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ORIGINAL PAPER

Organic acids, sugars, antioxidant activity, sensorial and other fruit characteristics of nine traditional Spanish *Citrus* fruits

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Abstract

The knowledge of the beneficial health properties of underutilised varieties of fruits is very valuable for the conservation of plant genetic diversity and agricultural development. The colour, weight, morphological parameters and total antioxidant activity (TAA) of the edible tissues of nine traditional *Citrus* fruits, three mandarin varieties, three lemon varieties, 'Dulce' lime, 'Cimboba' and 'Blanco' grapefruit was quantified. In addition, other fruit quality properties, such as organic acids and sugar concentrations in the *Citrus* juices were analysed, and the evaluation of organoleptic attributes, such as sweetness, aroma, firmness, lack of bitterness, overall impression and notable feature of fruits, was performed by a sensory panel. Results show significant differences among *Citrus* species and varieties of the analysed parameters. Analysis of the weight of the whole fruit and its edible tissues showed that the relative proportion of each fruit tissue was similar for all the studied *Citrus* species and varieties. on the other hand, 'Autóctona' mandarin and 'Fino' and 'Sanguino' lemons showed the highest TAA, while the ones most appreciated by consumers according to the sensory panel results were 'Dulce' lime followed by 'Sanguino' lemon, which could be due to their high fructose concentration and original colour, respectively. The utilisation of certain traditional *Citrus* species and varieties, such as'Mandarin' and 'Autoctona' mandarins, 'Sanguino' lemon and 'Dulce' lime, of the south-east of Spain in future breeding programmes to increase agricultural biodiversity. In addition, the consumption of traditional varieties of *Citrus* fruits with high antioxidant activity would improve the beneficial effect of fruits in human health.

Keywords Organics acid · Sugars · Antioxidant activity · *Citrus* fruits

Introduction

It is widely accepted that the adaptation of agricultural systems to climatic change is the most important challenge that human beings must face in the coming years [1, 2]. In this sense, the conservation and use of plant genetic diversity are essential for satisfying the needs of future world development. That is, the use of traditional varieties and their wild relatives for plant breeding is highly valuable for agricultural development, since these plants have acquired many desirable characteristics as a consequence of their long exposure to natural selection. Promising crops, including species and varieties with an important role in traditional food and agriculture, but nowadays negligible due to political or socio-economic reasons, are regarded as an important tool to face the new climatic scenery [2–4]. In the last few years, some of these promising crops have been re-valorised and have proved their important role in facing the problems related to climactic change, food and nutrition safety and environmental degradation, as well as in increasing the income in rural areas and even fighting poverty, hunger and malnutrition in developing countries [5–8].

In addition, traditional varieties and wild species are also appreciated for their nutritional value, versatility of use and also health benefits [9–11]. On the other hand, they are responsible for the unique taste of the local cuisine and have high economic importance in rural and urban areas [2, 7, 8]. Recently, these traditional plants are being used in restaurants to make delicatessen recipes [8, 10, 12].

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Spain is one of the major producers and exporters of *Citrus* fruits, especially lemons and oranges, although intensive agricultural practices have reduced the number of grown varieties to a few ones, as in most of the cultivated plant species, with the concomitant risk of genetic variability loss. However, several traditional *Citrus* species and varieties that have been cultivated for a long time still survive in the orchards of the south-east of Spain and are very well adapted to Mediterranean climatic conditions and highly valued due to their excellent quality [13, 14].

Organic acids and sugars are among the major nutritional compounds in *Citrus* fruits and are also of interest because of their important influence on the sensory properties of fruit juices. These are regarded as significant quality factors by both consumers and the food industry [15–18]. *Citrus* fruits not only have delicious flavours, but also have antioxidant capacity, with associated health benefits. It has been strongly demonstrated that the increasing trend in obesity is accompanied by a growing incidence of diabetes. In this sense, *Citrus* fruits represent a good source of bioactive compounds with antidiabetic and lipolytic effects [19] and also promising prospect in preventing cardiovascular and neurodegenerative diseases and certain types of cancer, which are being studied nowadays with increasing interest [15, 17, 19–23]. The antioxidant activity and organic acid and sugar content vary depending on several factors, such as species, varieties, irrigation and climatic conditions, among others [13, 17, 24–27]. The total antioxidant capacity of *Citrus* fruits has been mainly attributed to ascorbic acid and phenolic compounds, though there have been some divergences as to which compound was the major contributor [28–30].

The aim of this study was to evaluate the TAA of the edible tissues (juice, albedo and carpelar membrane) of nine traditional *Citrus* fruits in the south of Spain. In addition, other fruit properties, such as organoleptic attributes, organic acids and sugars in the fruit juice, were analysed. This knowledge could be useful for reconsidering the utilisation of certain traditional *Citrus* species and varieties in future breeding programmes to help in the efficient conservation of an important part of the agricultural biodiversity of the *Citrus* group species and cultivars. In addition, the use of traditional varieties or species with high antioxidant activity would improve the health beneficial effects of *Citrus* fruit consumption.

Materials and methods

Plant material

The *Citrus* fruits used in this research are autochthonous of the south-east of Spain, and they were three mandarin varieties (‘Mandarina’, ‘Autóctona’ and ‘Clementina’), three lemon varieties (Dulce’, ‘Fino’ and ‘Sanguino’), ‘Dulce’ lime, ‘Cimboba’ and ‘Blanco’ grapefruit. These species and varieties of *Citrus* fruits have been classified according to Rivera et al. [14] (Table 1). ‘Clementina’ mandarin and ‘Fino’ lemon are *Citrus* varieties still commercially used in Spain. However, as they have been cultivated for more than 50 years they are in fact considered as traditional ones, according to Ribera et al. [14]. For each *Citrus* species or variety, 60 fruits were harvested at the ripe stage, when skin colour was fully developed and taken to the laboratory. Then, 40 fruits, homogeneous in size and colour, were selected and divided into four lots; three of them (or replicates) were used

Table 1 Identification of the nine species and varieties of underutilised Spanish *Citrus* fruits

Citrus group	Botanical name	Local name
Mandarins	*Citrus reticulata* Blanco subsp. *deliciosa* (Riso) nov. comb. et stat.	‘Mandarina’
	Citrus reticulata Blanco subsp. *deliciosa* (Ten.) D. Rivera y cols. etnovar. “Mandarina del terreno”	‘Autóctona’
	Citrus clementina Hort. Ex.tan.	‘Clementina’
Lemons	*Citrus* × *limodulcis* D. Rivera y cols. etnovar. “Limón dulce”	‘Dulce’
	Citrus limón (L.) Burn. etnovar. “Fino”	‘Fino’
	Citrus × limoroseus D. Rivera y cols. etnovar. “Sangrino” (= Pink Flesh)	‘Sanguino’
Other *Citrus* fruits	*Citrus limetta* Risso subsp. *Limetta* etnovar. “Lima dulce del país”	‘Lima Dulce’ (‘Dulce’ lime)
	Citrus máxima (Burm.) Merrill etnovar. “Cimboa”	‘Cimboba’
	Citrus × paradisi Macfad. In hook. Etnovar. “Pomelo”	‘Pomelo Blanco’ (‘Blanco’ grapefruit)

for analytical determinations and the remaining for sensory analysis.

