

## Characterization and Stability of ‘de Russas’ Orange Juice From Organic Cultivation

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### Abstract

The objective of this study was to elaborate whole juice from the varieties of BRS ‘Russas 01’ and BRS ‘Russas 02’ from the orange tree ‘de Russas’, with organic certification and to analyse its quality characteristics. Twenty fruits of each variety were used to prepare the juice, with physicochemical analysis of pH, soluble solids, titratable acidity and the soluble solids/titratable acidity ratio (Ratio). The content of citric acid and vitamin C by high performance liquid chromatography using a UV detector. The stability of vitamin C during three days of storage at 4 °C. The volatile organic compounds by gas chromatography coupled with mass spectrometry. Data were evaluated by analysis of variance and Student’s t-test at 5% significance level. The pH presented an average higher than 4.00, an average content of soluble solids above 10 °Brix and Ratio 7, in compliance with the legislation. The vitamin C was maintained at the end of storage at 79.33% for variety ‘01’ and 86.40% for variety ‘02’. Thirty compounds were identified in the volatile fraction for variety ‘01’ and 61 compounds for variety ‘02’. Thus, it is possible to conclude that the variety BRS ‘Russas 02’ has greater potential to produce whole juice.

**Keywords:** *Citrus × sinensis* (L.) Osbeck (Rutaceae), family farming, gas chromatography, high performance liquid chromatography, organic acids, volatile organic compounds.

### 1. Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), Brazil leads the orange production world ranking (*Citrus × sinensis* (L.) Osbeck (Rutaceae), as specified by the Global Biodiversity Information Facility—GBIF), and oranges occupy the 6th position of the country’s top ten commodities, with a total production of 16,707,897 tons in 2020 (FAO, 2021). Oranges give Brazil not only the title of the world’s largest producer of the fruit, but also the one of the largest producer and exporter of frozen concentrated orange juice 65 °Brix (FCOJ) (Kist et al., 2021).

The Northeast Region is the second largest orange producer in Brazil, contributing, so far, with 1,179,973 tons in the 2022 harvest, preceded by the Southeast Region, which detains more than 80% of the production (Instituto Brasileiro de Geografia e Estatística [IBGE] [*Brazilian Institute of Geography and Statistics* in English], 2022).

Several factors negatively influence the expansion of northeastern citriculture, such as the hot and dry climate; the difficulty in acquiring cultivars that adapt to biotic and abiotic stresses; the seedlings with genetic and phytosanitary quality; the lack of fertile soils, the lack of appropriate water for irrigation, the lack of technical assistance and rural extension; and the farmers lack of access to rural credit (Sombra et al., 2018a).

Despite these obstacles, the state of Ceará, the fourth largest producer of oranges in the Northeast Region (IBGE, 2021), has potential for regeneration and expansion of the production chain, in the face of the absence or of the control of the main diseases and pests of economic importance and diseases that affect citriculture worldwide (Sombra et al., 2019).

In the state of Ceará (Brazil), the Vale do Jaguaribe region has a variety of orange adapted to the semi-arid region, called 'de Russas' orange (*C. × sinensis*) which has been cultivated in the form of orange trees in the Vale do Jaguaribe region since the mid-1930s, making the municipality of Russas be recognized as the "orange land" (Passos et al., 2020).

This variety, which is considered sweeter than the 'Pera' orange (*C. × sinensis*) has been studied by the Empresa Brasileira de Pesquisa Agropecuária (Embrapa [*Brazilian Agricultural Research Corporation* in English]) Mandioca and Fruticultura in Cruz das Almas (Bahia/Brazil), and it has been gaining space on the consumer's table; moreover, it holds the potential to meet the market demand for table fruit in the state (Sombra et al., 2016).

Although there are already 8 varieties (BRS 'Russas 01', '02', '03', '04', '05', '06', '07', '08') identified, selected and registered in the Ministério da Agricultura, Pecuária e Abastecimento (MAPA [*Ministry of Agriculture, Livestock and Supply* in English]), the 'de Russas' orange is commercialized only in its *in natura* form, without using processing that adds value to the product, as well as there are no studies which detail its food characteristics (Passos et al., 2013).

The 'de Russas' orange currently represents an expressive example of family farming in the rural area of the state of Ceará (Passos et al., 2020), which is distinguished from others by the high differentiation of cultivars, copa and rootstock used; the fact that there are simultaneous activities to cultivate citrus on the same property; and the low incidence of key pests and diseases (Sombra et al., 2016).

Management in citrus farms located in the rural areas of the municipalities of Limoeiro do Norte, Quixeré and Russas is classified as conventional, organic or agroecological, with some properties showing greater predisposition for organic production, requiring only technical guidance, since 50% of the orchards do not use pesticides or fertilizers (Sombra et al., 2018a).

