

ORIGINAL ARTICLE

# Determination of organic acids and carbohydrates in 'Salustiana' orange fruit from different rootstocks

*Determinação de ácidos orgânicos e carboidratos em frutos de laranjas 'Salustiana' provenientes de diferentes porta-enxertos*

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## Abstract

This study aimed to determine carbohydrates and organic acids in fruit from 'Salustiana' orange tree [*Citrus sinensis* (L.) Osb.] grafted onto different rootstocks using the HPLC technique, as well as to evaluate their physicochemical properties. As rootstocks, we tested 'Rangpur' lime, 'Cleopatra' mandarin, 'Sunki' mandarin, 'Swingle' citrumelo, and 'C-13' citrange. Fully mature fruit was first characterized according to their physicochemical parameters as fruit mass, longitudinal and equatorial diameters, peel color, juice yield, soluble solids, titratable acidity, and ratio. Then, freshly squeezed juice was used to determine and to quantify organic acids and carbohydrates by the HPLC system. All analyses were performed in triplicate complete randomized with five treatments (rootstocks). Fruit from all evaluated rootstocks presented adequate physicochemical attributes, meeting the quality standards of the citrus industry. 'C-13' citrange induced in a production of large-sized fruit whereas 'Rangpur' lime promoted higher soluble solids content in its juice. Significant differences were not observed between the treatments with regards to organic acids and carbohydrates concentrations. Citric and ascorbic acids were identified and quantified in the juice samples. The sugars identified were sucrose, glucose, and fructose. Organic acids and carbohydrates concentrations are consistent with those reported in the literature for citrus juice, providing information about nutrition and quality of 'Salustiana' sweet orange produced onto different rootstocks.

**Keywords:** *Citrus* spp.; Fruit quality; Orange juice; HPLC; Organic acids; Sugars.

## Resumo

Este estudo objetivou determinar carboidratos e ácidos orgânicos presentes em frutos de laranja 'Salustiana' [*Citrus sinensis* (L.) Osb.], enxertada sobre diferentes porta-enxertos utilizando técnicas de HPLC, assim como suas propriedades físico-químicas. Como porta-enxerto, foram testados limão 'Cravo', tangerina 'Cleopatra', tangerina



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'Sunki', citrumelo 'Swingle' e citrange 'C-13'. Os frutos completamente maduros foram caracterizados de acordo com seus parâmetros físico-químicos, como massa de fruto, diâmetros longitudinal e equatorial, cor da casca, rendimento de suco, sólidos solúveis, acidez titulável e *ratio*. Em seguida, o suco fresco extraído foi utilizado para determinar e quantificar os ácidos orgânicos e carboidratos pelo sistema de HPLC. Todas as análises foram realizadas em triplicata inteiramente casualizada com cinco tratamentos (porta-enxertos). Os frutos de todos os porta-enxertos avaliados apresentaram parâmetros físico-químicos adequados, atendendo aos padrões de qualidade da indústria de citros. O citrange 'C-13' induziu a produção de frutos de tamanho maior enquanto o limão 'Cravo' promoveu maior teor de sólidos solúveis em seu suco. Não foram observadas diferenças significativas entre os tratamentos em relação às concentrações de ácidos orgânicos e carboidratos. Os ácidos cítrico e ascórbico foram identificados e quantificados nas amostras de suco. Como açúcares, foram identificadas sacarose, glicose e frutose. As concentrações de ácidos orgânicos e carboidratos são consistentes com àquelas reportadas na literatura para sucos cítricos, fornecendo informações sobre nutrição e qualidade de frutos da laranjeira 'Salustiana' sobre diferentes porta-enxertos.

**Palavras-chave:** *Citrus* spp.; Qualidade de fruto; Suco de laranja; HPLC; Ácidos orgânicos; Açúcares.

## 1 Introduction

Brazil leads the worldwide production of oranges, which ensured an annual production of 17.3 million tons in 2016, responsible for 25% of the total production (Food and Agriculture Organization Corporate Statistical Database, 2018). About 80% of its production goes to the industry, mainly to process frozen concentrated orange juice (FCOJ) for exportation and the remaining (20%) is destined to supply the Brazilian fresh market. For this reason, the production of orange cultivars intended for the fresh and processing purpose has increased, because of the advantage of dual decision making and flexibility to the citrus growers.