Analytical determinations

Colour was determined as previously reported [31] in two opposite points of the equatorial diameter of each fruit by a Minolta colorimeter (CRC200, Minolta Camera Co., Japan), using the CIELAB coordinates. L^*, a^* and b^* parameters were measured and colour expressed as *Citrus* colour index ($CCI = a^*1000/L^*b^*$). Fruit diameter (*D*), length (*L*) and weight were also measured in each fruit.

Ethylene production and respiration rate were measured according to Díaz-Mula et al. [31] by sealing ten fruits of each subsample or replicate for 1 h in a 3 L or 30 L jars for Cimboba fruits, fitted with a silicon septum. After this time, 1 mL of the jar atmosphere was withdrawn and the ethylene concentration determined using a Hewlett-Packard gas chromatograph model 5890 (Wilmington, DE, USA) equipped with a flame ionisation detector and a stainless steel column packed with 80/100 mesh activated alumina. The carrier gas was nitrogen, at a flow rate of 30 mL min^{-1}, column temperature was 90 °C, and the injector and detector temperatures were 150 °C. The results were expressed as nanolitres of ethylene evolved per gram of fruit per hour (nL g^{-1} h^{-1}). Another 1 mL gas sample was used to determine the CO_2 concentration using a Shimatzu GL 14A gas chromatograph (Kyoto, Japan) equipped with a catarometric detector and a molecular sieve 5A column, 80–100 mesh (Carbosieve SII, Supelco Inc., Bellefonte, USA), of 2 m length and 3 mm i.d. Oven and injector temperatures were 50 and 110 °C, respectively. Helium was used as carrier gas at a flow rate of 40 mL min^{-1}. Respiration rate was expressed as milligrams of CO_2 evolved per kg of fruit per hour (mg kg^{-1} h^{-1}).

After that, the fruits of each replicate were separated into their edible portions (albedo, carpelar membranes and juice vesicles), which were weighed. Carpelar membrane and albedo were lyophilised to obtain a homogeneous sample of each replicate and stored at − 20 °C until total antioxidant activity (TAA) was measured. The number of seeds per fruit was recorded and the vesicle juices from the ten fruits of each lot were ground to obtain the juice, which was centrifuged at 10,000*g* for 10 min and the supernatant used for organic acids, sugars and TAA analysis.

Organic acid and sugar concentrations in juice vesicles were quantified in duplicate in each sample as previously reported [32] by using an HPLC system (Hewlett-Packard, series 1100, Waldbrom, Germany) equipped with a SUPELCOGEL C-610H (30 cm × 7.8 mm) column (at 30 °C), an absorbance detector (210 nm UV, for acid analysis) and a refractive index detector (for sugar analysis). The elution system was 0.1% H_3PO_4, running isocratically at a flow rate of 0.5 mL min^{-1}. Organic acids were quantified from the absorbance peaks at 210 nm and using calibration curves performed with citric, malic, succinic and ascorbic acid standards from Sigma (Poole, Dorset, England). Results were expressed as grams per 100 g of fresh weight (g 100 g^{-1} FW) for citric, malic and succinic acids and as miligrams per 100 g of fresh weight (mg 100 g^{-1} FW) for ascorbic acid. Sugars were quantified by comparison of refractive index peaks with those of standards of glucose, fructose and sucrose from Sigma (Poole, Dorset, England) and results were expressed as g 100 g^{-1} FW.

Total antioxidant activity (TAA) was determined on duplicate in each sample according to Pretel et al. [13] using the enzymatic system composed of the chromophore 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), the horse radish peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide), in which $ABTS^{\cdot+}$ radicals are generated. The reaction mixture contained 1.5 mM ABTS, 15 µM hydrogen peroxide and 0.25 µM HRP in 50 mM glycine–HCl buffer (pH 4.5) in a total volume of 2 mL. The absorbance was measured at 414 nm using a UNICAM Helios α spectrophotometer (Cambridge, UK). The fruit extract samples were added and their antioxidant compounds regenerated the ABTS. The absorbance was measured at 414 nm after 3 min. The assay temperature was 25 °C. A calibration curve was performed with L-ascorbic acid (0–20 nmol) from Sigma (Poole, Dorset, England), and the results are expressed as mg of L-ascorbic acid equivalent per 100 g of fresh weight (mg 100 g^{-1} FW).

Sensory evaluation

Sensory analysis of *Citrus* fruits species and varieties was carried out by ten trained adults, aged from 25 to 40 (5 female and 5 male) years. The panel was trained in a pre-test in which *Citrus* fruits with extremely low or high attributes (sweetness, aroma, segment firmness and lack of bitterness) were evaluated. In addition, the panel evaluated the overall impression and notable feature of fruits [12, 13]. A laboratory of sensory analyses with an individual booth for each panellist was used. Every panellist was randomly served one segment from each of the ten different fruits for each species and variety to evaluate the above-mentioned attributes on a ranked scale from 1 (very low) to 5 (very high).

Statistical analysis

Data from analytical determinations are mean ± SE of the measures made in three replicates of ten fruits along with standard errors. For sensory analysis, data are the mean ± SE of scores given by ten judges. Correlation and regression analyses were performed to evaluate the relationship between the different determined parameters using Sigma

Plot 11.0. The significant differences among species and varieties were calculated by Duncan's multiple-range test ($p<0.05$) using SPSS software package v. 21.0 for Windows. Principal component analysis (PCA) was performed using the package NTSYSpc 2.0 for Windows.