The production of organic food has shown rapid growth in recent years in the world and in Brazil. This increase is associated, in principle, with the rejection of the conventional model, in which phytosanitary products are used, which directly affect the consumer's health; the constant search for new ways to consume these foods; and the advance of scientific research with innovative tools and resources aiming at being used in organic productivity (Candioto, 2018; Watanabe et al., 2020).

According to the statistical yearbook *The World of Organic Agriculture*, in 2019, 3.1 million organic producers were registered worldwide, and the global organic food market reached 106 billion euros (Research Institute of Organic Agriculture [FiBL]; International Federation of Organic Agriculture Movements [IFOAM], 2021).

Data from MAPA's National Register of Organic Producers (CNPO) show that Brazil has, in 2022, more than 25,000 registered organic producers. The state of Ceará, specifically, has 1,122 registered organic producers, representing an increase of over 72% compared to 2019 (Brasil, 2022).

It can be seen that organic cultivation is one more way to preserve family farming in the semi-arid region of Ceará, reducing hunger and social inequality, thus contributing to the expansion of citriculture in the state and valuing the traditional variety of sweet oranges, the 'de Russas' orange (A. T. de Silva & S. T. de Silva, 2016; Sombra et al., 2019).

Furthermore, in order to meet the demands of the consumer market of citrus and its derivatives, there is a need for studies to verify the differences (stability, composition) among the 'de Russas' orange varieties when submitted to the elaboration of products, as well as the dissemination of information to the academic community and to the industry, considering that only its physicochemical characteristics were evaluated (Sombra et al., 2018b).

As an example of these characteristics, aroma and flavor are very important quality parameters. They appeal to consumers, especially in the case of juice, because they are closely related to the aroma of the fruit *in natura*, as it is the case of orange juice (Fan et al., 2009).

The constitution of the fruit aroma is derived from the release of volatile compounds of low molecular weight, which provides odors that are captured by the human olfactory sensory system. The aroma, associated with color, texture and size parameters of a fruit, contributes to the consumer's propensity to purchase it, and it also allows differentiating varieties of the same species and characterizing their quality (Mariano et al., 2022).

For these reasons, aroma has become an essential characteristic for the food industry. Given its properties of interest, new alternatives have been studied to retain the volatile compounds while maintaining the aromatic stability of the food (Belmeskine & Kaced, 2018; Vukoja et al., 2020).

The frequent consumption of fruits and vegetables helps to reduce the risk of cancer, cardiovascular diseases, hypertension, and stroke. Citrus fruit juice is an important source of bioactive compounds that provide health benefits due to the high content of vitamins, carotenoids, and phenolic compounds (Igal et al., 2021).

Orange has a high content of vitamin C, folic acid, potassium and pectin, and high concentrations of phytochemical substances, which contribute to the antioxidant action. During juice processing, heat treatment is applied with the purpose of increasing its shelf life, ensuring food safety, at a low cost. However, changes also occur in the nutritional and sensory properties and freshness of the product (Lee et al., 2021).

In this perspective, it is observed the increasing search of consumers for whole fruit juice (without added sugar and in its natural concentration), because, despite the short storage period compared to the commercial one, the substances are more easily preserved during its obtaining, and its acceptance is superior to pasteurized juice, such as orange juice (Ephrem et al., 2018; Olmedilla-Alonso et al., 2022; Wang & Xu, 2022).

Therefore, the aim of this study was to elaborate a whole orange juice from the orange tree 'de Russas' varieties (BRS 'Russas 01' and BRS 'Russas 02') with organic certification, as an incremental innovation, and to analyze its quality characteristics, correlating the results between the varieties to ascertain potential uses.

This study evaluated the compliance of the juice's physicochemical characteristics with the Brazilian current legislation; it also analyzed the maintenance of the ascorbic acid in the food during the consumption period, since its commercialization occurs at domestic level; and it characterized the juice's volatile fraction in order to convey the information to the academic community and to the industry, contributing to the dissemination of the culture's knowledge, to local sustainability, and to the market supply of this by-product.

For this purpose, the physicochemical characteristics of the juice were evaluated. The stability of the ascorbic acid present in the juice was analyzed during three days of storage after extraction, through high performance liquid chromatography with ultraviolet detection. Method validation was performed to verify its performance, correlating with the results obtained among the varieties. The volatile organic compounds were extracted and characterized by gas chromatography coupled to mass spectrometry.

## 2. Material and Methods

The research was carried out at the Laboratório de Físico-Química de Alimentos (LFQA [*Laboratory of Physical Chemistry of Foods* in English]) and at the Laboratório de Química Instrumental (LQI [*Laboratory of Instrumental Chemistry* in English]) of the Núcleo de Tecnologia e Qualidade Industrial do Ceará (NUTEC [*Nucleus of Technology and Industrial Quality of Ceará* in English]), located in Fortaleza (Ceará/Brazil).