By this context, the sweet orange cv. Salustiana [*Citrus sinensis* (L.) Osb.] has excellent performance for both purposes, but mainly for in natura consumption because its fruit is seedless, very taste (Castle & Baldwin, 2011) and has suitable physicochemical characteristics according to the fruit quality standards (Organisation for Economic Co-operation and Development, 2010; Companhia de Entrepostos e Armazéns Gerais de São Paulo, 2011). Nevertheless, physicochemical quality may appear divergent in their values according to the rootstock used for citrus cultivation.

Some authors have reported this influence on fruit quality in several citrus cultivars (Cantuarias-Avilés et al., 2010; Gonzatto et al., 2011; Legua et al., 2011; Silva et al., 2013; Tazima et al., 2014; Bacar et al., 2017). Similarly, Castle (2010) confirms that rootstocks are highly related to many fruit attributes such as fruit size, and juice quality in which sugars and acids are affected, being a major factor for growers of fresh and processing fruit. In this way, it is important to assay new alternatives of rootstocks to diversify citrus groves and verify their influence on fruit quality.

As far as the physicochemical quality of citrus is important for commercialization and consumption, the description of their compounds profile is required, because the general composition and antioxidant properties of foodstuff are critical to determine consumer acceptance and preference. Carbohydrates and organic acids are among the major compounds present in citrus fruit, and their nature and concentration largely affect taste characteristic and organoleptic quality (Kelebek et al., 2009).

According to Lado et al. (2014), the total soluble solids content present in citrus fruit is composed of about 80% of carbohydrates, 10% organic acids, and 10% nitrogenous compounds. Thus, sweet oranges present a high amount of sugars and their sweetness is intrinsic to their composition (Kelebek et al., 2009). They are compounded mainly by sucrose, the major sugar component, glucose, and fructose, and their ratio is usually 2:1:1 respectively (Lee & Coates, 2000).

Organic acids are the second major group of natural compounds present in oranges. Their nature and concentration in fruit are important to the fresh market and industry, because they greatly influence the

organoleptic properties and stability of fruit juices, being an important index to determine fruit authenticity (Kelebek et al., 2009; Scherer et al., 2012). However, their profile and concentration in citrus fruit are related to sugar content and are dependent on citrus varieties, soil, climate, and stress conditions (Jones, 1998; Lado et al., 2014). In general, citric, ascorbic, and malic acids are the most abundant organic acids found in sweet orange. According to Scherer et al. (2012) citric and malic acids are commonly used as acidulants to enhance beverages and ascorbic acid as an antioxidant.

By this context, this study aimed to determine carbohydrates and organic acids in fruit from 'Salustiana' orange tree grafted onto different rootstocks using HPLC techniques, as well as, to analyze other physicochemical characteristics.

## 2 Materials and methods

### 2.1 Chemicals

We used ultra-pure water, Milli-Q quality (Simplicity 185, Millipore, USA) to prepare samples and solutions. For organic acids, we used ascorbic and citric acid standards (Sigma-Aldrich, Saint Louis, USA) with purity > 98%; while carbohydrate standards were: sucrose (Synth, São Paulo, Brazil), D-(+)-glucose and D-(-)-fructose (Merck, Darmstadt, Germany) with purity levels of 98.9%, 99.5%, and 99.9%, respectively. And all other chemicals were HPLC quality.

### 2.2 Sample collections

Fully mature oranges from the mid-season cv. Salustiana [*Citrus sinensis* (L.) Osb.] grafted onto five rootstocks were manually harvested in July 2017 from the experimental station of the Instituto Agronômico do Paraná (Londrina-PR, Brazil), when fruit exhibited suitable maturation index, superior to the minimum index (9.5) established by the domestic marketing (Companhia de Entrepósitos e Armazéns Gerais de São Paulo, 2011), indicating the harvest point. As rootstocks, we tested 'Rangpur' lime (*C. limonia* Osb.), 'Cleopatra' mandarin (*C. reshni* Hort. ex Tanaka), 'Sunki' mandarin (*C. sunki* Hort. ex Tanaka), 'Swingle' citrumelo [*C. paradisi* Macf. cv. Duncan × *Poncirus trifoliata* (L.) Raf.] and 'C-13' citrange [*C. sinensis* (L.) Osb. × *P. trifoliata* (L.) Raf.]. 'Salustiana' oranges (10 fruit per tree) were collected from different trees (12 year-old), packed in plastic boxes (industrial standard: maximum capacity of 40.8 kg), and transported immediately to a cold chamber to pre-cooling for 12 h at  $4 \pm 1$  °C and 90% RH in the Agricultural Science Department of the Universidade Estadual de Londrina (Londrina-PR, Brazil). After pre-cooling, the juice samples were extracted using an industrial extractor (ES4EA-B60000 Croydon, Duque de Caxias, Brazil) to perform the chemical and chromatographic analyses.