Results and discussion

Morphological fruit characteristics

The fresh weight of *Citrus* fruits varied significantly among species and varieties, ranging between 35.83 ± 2.19 g in 'Clementina' mandarin and 1243.67 ± 95.44 g in 'Cimboba', which together with 'Blanco' grapefruit were the species with the highest juice vesicle weight, 769.82 ± 49.06 g and 130.11 ± 42.02, respectively. Albedo weight ranged between 1.18 ± 0.12 g per fruit in 'Clementina' mandarin and 250.65 ± 25.53 g per fruit in 'Cimboba', whereas the carpelar membrane weight ranged from 3.23 ± 0.51 to 69.65 ± 7.843 g per fruit in the same fruits (Fig. 1). Thus, results show that *Citrus* fruit species and varieties differed significantly in fruit weight, juice content and albedo and carpelar membrane weight. However, a positive correlation was found between fruit weight and juice vesicle weight ($y=0.619x+0.527$, $r^2=0.99$) in the different *Citrus* fruits, as well as between fruit vesicle weight and albedo weight ($y=0.33x-10.45$, $r^2=0.99$), juice vesicle weight and carpelar membrane weight ($y=0.088x+1.93$, $r^2=0.99$) and albedo and carpelar membrane weight ($y=0.259x+4.71$, $r^2=0.99$). These correlations show that that the relative proportion of each fruit tissue is similar for all the *Citrus* varieties studied. On the contrary, in previous studies no significant correlations were obtained between fruit weight and juice weight or albedo or carpelar membrane weight in a wide range of orange cultivars [13]. In these orange varieties, the relative proportion of each tissue was different for each one and independent of fruit size. However, these relative proportions do exist among *Citrus* species and varieties used in the present study. According to fruit weight values, the highest values of length and diameter were found in 'Cimboba' and the lowest in 'Clementina' mandarin (Fig. 2). Flavedo and albedo tissues could be considered as by-products of these *Citrus* fruits and as a source for volatile oils and fibre extraction, respectively. In this sense, 'Cimboba' could be an interesting variety, since the weights of flavedo and albedo tissues were ca. 150 and 250 g per fruit, respectively.

The *Citrus* colour index was highest in mandarins than in the other *Citrus* fruits, with the highest value being found in 'Autóctona' mandarin due to deeper colour change (Fig. 2). Lemon varieties, 'Cimboba' and 'Blanco' grapefruit had CCI close to 0, showing they had light yellow-coloured skin. In addition, important differences were also found in juice vesicle colour, as can be observed in Fig. 2. Significant differences were also found among fruit species and varieties in the number of seeds per fruit, ranquing from 1 in `Cimboba' to 24 in `Mandarina´ mandarin.

Respiration rate and ethylene production

Respiration rate at harvest was significantly different depending on the *Citrus* species and varieties, with values ranging from 9.68 ± 1 to 25.4 ± 0.98 mg $Kg^{-1}h^{-1}$ in 'Sanguino' lemon and 'Mandarina' mandarin, respectively

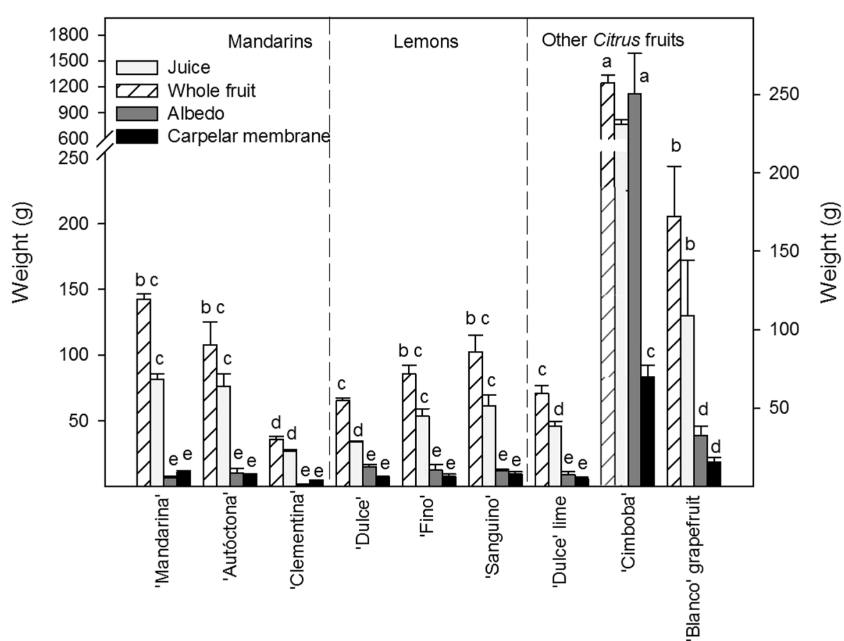

Fig. 1 Fruit and juice vesicle weight (*y* left scale) and albedo and carpelar membrane weight (*y* right scale) of different *Citrus* fruits. Data are the mean ± SE of three replicates of ten fruits. For each parameter, different letters show significant differences among *Citrus* species and varieties