### 2.1 Obtaining 'de Russas' Oranges

The fruits of the orange tree 'de Russas' which belong to the varieties of greater cultivation in region (BRS 'Russas 01' and BRS 'Russas 02') (Figure 1), grafted on clove lemon (according to the GBIF - *Citrus limonia* Osbeck) (Rutaceae), were acquired through a partnership with a family farmer from Sítio Pocinhos, in the rural area of Limoeiro do Norte-CE, located at 5°4'50.46"S latitude, 38°2'8.45"W longitude and 21 meters above sea level. Each orchard consists of 128 (0.32 ha) citrus plants, with 5 × 5 m spacing in double rows in a micro-sprinkler irrigation system, with two emitters per plant with a blade of 9.6 mm, twice a week, and a flow rate of 60 L/h.

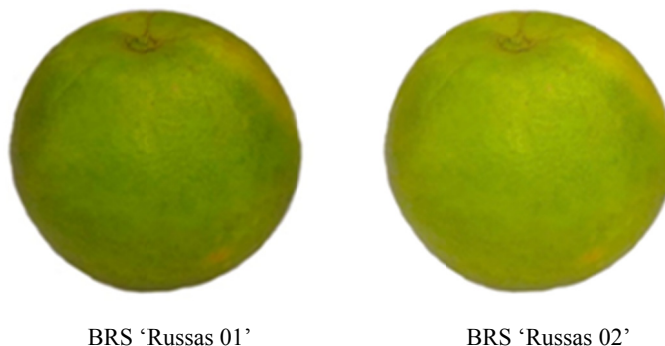


Figure 1. Fruits of 'de Russas' orange varieties

The climate of the region is BSw'h', according to the Köppen classification, characterized as hot and semi-arid. Between the period of August 2020 to June 2021, it had average rainfall of 55.02 mm, average temperature of 27.1 °C and average humidity of 786 mm (Estação Meteorológica da Unidade de Ensino, Pesquisa e Extensão [Meteorological Station of the Teaching, Research and Extension Unit in English]—5°10'55"S; 38°00'45"W 144.5 m a.s.l.).

The harvest was carried out manually at mid-height (on average 2 m) in the four quadrants of the canopy. The fruits were selected still in the field (July 2021), 180 days after flowering, in complete maturation, according to the 'Normas de Classificação de Citros de Mesa' [Classification Standards for Table Citrus in English] (CEAGESP, 2011) with skin coloration evaluated by visual scale, and fruit size evaluated by diameter measurement (mm) in (large, medium, or small).

The experimental design used was of randomized blocks, consisting of two orange varieties (BRS 'Russas 01' and BRS 'Russas 02') with four repetitions, totaling 8 experimental plots. Each plot had eight trees, totaling 64 trees (biological sample), planted at a spacing of five meters between planting lines and five meters between plants in the row, resulting in a usable area per plant of 25 m<sup>2</sup>. A total of 128 fruits were randomly collected from each variety (biological sample). For juice preparation, 20 fruits from each variety were selected (experimental sample).

The cultural practices of the farm consist of eliminating thieving shoots and verticalized branches, fertilizing bovine manure at will, performing weeding and crowning (catch basins) around the plant, piling up the soil near the stem, adding mulch, performing pruning and flower inductions (water stress), and periodic phytosanitary management due to the seasonality of pests and diseases; these activities do not have a defined frequency to be performed by the farmers in the region, nonetheless, in general, they occur weekly (Sombra et al., 2018a).

As for the occurrence of pests, the citriculture developed in the region presents low incidence of key pests and diseases. For the control, natural pesticides based on plants with insecticidal and/or repellent characteristics are used, such as tobacco grout and Indian Neem (according to the GBIF, *Azadirachta indica* A. Juss.) (Meliaceae), in the coexistence with the pest.

The soils in the orange-growing region 'de Russas' are predominantly 'Fluvic Neosols (RY)', defined as non-hydromorphic mineral soils formed by overlapping layers of alluvial sediments.

The collected 'de Russas' oranges had an average weight of 187.52 g and 178.97 g for 'Russas 01' and 'Russas 02' varieties, respectively. The adult plant of the 'de Russas' orange is grafted on the citrus 'Swingle' *Citrus paradisi* Macfad. (Rutaceae) × *Poncirus trifoliata* (L.) Raf (Rutaceae) (according to the GBIF). For this reason, there is still no precise classification for fruit size, because they are not considered navel, common or low acidity oranges, according to CEAGESP (2011).

Immature fruits, with skin defects, shape defects, field defects, mechanical damages, damages associated with wounds and post-harvest injuries, disease and pest attacks and/or advanced maturation stage - senescence (soft texture, peculiar odor and typical flavor change) were excluded. The varieties selected were packed in plastic bags and taken to the laboratory in thermal bags.

The oranges have the organic quality seal (Brazilian, American and European) certified by the Instituto Biodinâmico (IBD [Biodynamic Institute in English])—CE 154 and the fair-trade seal (Fair for Life), which is accompanied by the company Nat'Organico.