### 2.3 Physicochemical analysis

The physical characteristics of fruit were evaluated, determining the fruit mass (g) with a semi-analytical scale (M505 Bel, Piracicaba, Brazil). A digital caliper (ABS Mitutoyo, Kawasaki, Japan) measured the longitudinal diameter (mm) and equatorial diameter (mm). Peel color was obtained by the Hue angle ( $h^\circ$ ) means for each sample, using mean values for red-green ( $a^*$ ) and yellow-blue ( $b^*$ ) measured with a digital colorimeter (CR-10 Konica Minolta, Tokyo, Japan), considering three measure points in the equatorial surface of each fruit. Hue angle was determined according to McGuire (1992). Then, the juice yield was obtained by the ratio between juice mass and fruit mass (Tazima et al., 2014).

Fruit samples were also characterized according to their chemical profile. A digital refractometer with automatic temperature compensation (DR301-95 Krüss Optronic, Hamburg, Germany) was used to determine soluble solids (SS) contents, the result was expressed as °Brix. Titratable acidity (TA) was assessed by titration with a standard 0.1 N NaOH solution in a semi-automatic titrator, the results were expressed in

percentage of citric acid (Gaithersburg & Horwitz, 1990). The ratio was determined by the relation between soluble solids (SS) and titratable acidity (TA).

## 2.4 Chromatographic analysis

For HPLC analysis, 5 ml of freshly squeezed juice mixed with 15 ml of ultra-pure water (Milli-Q) were centrifuged at 50000 rpm in a centrifuge (CP100WX Hitachi, Tokyo, Japan) for 10 min, the supernatant was then filtered through 0.22 µm pore size membrane filters (Sartorius Stedim Biotech, Goettingen, Germany).

The determination of organic acids and carbohydrates were performed by HPLC instrumental system (LC-20 A Shimadzu, Kyoto, Japan) equipped with a high-pressure pump system (LC-20AT), the automatic injector (SIL-20AC HT), refractive index detector (RID-10A), photodiode array (SPD-M20A), column oven (CTO-20A) and control module (CBM-20A). Data collection and integration of chromatographic peaks were computed processed by the LC Solutions software (Shimadzu, Kyoto, Japan).

### 2.4.1 Organic acids

The centrifuged and filtered samples were vialled and injected into the HPLC system. The injection volume used was 20 µL. Organic acids were analyzed onto a Phenomenex C18 column (250 × 4.6 mm, particle size 5 µm) (Phenomenex, Torrance, USA). The mobile phase consisted of 25 mM sodium phosphate buffer solution, and pH adjusted to 2.4 with phosphoric acid at a flow of 1.0 mL min<sup>-1</sup>. The column was kept at 30 °C with refractive index detection (RID -10A).

### 2.4.2 Carbohydrates

Samples of 2 mL of orange juice were used to determine carbohydrates, previously centrifuged, and filtered. Solid-phase extraction was performed passing samples through a Sep-Pak C18 500 mg cartridge after activated with 2 mL of methanol and 4 mL of ultra-pure water (Milli-Q). The eluate was collected and filtered through a 0.22 µm membrane filter (Sartorius Stedim Biotech, Goettingen, Germany). The HPLC system received 20 µm of the sample. Sugars were analyzed using a Shim-pack CLC-NH2 (M) column (250 × 4.6 mm, particle size 5 µm) (Shimadzu, Kyoto, Japan) kept at 30 °C (CTO-10AS VP). The used mobile phase was a mixture of acetonitrile: water 75:25 (v/v) at a flow of 1.0 mL min<sup>-1</sup>, according to Pauli et al. (2011).

## 2.5 Statistical analysis

All analyses were performed in triplicate in a complete randomized design with five treatments (fruit from different rootstocks). Data were processed by the R 3.4.1 version (The R Foundation for Statistical Computing, Vienna) and assayed according to the experiment design, using analysis of variance followed by Tukey's test ( $p \leq 0.05$ ).