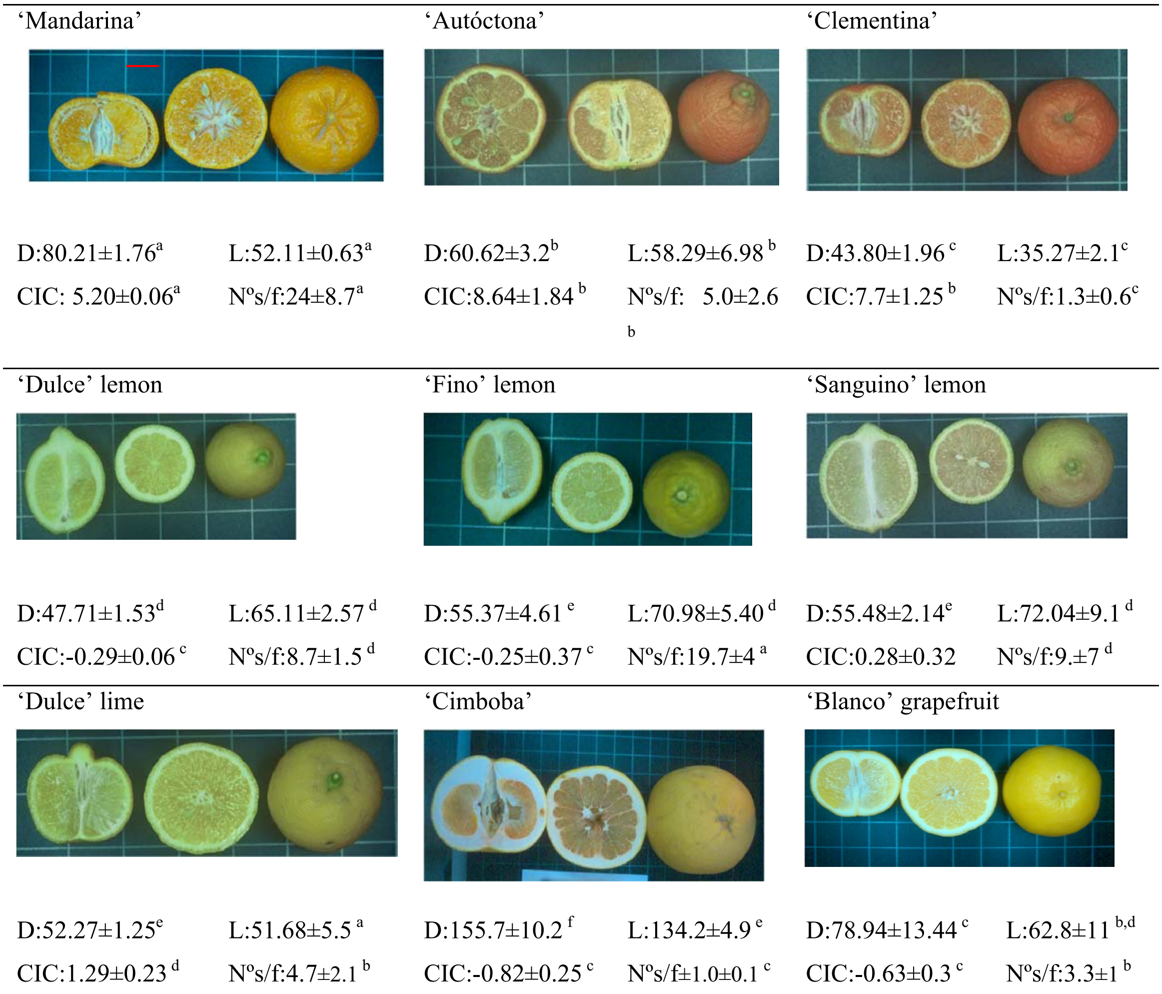

Fig. 2 Photographs of the different *Citrus* varieties. Values of fruit diameter (*D*, mm), length (*L*, mm), *Citrus* index colour (CIC) and number of seeds per fruit (No s/f). Data are the mean ± SE of tree replicates of ten fruits. For each parameter, different letters show significant differences among *Citrus* species and varieties

(Fig. 3). These values are relatively low as compared to other fresh fruits, since respiration rate in *Citrus* fruits, as in other non-climacteric fruits, decreases with fruit development on tree [33]. Similarly, ethylene production was also low during ripening of non-climacteric fruit, although significant differences were found among the studied *Citrus* fruits. Thus, 'Blanco' grapefruit showed the lowest ethylene production, ≈0.01 nL g^{-1} h^{-1}, while the highest was found in 'Autóctona' mandarin with values of 0.33 ± 0.06 nL g^{-1} h^{-1}. *Citrus* fruits are non-climacteric fruits in which ethylene production is very low and ethylene is not required for the coordination and completion of the ripening process. However, these fruits have a high sensitivity to exogenous ethylene, which can affect some of their quality parameters during postharvest storage, such as enhanced pathogen susceptibility, physiological disorders and senescence, with a net reduction in postharvest life [34]. Thus, it is interesting to know the ethylene production of these fruits to know how much ethylene could be accumulated in the storage chambers and, in turn, would induce detrimental effects in these non-climacteric fruits.

Organic acid and sugar content

Organic acids, ascorbic acid and sugars are the major components of the juice of *Citrus* fruits, although their profile and concentration depend on the fruit species and varieties, and also their concentration is affected by ripening stage, environmental conditions and agronomical practices [17, 24]. The main organic acid in the studied *Citrus* fruits was citric acid, and as expected the highest concentrations of citric acid, 6.78 ± 0.20 and 5.44 ± 0.22 g 100 g^{-1} FW, were obtained in 'Fino' and 'Sanguino' lemons, respectively, followed by 'White' grapefruit and 'Cimboba' with 3.09 ± 0.11

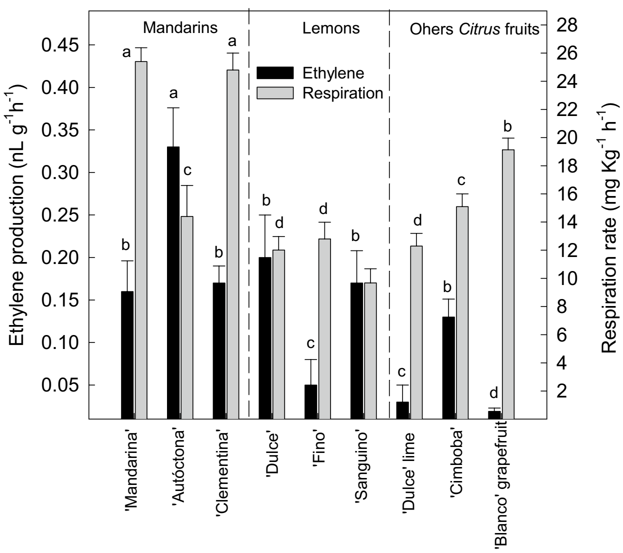

Fig. 3 Ethylene production and respiration rate of the different *Citrus* fruits. Data are the mean ± SE of three replicates of ten fruits. Different letters in bars of ethylene and respiration rates show significant differences among *Citrus* varieties

and 2.47 ± 0.13 g 100 g^{-1} FW, respectively. However, in 'Dulce' lemon and 'Dulce' lime, the citric acid concentration was very low, 0.11 ± 0.01 and 0.14 ± 0.01 g 100 g^{-1} FW, respectively (Fig. 4). Malic acid was the second major acid present in most of the *Citrus* species and varieties, and it was found at concentrations ranging from 0.21 ± 0.01 to 0.51 ± 0.03 g 100 g^{-1} FW in 'Blanco' grapefruit and 'Sanguino' lemon, respectively, and the major organic acid in 'Dulce' lemon and 'Dulce' lime (Fig. 4). These acidless fruits also had the highest concentration of succinic acid ca. 0.17 and 0.12 g 100 g^{-1} FW, respectively, while in the remaining *Citrus* fruits succinic acid concentration was lower than 0.1 g 100 g^{-1} FW (Fig. 4). Previously, it has been reported that citric is the main organic acid in all *Citrus* fruits belonging to several mandarin [17, 35] and lemon cultivars, either acidic or acidless ones [24, 36]. However, the present results show that in 'Dulce' lemon and 'Dulce' lime, the major organic acid was malic acid, while in the acidic fruits citric was the major acid. Finally, ascorbic acid concentration varied significantly among *Citrus* species and varieties, with the higher concentration being found in 'Clementina' mandarin, 74.83 ± 6.69 mg 100 g^{-1} FW, and the lowest in 'Dulce' lemon and 'Mandarina' mandarin, 4.75 ± 0.03 and 5.37 ± 0.64 mg 100 g^{-1} FW, respectively (Fig. 4).