## 2.2 Processing of the 'de Russas' Orange Whole Juice

Obtaining the whole orange juice from the BRS 'Russas 01' and BRS 'Russas 02' varieties comprised the stages of harvest, reception, selection (20 fruits from each variety), weighing, washing, sanitization, cutting, manual extraction and filtration, as described in Figure 2 (Brasil, 2009; Marques, 2019).

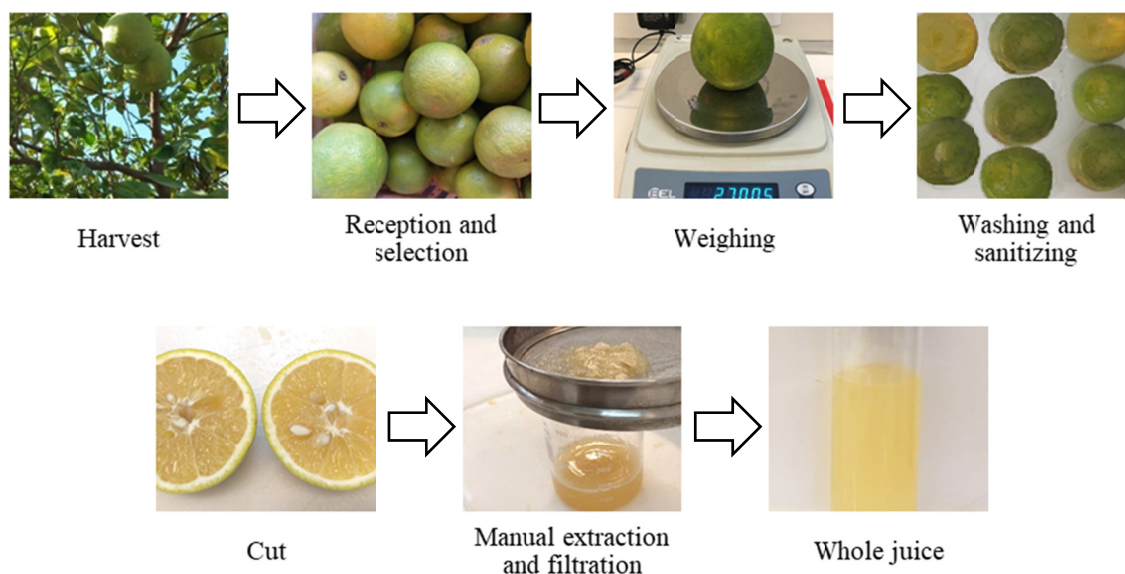


Figure 2. Flowchart of the steps for obtaining the whole juice of the 'de Russas' oranges

## 2.3 Physicochemical Analyses

The physicochemical analyses of the juice were performed using the methodology of the Official Methods of Analysis (Association of Official Analytical Chemists [AOAC], 2016b). The pH was measured in 10% (w/v) aqueous solutions of the samples in a pH meter (OHAUS<sup>®</sup>, USA, Starter 2100 Bench) (method 981.12). The soluble solids were measured by direct reading in a bench top analog refractometer (A. Krüss Optronic, Germany), with a scale from 0 to 95 °Brix, obtaining values with a precision of 0.1 °Brix at 20 °C (method 932.12). Titratable acidity was determined by titration with 0.1 N sodium hydroxide (Sigma-Aldrich<sup>®</sup>, USA) with the results expressed in g 100 g<sup>-1</sup> of citric acid (method 942.15). The sugar/acid ratio was calculated on the basis of the ratio between soluble solids and titratable acidity.

## 2.4 Analysis of Organic Acids

The analysis of organic acids was performed following the methodology adapted from Scherer et al. (2012). The stability of ascorbic acid was determined by means of high-performance liquid chromatography (HPLC) at time intervals counted in units of days, being 'T0' the day of the juice preparation; 'T1' the first day of refrigerated storage (4 °C); 'T2' the second day of refrigerated storage (4 °C); and 'T3' the third day of refrigerated storage (4 °C) (Brasil, 2004). The citric acid was quantified on the day of the juice preparation.

The determination of the acids was performed in a high efficiency liquid chromatograph, equipped with a binary pumping system (Gilson<sup>®</sup>, USA, model 321), reverse phase column C18 (Hichrom<sup>®</sup>, UK, 15 cm length × 4.6 mm diameter × 5 µm particle size), Ultraviolet-Visible (UV-VIS) detector (Gilson<sup>®</sup>, USA, model 152), automatic injector with 20 µl loop (Gilson<sup>®</sup>, USA, model 234), degasser (Gilson<sup>®</sup>, USA, model 864) and data analysis and processing system (Gilson<sup>®</sup>, USA, Software Unipoint 3.0).