## 3 Results and Discussion

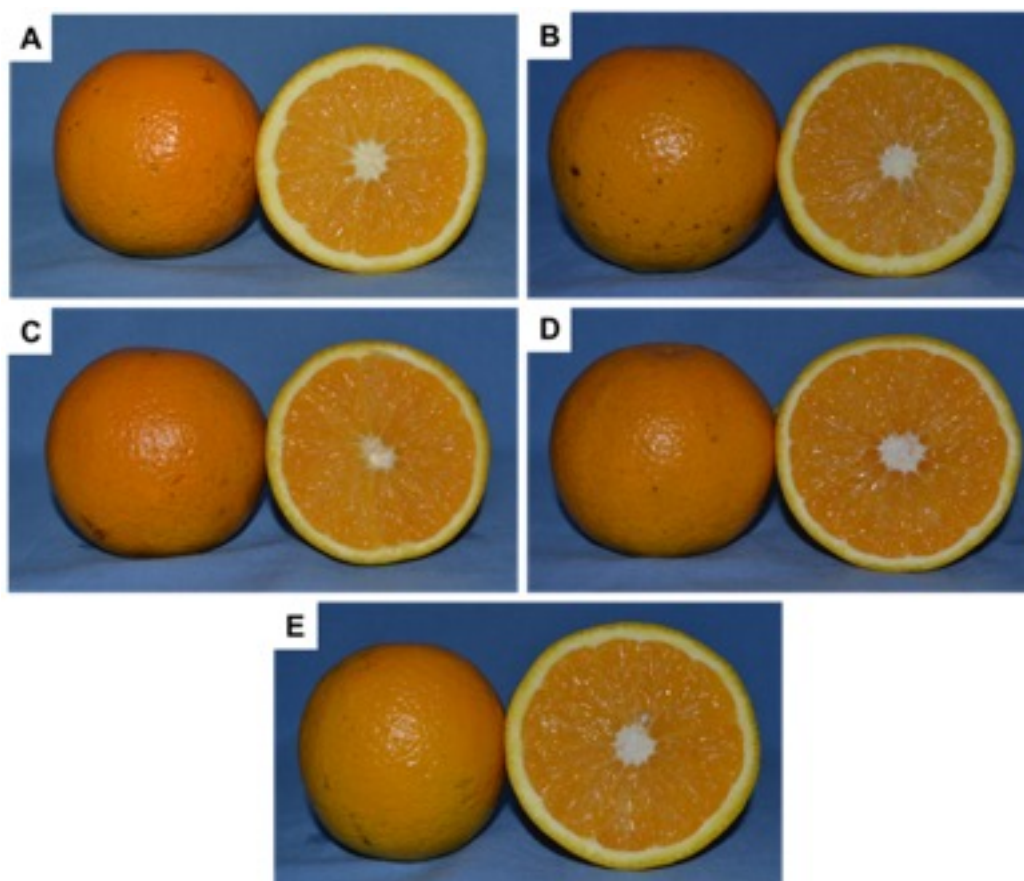
### 3.1 Physicochemical analysis

Fruit from 'Salustiana' sweet orange was first characterized according to its physical characteristics (Table 1 and Figure 1). The results showed that 'C-13' citrange rootstock induced production of large-sized fruit, presenting fruit mass of 222 g, longitudinal and equatorial diameters of 72 and 77 mm, respectively. These values were significantly greater than those noticed in oranges from 'Cleopatra' mandarin, which resulted in fruit of small caliper with 163 g per fruit and longitudinal and equatorial diameter of 65 and 68 mm, respectively.

**Table 1.** Fruit physical characteristics from ‘Salustiana’ sweet oranges tree grafted onto five rootstocks.

Rootstocks	Fruit physical characteristics Mean ± SD			
	Fruit mass (g) <sup>a</sup>	LD (mm) <sup>a</sup>	ED (mm) <sup>a</sup>	Color ( <i>h</i> <sup>o</sup> ) <sup>ns</sup>
‘Rangpur’ lime	186 ± 9.0 bc	69 ± 1.2 a	72 ± 1.5 bc	82.8 ± 1.4
‘Cleopatra’ mandarin	163 ± 6.5 c	65 ± 1.7 b	68 ± 0.8 c	84.9 ± 0.4
‘Sunki’ mandarin	200 ± 14.7 ab	70 ± 1.7 a	73 ± 2.7 ab	81.6 ± 1.6
‘Swingle’ citrumelo	196 ± 13.8 ab	69 ± 1.9 a	73 ± 1.9 ab	83.4 ± 2.4
‘C-13’ citrange	222 ± 7.8 a	72 ± 0.5 a	77 ± 1.4 a	82.7 ± 2.0
Mean	193	69	73	83.1
CV (%)	5.6	2.2	2.5	2.1

LD – Longitudinal diameter; ED – Equatorial diameter. <sup>a</sup>Means followed by the same letter in the columns do not differ statistically according to Tukey’s test ( $p \leq 0.05$ ). <sup>ns</sup>Non-significant at 0.05 level.