Thus, the profile of organic acids in *Citrus* fruits depends on the cultivar or variety, but not on its levels of total acidity. Citric acid is synthesised in the mitochondria of juice cells via the Krebs cycle and is stored in the vacuole throughout a mechanism involving a large influx of H^+, which is mediated by the H^+-ATPase of the tonoplast. This influx of protons reduces vacuolar pH and provides the driving force for additional citric acid uptake in acidic fruits [24, 37]. In this sense, the variation of organic acids between acidless and acidic fruits could be related to the absence of H^+-ATPase pump on the vacuolar membrane of acidless fruits [38].

Sucrose, glucose and fructose were found in all the *Citrus* fruits, although the major one was dependent on the fruit species and variety (Fig. 5). Thus, sucrose was the main sugar in mandarins, ranging between 9.51 ± 0.2 and 11.87 ± 0.89 mg 100 g^{-1} FW, in the ethnovarieties 'Autóctona' and 'Mandarina', respectively, followed by fructose (1.8–2.4 g 100 g^{-1} FW) and very low concentrations of glucose (less than 0.5 g 100 g^{-1} FW), according to previous reports in other mandarin cultivars [1, 17, 35]. However, in lemon fruits, the main sugar was different depending on the variety. Thus, in 'Sanguino' lemon, the major sugar was glucose, ca. 4 g 100 g^{-1} FW, while in 'Dulce' and 'Fino' lemons it was fructose with concentrations of 3.03 ± 0.23 and 2.69 ± 0.35 mg 100 g^{-1} FW, respectively. In 'Blanco' grapefruit and 'Cimboba', the major sugar was sucrose followed by fructose and glucose, while in `Dulce' lime the main sugar was glucose followed by sucrose and fructose.

Fructose is one of the most important dietary monosaccharides and it is known to be the sweetest of all naturally occurring carbohydrates [39], which together with the lack of acid content makes 'Dulce' lemon an excellent edible source to make delicious jams and conserves, especially for children, due to its sweetness and delicate pleasant flavour [14]. Albertini et al. [24] when comparing acidic with acidless varieties of lemon, lime and orange fruits, reported that fructose was the predominant sugar in acidless varieties, while in acidic ones it was glucose. However, this statement cannot be considered as general for all *Citrus* fruits, since our results, on one hand, showed that both 'Fino' and 'Dulce' lemon fruits, acidic and acidless varieties, respectively, had fructose as the major sugar and, on the other, 'Dulce' lime which is an acidless variety had glucose as the predominant sugar. Sucrose metabolism depends on the activities of β-fructosidase (invertase) leading to the formation of fructose and α-glusosidase, which accounts for glucose synthesis. Thus, the high ratio of glucose/fructose found in 'Sanguino' lemon and 'Dulce' lime could be due to the higher activity of α-glusosidase with respect to β-fructosidase. On the contrary, the high ratio of fructose/glucose in 'Dulce' and 'Fino' lemons ndicates a higher activity of β-fructosidase than α-glusosidase, showing that sucrose metabolism occurs in different ways in *Citrus* fruit species and varieties as recently proposed by Oustric et al. [36].