### 2.4.1 Preparation of the Mobile Phase Solution

The mobile phase used was composed of a buffer solution of monopotassium phosphate 0.2 M (KH<sub>2</sub>PO<sub>4</sub>) (BCBD5129) (Sigma-Aldrich<sup>®</sup>, USA) with pH adjustment to 2.4. After preparation, it was filtered in a complete vacuum filtration system with a 0.45 µm pore size membrane (Millipore<sup>®</sup>, USA), stored in a glass vial and then degassed in a sonicator (Branson<sup>®</sup>, USA, 2800-MH Ultrasonic Cleaner) for complete removal of the gases dissolved in the solution.

#### 2.4.2 Preparation of the Standard Solutions and Obtaining of the Calibration Curves

The second step of the analysis consisted in the preparation of the mother solutions of the analytical standards of ascorbic acid 99% (BCCF4919) and citric acid 99.5% (BCCD1540) (Sigma-Aldrich<sup>®</sup>, USA), with concentration of 1000 mg L<sup>-1</sup>; from the primary standard solution, the necessary dilutions were made to reach the desired concentrations (100, 250, 500 and 750 mg L<sup>-1</sup>). The preparation of the calibration curves was protected from light.

After preparation, the solutions were filtered through a Millex membrane (Millipore<sup>®</sup>, Brazil, 0.45 µm pore size, 13 mm) to remove all impurities. They were then transferred to the vials of the automatic injector and an instrumental quantification was performed by HPLC-UV, obtaining the calibration curves of the standards (Figure 3).

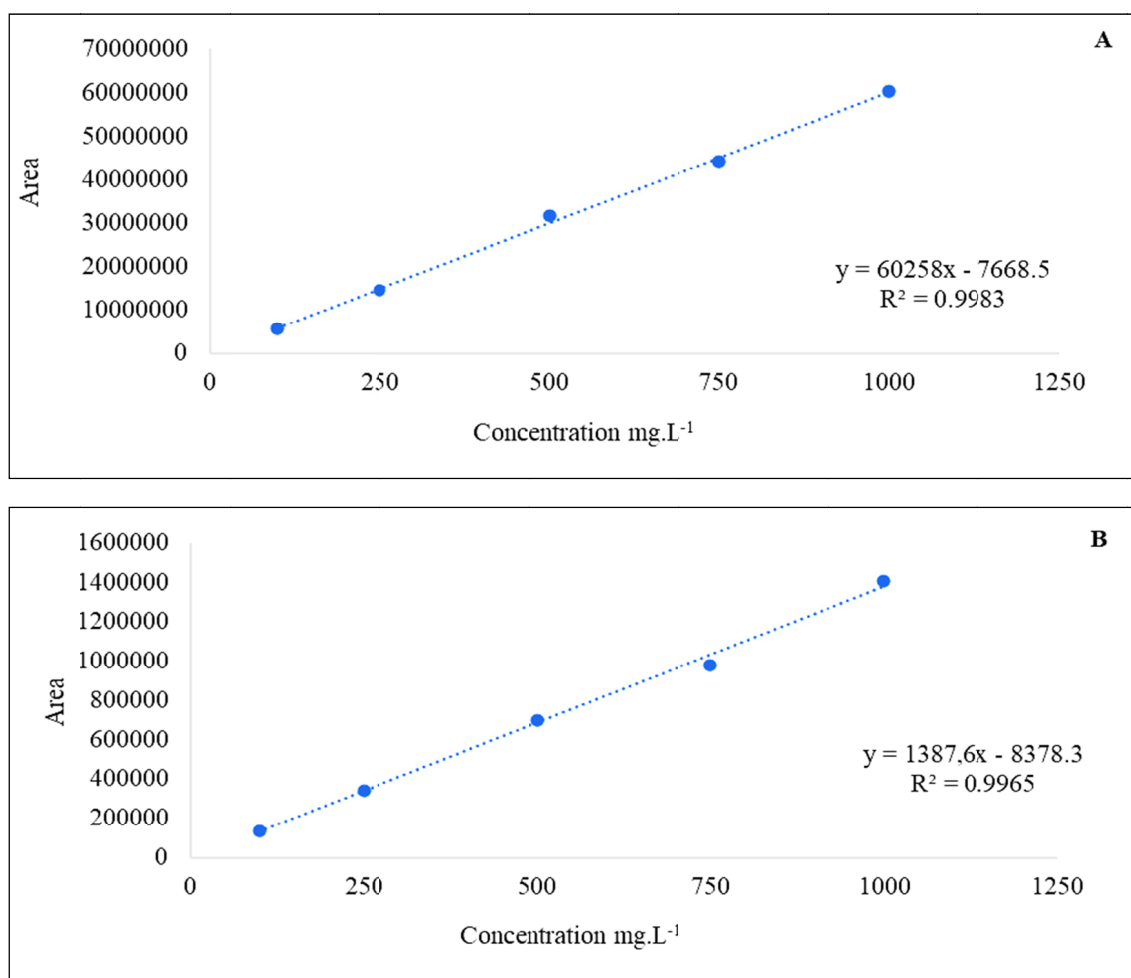


Figure 3. Standard calibration curves—ascorbic acid (A) and citric acid (B)

#### 2.4.3 Validation of the Ascorbic Acid Method by High Performance Liquid Chromatography with Ultraviolet Detection (HPLC-UV)

Analytical validation was performed using the following parameters: selectivity, linearity, precision, accuracy, detection and quantification limits, and measurements were performed in triplicate (AOAC, 2016a; Brasil, 2017; Instituto Nacional de Metrologia, Qualidade e Tecnologia [INMETRO] [*National Institute of Metrology, Quality and Technology* in English], 2020; Ribani et al., 2004).