**Figure 1.** Fruit from ‘Salustiana’ oranges tree [*Citrus sinensis* (L.) Osb.] grafted onto different rootstocks: (A) ‘Rangpur’ lime (*C. limonia* Osb.); (B) ‘Cleopatra’ mandarin (*C. reshni* Hort. ex Tanaka); (C) ‘Sunki’ mandarin (*C. sunki* Hort. ex Tanaka); (D) ‘Swingle’ citrumelo [*C. paradisi* Macf. cv. Duncan × *Poncirus trifoliata* (L.) Raf.]; and (E) ‘C-13’ citrange [*C. sinensis* (L.) Osb. × *P. trifoliata* (L.) Raf.].

These values are greater than those observed by Siqueira et al. (2007) and Bini et al. (2009) evaluating the same cultivar in Valencia (Spain) and Uruguaiana (Brazil). Siqueira et al. (2007) recorded 46 g per fruit with 46 mm of diameter at the end of their experiment; while Bini et al. (2009) noticed fruit weight varying from

128 to 139 g with diameters between 64 and 68 mm, depending on the crop season. On the other hand, our findings were related to those found by Santos et al. (2010), characterizing eight seedless varieties of citrus produced in Viçosa (Brazil), in which ‘Salustiana’ orange fruit ranked 160 g with diameters between 68 and 70 mm. This was similar to other authors who assessed the influence of rootstocks under different citrus cultivars as ‘Valencia’ orange (Auler et al., 2008), ‘Okitsu’ Satsuma mandarin (Tazima et al., 2013), and ‘Jaffa’ orange (Bacar et al., 2017). As observed in the present study, larger and heavier fruit may present greater demand in the market since Brazilian consumers have a preference for large size fruit (Pio et al., 2001).

Concerning peel color, the tested rootstocks did not influence this aspect. Flavedo color was based on the *Hue* angle that demonstrates its changes, values closest to 90  $h^\circ$  indicates a high yellowish content in the peel, as observed for this cultivar which ranged from 81.6 to 84.9  $h^\circ$  according to the rootstock. This color change occurs due to the chlorophyll degradation and synthesis of carotenoids, responsible for the yellow or orange color of the flavedo (Ladaniya, 2008; Santos et al., 2010). In general, all treatments presented fruit with adequate peel color based on the international and domestic standards of table citrus (Organisation for Economic Co-operation and Development, 2010; Companhia de Entrepostos e Armazéns Gerais de São Paulo, 2011).

Table 2 shows the juice yield and chemical composition of ‘Salustiana’ orange juice. We observed a significant difference between juice yield and soluble solids contents parameters. ‘Swingle’ citrumelo and ‘Sunki’ mandarin rootstocks induced higher juice content for this cultivar, as reported by Santos et al. (2010) in fruit from ‘Salustiana’ orange and ‘Swingle’ citrumelo combination. In addition, the evaluated treatments were superior in juice yield than those observed by Bini et al. (2009), 39%; and also, higher than the minimum juice content required by the Organisation for Economic Co-operation and Development (2010) and the Companhia de Entrepostos e Armazéns Gerais de São Paulo (2011), 35 and 45% respectively.

**Table 2.** Juice yield and fruit chemical composition from ‘Salustiana’ sweet orange tree grafted onto five rootstocks.