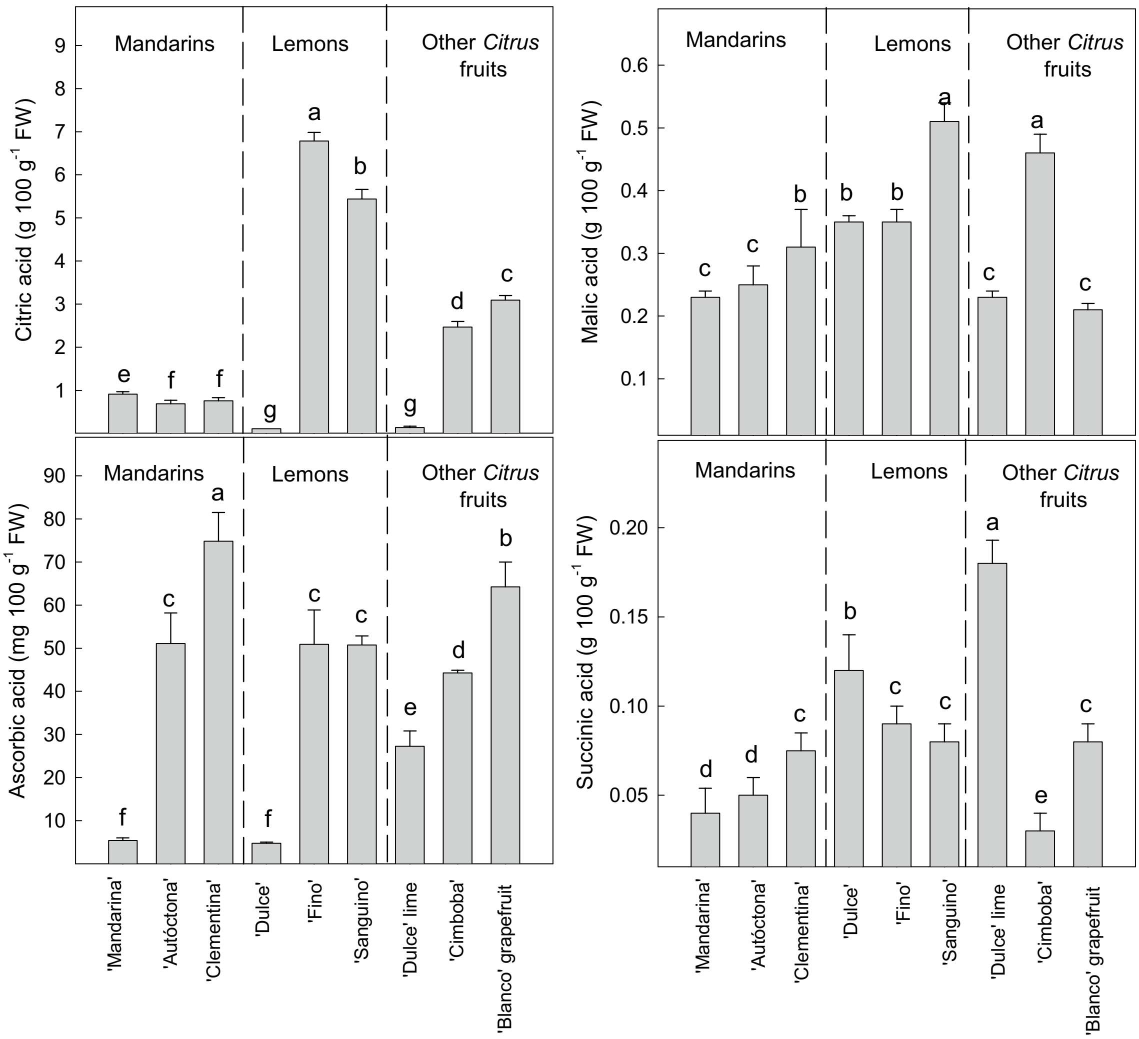

Fig. 4 Citric, malic, succinic and ascorbic acid concentrations in juice vesicles of the different *Citrus* fruits. Data are the mean ± SE of three replicates. Different letters in bars show significant differences among *Citrus* species and varieties

Antioxidant activity

The ABTS assay was used to measure the total antioxidant activity (TTA) which has been reported to be easy, accurate and rapid to apply to determine the antioxidant capacity in fruits and vegetables [40]. Significant differences in TAA in the juice of *Citrus* fruits were found among *Citrus* species and varieties, with 'Fino' and 'Sanguino' lemons and 'Autóctona' mandarin showing the greatest TAA levels, more than 95 mg 100 g^{-1}, and the 'Dulce' lemon the lowest, 1.01 ± 0.15 mg 100 g^{-1} (Fig. 6). However, in albedo and carpelar membrane, the TAA was very low as compared to TAA of fruit juice, and differences among species were not as strong as in juice TAA, according to a previous report in a wide range of orange cultivars [13]. Thus, TAA in albedo ranged between 2.32 ± 1.04 and 0.29 ± 0.09 mg 100 g^{-1} in 'Sanguino' lemon and 'Clementina' mandarin, respectively, and in carpelar membrane tissue between 0.02 ± 0.001 and 1.46 ± 0.01 mg 100 g^{-1} for 'Clementina' mandarin and 'Cimboba', respectively. No correlation was found between TAA in fruit juice and TAA in any of the other edible tissues (albedo and carpelar membrane) of these *Citrus* fruits. However, there was a high correlation between the TAA of the last two tissues ($y = 0.61x - 0.24$; $r^2 = 0.60$), according to previous report, in several orange traditional varieties [13]. On the contrary, considering the fresh weight of the edible tissues of each fruit and their TAA, 'Cimboba' was the fruit with the highest TAA (ca. 737.7 mg/fruit), followed by 'Blanco' grapefruit (137.5 mg/fruit). In previous reports, it has been reported that ascorbic acid accounts for 65–100% of the TAA [41]. However in the present experiment, a low correlation was found between ascorbic acid content and TAA ($y = 0.45x + 11.088$, $r^2 = 0.38$) and, in turn, other bioactive compounds such as carotenoids or phenolics might be

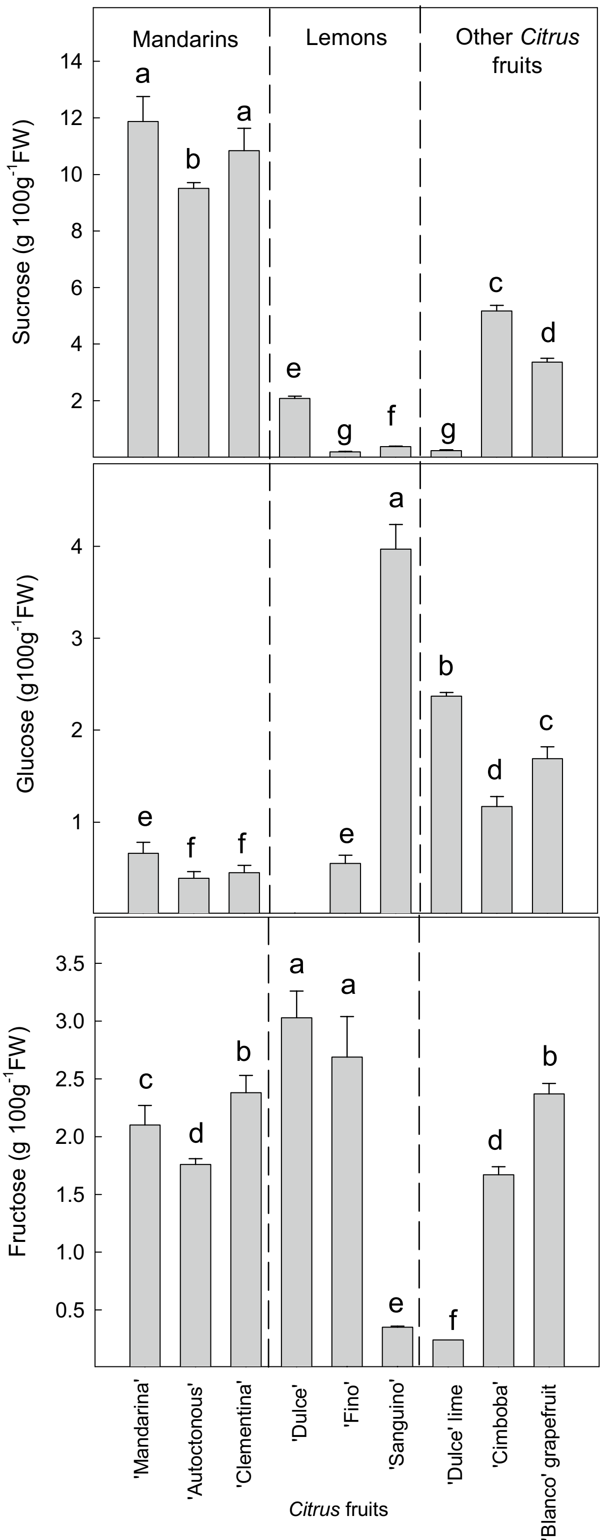

Fig. 5 Sucrose, glucose and fructose concentrations in juice vesicles of the different *Citrus* fruits. Data are the mean ± SE of three replicates. Different letters in bars show significant differences among *Citrus* species and varieties