##### (1) Selectivity

The selectivity of the method was observed through its capacity of identifying or of quantifying the analyte of interest without the interference of other compounds that could be present in the matrix studied.

## (2) Linearity

The linearity was verified from the linear regression equation of the calibration curve, constructed from the peak area of the compound under study *versus* the concentration of the analyte, using five concentrations of the standard solution.

## (3) Precision

Precision was evaluated in terms of repeatability (same analyst, same instrumentation and in a single run), and intermediate precision (analyst and different days, same instrumentation). The results were expressed as coefficient of variation (CV%).

## (4) Accuracy

The accuracy was determined through the recovery of the analyte by its quantification in the matrix under study. The samples were fortified at a concentration of 250 mg L<sup>-1</sup> of the ascorbic acid standard. The results were expressed in percentage (%).

## (5) Limits of detection (LOD) and quantification (LOQ)

The sensitivity of the method was defined through the limits of detection and quantification, based on the parameters of the analytical curve (Equations 1 and 2).

$$\text{LOD} = 3.3 \times s/S \quad (1)$$

$$\text{LOQ} = 10 \times s/S \quad (2)$$

Where, 's' is the linear coefficient of the equation and 'S' is the angular coefficient of the analytical curve.

### 2.4.4 Sample Preparation and Injection

Before the sample conditioning step, which was protected from light, the whole orange juices from the BRS 'Russas 01' and 'Russas 02' varieties were diluted in Milli-Q<sup>®</sup> (Millipore<sup>®</sup>, USA), water for ascorbic acid (1:2) and citric acid (1:10) quantification and filtered in black-band filter paper (Quanty<sup>®</sup>, Germany, 12.5 cm, 28 µm of porosity) to remove impurities.

Next, the solid-phase extraction (SPE) cartridge (Supelco<sup>®</sup>, USA, ENVI-18, 6 mL), stationary phase C18, was treated with a solution of Acetonitrile (Sigma-Aldrich<sup>®</sup>, USA): Water Milli-Q<sup>®</sup> (Millipore<sup>®</sup>, USA) (1:1). 10 mL of the sample was added to the cartridge, then the liquid was removed with Manifold (Supelco<sup>®</sup>, USA) and 5 mL was discarded. The remaining 5 mL was transferred to a 10 mL test tube, and it was filtered through a Millex membrane (Millipore<sup>®</sup>, Brazil, 0.45 µm pore size, 13 mm) to remove impurities.

After the conditioning step, the samples were placed in the vials of the automatic injector and analyzed by HPLC-UV, in duplicate, in isocratic elution mode, with a flow rate of 0.8 mL min<sup>-1</sup>, detector wavelength of 254 nm, in the UV region, for ascorbic acid, and 214 nm for citric acid, ambient column temperature, and a 10-minute run.

### 2.5 Analysis of Volatile Organic Compounds (VOCs) by Gas Chromatography Coupled to Mass Spectrometry (GC-MS)

The analysis of volatile organic compounds was performed in gas chromatography coupled to mass spectrometry (GC-MS), using the methodology adapted from Biasoto et al. (2015) and Mirhosseini et al. (2007).

#### 2.5.1 Extraction and Injection of the Analytes into the Chromatographic System

The analytes were extracted using the headspace solid-phase microextraction (HS-SPME) technique. In appropriate 40 mL flasks, aliquots of 10 mL of the juice and 3 g of sodium chloride were added, followed by their vortexing for 1 minute and then their extraction in a thermostatic bath. As extraction conditions, it was used fiber for SPME of polyacrylate (Supelco<sup>®</sup>, USA, polar, 85 µm of film thickness), time (30 minutes) and extraction temperature (65 °C).

The extracted volatile organic compounds were analysed on a GC-MS (Thermo Scientific<sup>™</sup>, USA, Focus GC, DSQII) equipped with a Quadrupole-type mass analyzer and a column: DB5-ms (Agilent Technologies J&W<sup>®</sup>, USA, 30 m length × 0.25 mm internal diameter × 0.25 µm film thickness). The injection temperature was 260 °C and the fiber was exposed in splitless mode. The helium 5.0 (99.999%) carrier gas flow rate was 1.0 mL min<sup>-1</sup>. The initial temperature programming was 60 °C held for 1 minute and at a rate of 5 °C.min<sup>-1</sup> until 210 °C, then 15 °C.min<sup>-1</sup> until 300 °C, held for 1 minute (total of 38 minutes). The temperature of the transfer line (interface) was maintained at 300 °C. The mass spectrometer was operated in electron impact (EI) mode with electron

energy of 70 eV at 250 °C. Full scan mass spectra were acquired in the mass range 50 to 400 (m/z). For data analysis and processing: the software Xcalibur™ 2.0.7 was used (Thermo Scientific™, USA).