Rootstocks	Fruit physical characteristics			
	Mean $\pm$ SD			
	Juice Yield (%) <sup>a</sup>	SS ( $^\circ$ Brix) <sup>a</sup>	TA (% citric acid) <sup>ns</sup>	Ratio (SS/TA) <sup>ns</sup>
‘Rangpur’ lime	47.2 $\pm$ 1.1 b	11.1 $\pm$ 0.3 a	0.75 $\pm$ 0.02	14.7 $\pm$ 0.3
‘Cleopatra’ mandarin	47.6 $\pm$ 1.1 b	9.3 $\pm$ 0.1 c	0.71 $\pm$ 0.04	13.1 $\pm$ 0.7
‘Sunki’ mandarin	48.9 $\pm$ 2.1 ab	9.9 $\pm$ 0.2 b	0.68 $\pm$ 0.04	14.6 $\pm$ 1.0
‘Swingle’ citrumelo	50.5 $\pm$ 1.6 a	10.3 $\pm$ 0.3 b	0.77 $\pm$ 0.09	13.3 $\pm$ 1.4
‘C-13’ citrange	46.9 $\pm$ 1.2 b	9.1 $\pm$ 0.2 c	0.70 $\pm$ 0.03	13.1 $\pm$ 0.8
Mean	48.2	9.9	0.72	13.8
CV (%)	1.7	2.2	7.2	6.6

SS – Soluble solids; TA – Titratable acidity. <sup>a</sup>Means followed by the same letter in the columns do not differ statistically according to Tukey’s test ( $p \leq 0.05$ ). <sup>ns</sup>Non-significant at 0.05 level.

Regarding soluble solids content, ‘Rangpur’ lime reached the highest value (11  $^\circ$ Brix) for ‘Salustiana’ orange fruit, differing from the other treatments. This characteristic agrees to Stenzel et al. (2006), who reported an early maturation in fruit from ‘Folha Murcha’ sweet orange tree onto ‘Rangpur’ lime, comparing to other rootstocks. The range between our treatments was 9 to 11  $^\circ$ Brix, indicating a suitable quality for harvest since fruit should present at least 9  $^\circ$ Brix as soluble solids content in its juice (Pereira et al., 2006). As soluble solids content, titratable acidity can also be used to predict the maturation degree of fruit. These

indices were similar among the treatments ranging from 0.68 to 0.77 and considered adequate according to Pereira et al. (2006). Because fully mature fruit of sweet oranges must present titratable acidity, expressed as citric acid, between 0.5 and 1.0%. The ratio (soluble solids:acidity), which indicates the fruit maturation index, was similar for all tested treatments (rootstocks) varying from 13.1 to 14.7. These indices are close to those reported by Bini et al. (2009) and Castle & Baldwin (2011) for the same cultivar, 11.5 and 12.8 respectively.

Environmental conditions and crop managements are among the major factors that influence fruit quality during its growth. For example, high temperatures contribute to the chlorophyll degradation and accumulation of soluble solids in the pulp (Ladaniya, 2008; Santos et al., 2010), while water availability regulates juice content and manages fruit production. Despite some differences, the evaluated rootstocks induced adequate physicochemical parameters for 'Salustiana' sweet oranges, meeting the minimum requirement of the international and domestic fresh market (Organisation for Economic Co-operation and Development, 2010; Companhia de Entrepósitos e Armazéns Gerais de São Paulo, 2011).

### 3.2 Organic acids composition in orange juice

Table 3 describes the determination and quantification of organic acids. We did not observe significant differences among the treatments. Organic acids showed a total average of 737 mg 100 mL<sup>-1</sup> of the juice of 'Salustiana' sweet orange, in which citric acid was the major organic acid identified in the juice ranging from 602.4 to 735.1 mg 100 mL<sup>-1</sup> of orange juice, depending on the rootstock. These findings agree to Kelebek et al. (2009), Nour et al. (2010), Kafkas et al. (2011), Kelebek & Selli (2011) and Scherer et al. (2012), who quantified citric acid as the major organic acid in sweet orange juices [*C. sinensis* (L.) Osb.].

**Table 3.** Organic acids composition in fruit from 'Salustiana' sweet orange tree grafted onto five rootstocks.

Rootstocks	Organic acids (mg 100 mL <sup>-1</sup> of orange juice)		
	Mean ± SD		
	Citric acid <sup>ns</sup>	Ascorbic acid <sup>ns</sup>	Total <sup>ns,a</sup>
'Rangpur' lime	735.1 ± 52.5	58.4 ± 5.4	799.0 ± 57.4
'Cleopatra' mandarin	602.4 ± 23.1	56.3 ± 6.3	663.9 ± 27.8
'Sunki' mandarin	659.4 ± 63.6	60.9 ± 1.8	725.9 ± 65.6
'Swingle' citrumelo	665.4 ± 89.9	56.2 ± 5.0	726.7 ± 89.2
'C-13' citrange	733.6 ± 39.4	58.4 ± 8.3	797.4 ± 47.1
Mean	679.2	58.0	737.2
CV (%)	8.6	9.9	8.2

<sup>ns</sup>Non-significant at 0.05 level; <sup>a</sup>Total of organic acids expressed as citric acid equivalent.