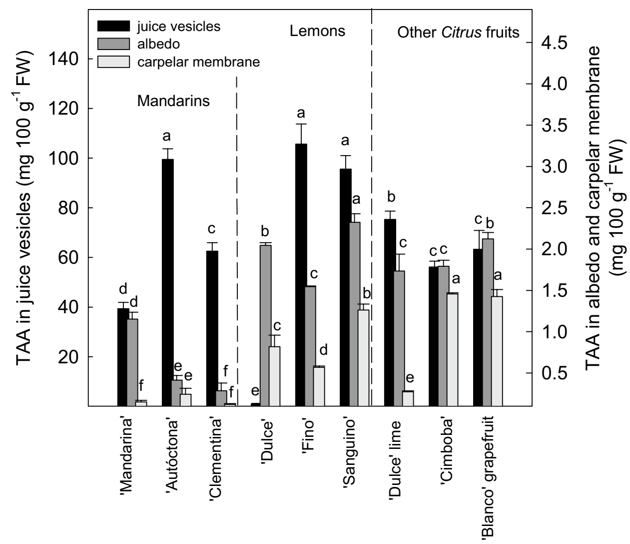

Fig. 6 Total antioxidant activity (TAA) in juice vesicles (left *y* axis) and albedo and carpelar membranes (right *y* axis) of the different *Citrus* fruits. Data are the mean ± SE of three replicates. Different letters in the bars of the TAA of juice vesicles and albedo and carpelar membrane show significant differences among the *Citrus* species and varieties

also responsible for their TAA as has been reported for other *Citrus* fruits [29]. In this sense, the phenolic concentration correlated with antioxidant capacity measured by ABTS and DPPH assays of juices from 15 *Citrus* varieties [42] as well as with the antioxidant capacity measured by the FRAP and ABTS assays of lemon peel [43].

Sensorial analysis showed significant differences among *Citrus* fruits in sweetness, aroma, firmness and lack of bitterness of segments (Table 2). The *Citrus* fruits with the highest scores for sweetness were 'Dulce' lime (4.9 ± 0.3) and 'Dulce' lemon (4.8 ± 0.4) followed by 'Clementina' mandarin (4.0 ± 0.4), while those with the lowest scores were 'Fino' (1.1 ± 0.3) and 'Sanguino' (1.2 ± 0.4) lemons. The fruits with the highest scores for aroma were 'Dulce' lime and 'Blanco' grapefruit (4.9 ± 0.3). The three mandarin varieties as well as 'Dulce' lime and 'Dulce' and 'Sanguino' lemons had a low bitterness degree, that is, high values of lack of bitterness (close to 5), while 'Blanco' grapefruit and 'Fino' lemon had the highest bitterness with scores of lack of bitterness of 2.3 ± 0.6 and 2.5 ± 0.5, respectively, followed by 'Cimboba' fruit (3.9 ± 0.7). With respect to segment firmness, higher scores were given to lemon varieties and 'Dulce' lime (close to 5). The lowest scores of firmness were for mandarin varieties (less than 4). In relation to the overall impression, judges gave high scores for all the quality parameters to 'Dulce' lime while 'Mandarin' mandarin was highly scored for aroma and 'Sanguino' lemon for colour and aroma. Thus, the *Citrus* fruits evaluated in the present study had a wide range of aroma, sweetness, bitterness and

Table 2 Scores from the sensory analysis for sweetness, aroma, firmness, lack of bitterness, overall impression and notable feature of underutilised Spanish *Citrus* fruits

Local names	Sweetness	Aroma	Firmness	Lack of Bitterness	Overall impression	Notable feature
'Mandarina'	3.4 ± 0.5^{b}	4.4 ± 0.5^{a}	3.5 ± 0.5^{b}	5.0 ± 0^{a}	4.5 ± 0.5^{a}	Aromatic
'Autóctona'	3.5 ± 0.7^{b}	1.9 ± 0.4^{c}	2.3 ± 0.5^{c}	5.0 ± 0^{a}	3.2 ± 0.9^{b}	None
'Clementina'	$4.0 \pm 0.4^{a,b}$	2.5 ± 0.5^{b}	3.5 ± 0.5^{b}	5.0 ± 0^{a}	3.8 ± 0.7^{b}	Sweet
'Dulce' lemon	4.8 ± 0.4^{a}	2.8 ± 0.7^{b}	4.8 ± 0.4^{a}	5.0 ± 0^{a}	3.3 ± 0.5^{b}	Original
'Fino' lemon	1.1 ± 0.3^{d}	4.1 ± 0.3^{a}	4.8 ± 0.4^{a}	2.5 ± 0.5^{c}	$4.1 \pm 0.3^{a, b}$	Aromatic
'Sanguino' lemon	1.2 ± 0.4^{d}	4.2 ± 0.6^{a}	5.0 ± 0^{a}	5.0 ± 0^{a}	4.8 ± 0.4	Colour
'Dulce' lime	4.9 ± 0.3^{a}	4.9 ± 0.3^{a}	4.9 ± 0.3^{a}	4.9 ± 0.3^{a}	4.9 ± 0.3	All of them
'Cimboba'	2.5 ± 0.5^{c}	3.1 ± 0.3^{b}	5.0 ± 0^{a}	3.9 ± 0.7^{b}	2.3 ± 1.1	Too big
'Blanco' grapefruit	2.9 ± 0.5^{b}	4.9 ± 0.3^{a}	4.1 ± 0.3^{b}	2.3 ± 0.6^{c}	4.5 ± 0.5	Aromatic

Data are the mean ± SE of ten replicates

Different letters show significant differences ($p < 0.05$) among Spanish *Citrus* fruits