### 2.5.2 Identification of Volatile Compounds

The majority of the volatile organic compounds (VOCs) were identified by comparing their mass spectra with the mass spectra of the standards contained in the library of the National Institute of Standards and Technology (NIST, 2022). The other compounds present in the volatile matrix of the orange juice ‘de Russas’ were not identified due to the low similarity with the mass spectra listed in the library.

### 2.6 Statistical Analysis

A randomized block design was used in which the treatments were composed of the varieties BRS ‘Russas 01’ and BRS ‘Russas 02’ with 4 repetitions of 5 fruits per experimental unit (plot), which comprised 20 fruits of each variety (experimental sample).

The major volatile organic compounds identified in the juices of the BRS ‘Russas 01’ and BRS ‘Russas 02’ varieties were evaluated by principal component analysis (PCA) to verify differences between the varieties, as well as to obtain the varieties’ profiles.

The data from the analyses are presented as average±standard deviation. They were evaluated by analysis of variance (ANOVA) followed by Student’s t test at 5% significance level. STATISTICA™ version 10 (Statsoft, 2011) was the software which was used.

## 3. Results and Discussion

### 3.1 Physicochemical Characterization

The pH values of the orange juices of the studied ‘Russas 01’ and ‘Russas 02’ varieties presented an average 4.15a±0.02 and 4.06b±0.03 respectively, with a significant difference among the varieties (Table 1). The pH for orange juice is not established by the legislation (Brasil, 2018).

Table 1. Physicochemical characterization of whole orange juice from ‘de Russas’ oranges

Whole juice	Physicochemical composition			
	Parameters			
	pH	Soluble solids (SS) (°Brix)	Titrate acidity (TA) (g 100 g <sup>-1</sup> )	Relation SS/TA (Ratio)
‘Russas 01’	4.15a±0.02	10.05a±0.09	0.42a±0.02	23.81a±1.34
‘Russas 02’	4.06b±0.03	10.73a±0.46	0.40a±0.01	26.72a±1.50
Legislation*	-	Minimum 10	-	Minimum 7

Note. Means±standard deviation followed by the same letters in the column did not differ significantly ( $p \geq 0.05$ ) by Student’s t test.

\*Normative Instruction n° 37 (Brasil, 2018).

Source: Research data.

Sombra et al. (2018b) carried out the physicochemical characterization of the fruits of the orange tree ‘de Russas’ in the city of Limoeiro do Norte (Ceará/Brazil), comparing them with fruits of the variety BRS ‘Russas 02’ grafted on citrandarin San Diego (according to the GBIF—*Citrus sunki* hort. ex *Tanaka* × *Poncirus trifoliata* (L.) Raf.) (Rutaceae), obtaining average pH value of 4.40 for the orange juice of the variety BRS ‘Russas 02’. It is worth mentioning that the varieties analyzed in this study were grafted on clove lemon (according to the GBIF—*C. limonia* Osbeck), which can influence the differences in pH (among other factors, such as luminosity, irrigation, soil), even though they are from the same variety.

In Brazil, the varieties destined for direct consumption and juice production are: ‘Pera’, ‘Valencia’, ‘Natal’ and ‘Folha Murcha’ (*C. × sinensis*) (Bastos et al., 2014). The results obtained in this study for the pH in the juice from the fruits BRS ‘Russas 01’ and BRS ‘Russas 02’ varieties (ranging from 4.06 to 4.15) were similar to those reported by Spira et al. (2018) in non-processed juice (4.06) of the fruits of the ‘Pera’ orange (*C. × sinensis*) grown in Bauru, São Paulo, Brazil.

The results obtained in this study for the pH of the fruit juice of the BRS ‘Russas 01’ and BRS ‘Russas 02’ varieties (4.06 to 4.15) were higher than those reported by Habibi et al. (2021) in juices from four blood orange cultivars (3.00 to 3.40) (‘Moro’, ‘Tarocco’, ‘Sanguinello’ and ‘Sanguine’) (*C. × sinensis*), grafted onto the ‘C-35’



citrange rootstock (*Citrus × sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf. (Rutaceae) (according to the GBIF), harvested in the Mazandaran province (Iran).

The soluble solids of the orange juices of the studied 'Russas 01' and 'Russas 02' varieties presented an average  $10.05a \pm 0.09$  and  $10.73a \pm 0.46$  respectively; furthermore, it was not observed a difference among the varieties (Table 1). The values of soluble solids complied with that established by the legislation (Brasil, 2018) for orange juice (minimum 10 °Brix).