Kelebek et al. (2009) found 1266 mg of citric acid in 100 mL of orange juice produced in Turkey, close to those reported by Nour et al. (2010) notifying a concentration of 1392 mg 100 mL<sup>-1</sup> in Romania. Contrasting these values, Scherer et al. (2012) reported concentration of citric acid between 517 and 529 mg 100 mL<sup>-1</sup> of orange juice produced in Brazil, close to the values obtained in the present study (602 to 735 mg 100 mL<sup>-1</sup>) indicating that the concentration of organic acid, like other components, is dependent of the local environment (Lado et al., 2014).

Now, ascorbic acid levels ranged from 56.2 to 60.9 mg 100 mL<sup>-1</sup> of orange juice, similar to the values quantified by Kelebek et al. (2009) and Nour et al. (2010) for sweet orange, 49.0 and 63.6 mg 100 mL<sup>-1</sup> respectively. But higher than those noticed by Meléndez-Martínez et al. (2007) who described an average of

ascorbic acid equal to 39.1 mg 100 mL<sup>-1</sup> in ‘Valencia’ orange juice produced in Spain; and Legua et al. (2014) describing concentrations of 27 mg 100 g<sup>-1</sup> of fresh weight of fruit from ‘Clemenules’ mandarin tree grafted onto ‘Cleopatra’ mandarin and ‘Swingle’ citrumelo cultivated in Portugal.

However, the level of ascorbic acid appears to be variable in citrus fruit due to the variability between cultivars and species. Fruit of ‘Mexerica do Rio’ produced in Viçosa (Brazil) presented high amount of ascorbic acid in its juice, about 115 mg 100 g<sup>-1</sup> of juice (Silva et al., 2011); superior to the level mentioned above and those reported by Machado et al. (2017) for fruit from ‘Tahiti’ acid lime trees grafted on 12 rootstocks in Jaíba (Brazil), which ranged from 27 to 33 mg 100 g<sup>-1</sup> of juice, being among the evaluated rootstock the ‘Rangpur’ lime, ‘Swingle’ citrumelo, ‘Cleopatra’ mandarin, and ‘Sunki’ mandarin. It indicates that the quantification of ascorbic acid is dependent on the cultivar associated with the managements and local environment. Thus, it is essential knowing the concentration of this organic acid in orange, because it is an important antioxidant and its concentration may indicate the quality of citrus juice (Kelebek et al., 2009; Legua et al., 2014), being the main biologically active form of vitamin C and can eliminate several free radicals (Davey et al., 2000; Scherer et al. 2012).

### 3.3 Sugar composition in orange juice

Despite the differences verified in the total soluble solids content of the evaluated treatments (Table 2), we did not notice significant differences among them for the concentration of carbohydrates (Table 4). There were three major sugar components in ‘Salustiana’ orange juice samples: sucrose, glucose, and fructose, just as described in previous studies regarding different citrus varieties (Cancalon, 1994; Kelebek et al., 2009; Kafkas et al., 2011; Kelebek & Selli, 2011).

**Table 4.** Sugar composition in fruit from ‘Salustiana’ sweet orange tree grafted onto five rootstocks.

Rootstocks	Carbohydrates (mg.100 mL <sup>-1</sup> of orange juice)				
	Mean ± SD				
	Sucrose <sup>ns</sup>	Glucose <sup>ns</sup>	Fructose <sup>ns</sup>	Ratio <sup>ns,a</sup>	Total <sup>ns,b</sup>
‘Rangpur’ lime	1654.8 ± 151	819.3 ± 48	949.7 ± 42	0.86 ± 0.01	5016.0 ± 322
‘Cleopatra’ mandarin	1568.7 ± 95	746.0 ± 45	882.3 ± 51	0.85 ± 0.01	4662.7 ± 277
‘Sunki’ mandarin	1528.8 ± 102	784.8 ± 26	913.2 ± 24	0.86 ± 0.01	4755.2 ± 190
‘Swingle’ citrumelo	1686.0 ± 35	775.9 ± 56	893.5 ± 65	0.87 ± 0.01	4858.1 ± 256
‘C-13’ citrange	1704.4 ± 76	820.4 ± 50	943.5 ± 63	0.87 ± 0.05	5056.0 ± 130
Mean	1628.5	789.3	916.4	0.86	4869.6
CV (%)	6.1	5.9	5.6	3.0	5.0

<sup>ns</sup>Non-significant at 0.05 level. <sup>a</sup>Glucose/fructose ratio. <sup>b</sup>Total of carbohydrates expressed as sucrose equivalent.