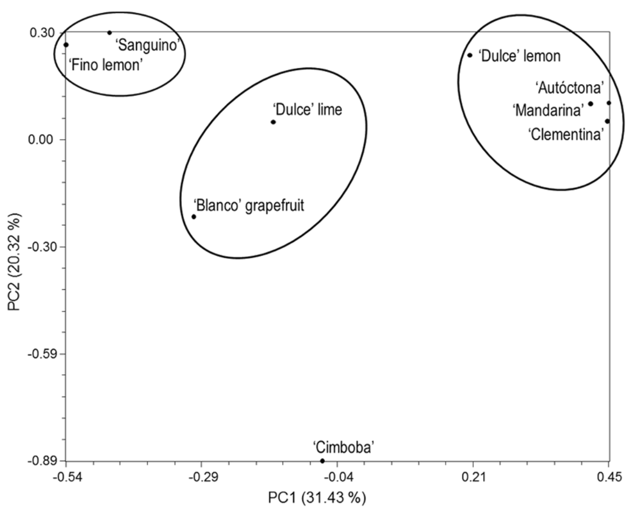

Fig. 7 Principal component analysis (PCA) using total antioxidant activity, organic acid and sugar concentrations in juice vesicles and sensory analysis

firmness values, showing their potential to be used in restaurants to make delicatessen food [8, 10–12] or in the agro food industry.

PCA was used to briefly evaluate the global relationship between the *Citrus* species and varieties studied (Fig. 7). PCA clearly grouped the three mandarin varieties ('Mandarina', 'Autóctona' and 'Clementina') as well as 'Fino' and 'Sanguino' lemons. However, 'Dulce' lemon was placed closer to mandarins than to the others lemon varieties.'Dulce' lime and 'Blanco' grapefruit formed another group, while the 'Cimboba' is the most different of all, and not grouped with any other. The first two main principal components (PC1 and PC2) explained the 31.43 and the 20.32% of the variability, respectively. The analysis of the correlations between data of the sensory analysis attributes, antioxidant activity and individual and organic acid concentration provided some interesting relationships (Table 3). Only significant correlations (both positive and negative) having a high level of significance have been commented. For instance, the panellist scores for overall impression were positively correlated with fruit aroma, while surprisingly sweetness was negatively correlated with citric acid concentration and sum of organic acids, but no correlations were found between sweetness and fructose, glucose or sucrose concentration or with the sum of sugars. Moreover, a negative correlation was found between glucose and fructose, while sucrose correlated with the sum of sugars. Finally, it is worth noting that fruit firmness negatively correlated with both sucrose and total sugar concentration, while no correlations were found between TAA and any of the remaining parameters.

Nowadays, citriculture around the world is based on a few cultivars selected by their fruit quality attributes, yield and tolerance against different environmental stresses, both biotic and abiotic. Thus, traditional varieties have disappeared or are in the verge of extinction. To solve this problem, the Food and Agriculture Organization (FAO) has a very active policy on the conservation of plant genetic resources, including traditional varieties in five continents [44]. In addition, many countries have national programmes for the conservation of plant genetic resources. The Spanish *Citrus* Germplasm Bank is part of this context and has become a fundamental objective of improvement programmes. This work provides useful information on different parameters, such as organic acids, sugars, antioxidant activity and sensorial properties of nine traditional *Citrus* species and varieties that could be useful for genetic improvement in future breeding programmes. Nevertheless, further work is required to establish if current results would be impacted by seasonal variation year to year.

Table 3 Correlations between sensory analysis attributes, total antioxidant activity (TAA) and individual sugar and organic acid concentrations

	Aroma	Firm.	Lack bitter.	Sweet.	Overall impres.	Ascorbic acid	Citric acid	Malic acid	Succinic acid	Sum of acids	Fructose	Glucose	Sucrose	Sum of sugars
TAA	−0.139	−0.230	−0.215	−0.607	−0.012	0.574	0.544	0.168	−0.241	0.539	−0.266	0.145	−0.014	−0.032
Aroma		0.499	−0.410	−0.213	0.761*	−0.164	0.346	−0.179	0.392	0.336	−0.349	0.565	−0.476	−0.427
Firmness			−0.248	−0.241	0.125	−0.200	0.414	0.562	0.440	0.434	−0.229	0.453	−0.807**	−0.787*
Lack of bitterness				0.506	−0.032	−0.386	−0.617	0.095	0.083	−0.602	−0.362	0.047	0.367	0.328
Sweetness					−0.073	−0.399	−0.945**	−0.561	0.444	−0.943**	0.074	-0.372	0.273	0.203
Overall impression						−0.031	0.211	−0.306	0.491	0.202	−0.406	0.564	−0.273	−0.222
Ascorbic acid							0.388	0.144	−0.238	0.389	−0.037	0.179	0.045	0.091
Citric acid								0.509	−0.166	0.999**	−0.037	0.390	−0.506	−0.439
Malic acid									−0.224	0.537	−0.221	0.391	−0.363	−0.327
Succinic acid										−0.156	−0.305	0.253	0.491	−0.684*
Sum of acids											−0.051	0.404	−0.521	−0.455
Fructose												−0.837**	0.288	0.291
Glucose													−0.508	−0.445
Sucrose														0.991**

Firm firmness, *Lack bitter* lack of bitterness, *Sweet* sweetness, *Overall impress* overall impression

*, **Significant correlation at $p < 0.01$ and $p < 0.05$, respectively

Conclusion

Results show significant differences among *Citrus* species and varieties on the analysed parameters. However, positive correlations were found among fruit weight and the weight of the different fruit tissues, showing that the relative proportion of each fruit tissue was similar for all the *Citrus* varieties studied. TAA in fruit juice was also different depending on the *Citrus* species and varieties, showing that 'Autóctona' mandarin and 'Fino' and 'Sanguino' lemons had the highest TAA. On the other hand, organic acid and sugar profiles and concentrations were dependent on fruit species and varieties, but not on their content of total acidity: that is to say, they do not depend on whether they are acidic or acidless fruits. The fruits most appreciated by consumers regarding the scores given by the sensory panel would be 'Mandarina' mandarin for its aroma, 'Sanguino' lemon for its original colour and 'Dulce' lime which had high scores for all the studied parameters. In addition, 'Autóctona' mandarin and 'Sanguino' lemon are also interesting due to the high TAA of their juices. Thus, this knowledge could be useful to reconsider the utilisation of certain traditional *Citrus* species and varieties in future breeding programmes, to increase agricultural biodiversity in the south-east of Spain. In addition, the consumption of *Citrus* fruits with high antioxidant activity would improve the health beneficial effect of these fruits.

Compliance with ethical standards

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

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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