اسیدی مربوط به گونه Mioro بود ( $12.50\text{ g L}^{-1}$ ) و مایعات Loretina و Mioro بالاترین میزان مواد جامد محلول را نشان دادند، به ترتیب  $12.77$  و  $12.57$ . Oronules و Loretina بالاترین میزان کل ترکیبات فنولیک، به ترتیب  $78.75$  و  $75.56$  میلی گرم را نشان دادند. ترکیب اصلی فرار، لیمون مونو ترپین بود و به دنبال آن غلظت  $\beta$ -myrcene بود ( $25\text{ }\mu\text{g L}^{-1}$ ). بهترین نوع در بین ۹ گونه مورد بررسی برای مصرف تازه، کلمنروبی "Clemenrubí" بود با توجه به محتوای بالای فنول و اسید اسکوربیک آن. عطر اصلی آب نارنگی لیمون بود و ارگانیزم کشت Mioro مشخصات متفاوتی نسبت به بقیه ارگانیزمهای کشت، با توجه به تجزیه و تحلیلهای مؤلفه های اصلی نشان داد.