According to Silva et al. (1999) and Lee & Coates (2000), these carbohydrates represent more than 80% of the total soluble solids content presented in orange juice. The ratio between these compounds is generally 2:1:1, sucrose: glucose: fructose respectively. Similar ratio was observed in our study for ‘Salustiana’ orange juice. It is considered typical for commercial orange juices according to Zhang & Ritenour (2016) and is consistent to those described in other reports for orange cultivars (Lee & Coates, 2000; Kelebek et al., 2009; Kelebek & Selli, 2011; Kafkas et al., 2011).

The average of the total amount of carbohydrates was 4870 mg 100 mL<sup>-1</sup> of orange juice. The major identified carbohydrate was sucrose ranging from 1528.8 to 1704.4 mg 100 mL<sup>-1</sup> of orange juice, just as observed by Kelebek et al. (2009) for ‘Kozan’ sweet orange. In general, sucrose was responsible for 48.8%



of the total sugar content followed by fructose (27.5%) and glucose (23.7%). The total sugar content is consistent with those reported by Lee & Coates (2000) for 'Valencia' sweet orange (50.5% sucrose, 23.7% glucose, and 25.8% fructose), and Kelebek & Selli (2011) for 'Dortyol' sweet orange (42.0% sucrose, 28.1% glucose, and 29.9% fructose). However, Legua et al. (2014) determined different sucrose:glucose:fructose ratio in fruit from 'Clemenules' mandarin trees grafted onto 'Cleopatra' mandarin (8:1:1) and 'Swingle' citrumelo (7:1:1), contrasting to the usual ratio described by Cancalon (1994) for fresh-squeezed juices (2:1:1).

The glucose:fructose ratio for the samples was also performed because it allows us to determine the quality and authenticity of orange juices (Kelebek & Selli, 2011). According to these authors, this ratio should present indices between 0.85 and 1.00 since orange fruit contains similar quantities of fructose and glucose, or even a slight increase in fructose, just as observed in our study for 'Salustiana' orange juice and for juice produced from different cultivars in the United States (Zhang & Ritenour, 2016). Thus, orange juice must present this pattern (0.85 – 1.00) as a sugar constituent for the citrus industry, if not, an adulteration should be considered (Lee & Coates, 2000).

The glucose:fructose ratio ranged from 0.85 to 0.87 in 'Salustiana' orange samples. These values were between those described by Zhang & Ritenour (2016) for 286 samples of orange juice produced in four States of the United States (0.8–0.9). They are also close to those observed by Kelebek & Selli, (2011) for 'Dortyol' oranges in Turkey (0.94), and lower than the maximum values required by the European Union legislation (1.03). It attends the orange juice requirement with regards to quality, authenticity, and identity because most of all produced juice in Brazil, as frozen concentrated orange juice (FCOJ), is exported to these countries (Lado et al., 2014; Kelebek & Selli, 2011).

In summary, despite the similarity among the evaluated treatments, the concentration, and composition of organic acids and carbohydrates may differ according to the citrus variety, soil-climate conditions, season, stress condition, fruit maturity, juice processing, crop and postharvest management as described in the literature (Cancalon, 1994; Jones, 1998; Rekha et al., 2012; Lado et al., 2014). However, the tested rootstocks do not appear to influence the organic acids and carbohydrates contents in 'Salustiana' sweet oranges.

## 4 Conclusions

'Salustiana' oranges produced on all evaluated rootstocks presents adequate physicochemical parameters, attending the quality standards for the citrus industry. 'C-13' citrange induces production of larger and heavier fruit. Significant differences were not observed between the treatments regarding organic acid and carbohydrate concentrations. As organic acids, citric and ascorbic acids were identified in the samples. The sugar composition consisted of sucrose, glucose, and fructose (1:1:2). The results regarding sugars and organic acids concentration characterize the evaluated cultivar, providing information about nutrition and the quality of fruit produced onto different rootstocks.

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