Sombra et al. (2018b) obtained an average content of 12.02 °Brix for the juice of the BRS 'Russas 02' variety in their study. The results obtained in this work for the soluble were similar to those reported by Coelho et al. (2021), when they evaluated 22 citrus fruits from the Brazilian semiarid region, obtaining levels of 11.6, 13.3, 12.8, 10.7 and 10.1 °Brix for the varieties 'Pera D12', 'Pera C21', 'Pera D09', 'Natal 112' and 'Valencia Tuxpan' (*C. × sinensis*), respectively.

The results obtained in this study for the soluble solids are also similar to those reported by Akinola et al. (2018), when they evaluated the chemical, microbiological and sensory characteristics of orange juice (*C. × sinensis*) samples chemically preserved in Akure (Nigeria), obtaining 9.40 °Brix for the control sample (without preservatives) on the first day of analysis.

The titratable acidity of the orange juices of the studied 'Russas 01' and 'Russas 02' varieties presented an average  $0.42a \pm 0.02$  and  $0.40a \pm 0.01$  respectively; once more, it was not observed a significant difference among the varieties (Table 1). A value for titratable acidity in orange juice (Brasil, 2018) has not been established.

The results obtained in this study for the citric acid in the juice of the fruits of the BRS 'Russas 01' and BRS 'Russas 02' varieties (ranging from 0.40 to 0.42 g 100 g<sup>-1</sup>) were similar to those reported by Sombra et al. (2018b) in the juice of the fruits of the variety BRS 'Russas 02' (0.46 g 100 g<sup>-1</sup>). Coelho et al. (2019) elaborated juice from 'Pera' orange (*C. × sinensis*) cultivated under organic and conventional systems in Juazeiro (Bahia/Brazil), obtaining 0.61 and 0.69 g 100 g<sup>-1</sup> for the organic juice and conventional juice ( $p \geq 0.05$ ), respectively.

The results obtained in this study for the citric acid were similar to those reported by Hayatullah et al. (2021) in whole tangerine juice (0.65) (*Citrus reticulata* Blanco) (Rutaceae) (according to the GBIF), manufactured in Peshawar (Pakistan).

Some studies have shown that the variation in the chemical composition of citrus fruits is associated with edaphoclimatic factors, maturation, and agricultural practices, even when they belong to the same variety, intervening in the quality and acceptance of the fruits. However, more studies are needed to evaluate the influence of the cultivation system, both organic and conventional, on the physical, chemical and sensorial characteristics of citrus fruits and their derivatives (Petry et al., 2015).

The soluble solids/tituable acidity ratio (Ratio) of the orange juices of the studied 'Russas 01' and 'Russas 02' varieties presented an average  $23.81a \pm 1.34$  and  $26.72a \pm 1.50$  respectively; there was not a significant difference among the varieties (Table 1). The values of Ratio complied with what is established by the legislation (Brasil, 2018) for orange juice (minimum 7).

It is noteworthy that the official standard used for comparison of the physicochemical analyses is for processed orange juice (Brasil, 2018). It is also worth noting that this is a local study, with the data compared to the Brazilian specifications for this type of product; nevertheless, these standards may differ according to the country in which the study is conducted.

The Ratio is the balance between sweet or acidic taste, which is between the sugars and the organic acids present in the fruit. When the sugar content increases and the acid content reduces, the Ratio also increases (Sampaio et al., 2019). The results of this parameter indicate that the orange juice from variety BRS 'Russas 02' (26.72) presents a higher degree of sweetness than the orange juice from variety BRS 'Russas 01' (23.81) (Table 1).

The results obtained in this work for the soluble solids/titratable acidity ratio in the juice of the variety BRS 'Russas 02' (ranging from 23.81 to 26.72) were similar to those reported by Sombra et al. (2018b) (25.98). Coelho et al. (2019) observed a significant difference for the soluble solids/tituable acidity ratio between the juice of 'Pera' orange (*C. × sinensis*) produced in conventional (16.35) and organic (18.76) systems. These results are associated to this study since the varieties from 'de Russas' oranges which were studied are cultivated in an organic system and presented a high soluble solids/tituable acidity relation.

The results obtained in this study for the soluble solids/titratable acidity ratio in the juice of the BRS 'Russas 01' and BRS 'Russas 02' varieties were higher than those reported by Kenge et al. (2015), who evaluated the quality

of five samples of fresh sweet orange (*C. × sinensis*) juices sold in Malaysia, obtaining averages ranging from 10.90 to 15.30 for the fresh juices.

Silva et al. (2022) evaluated the physicochemical profile of four orange varieties ('Valencia', 'Folha Murcha', 'Pera' and 'Iapar') (*C. × sinensis*) grown in Paranavaí (Paraná/Brazil) and obtained lower concentrations (7.59-19.90) than those found in the present study.

Therefore, it can be seen that the 'de Russas' orange presents significant advantages if compared to the fruits of other sweet orange varieties, due to its higher concentration of sugars (Ratio). The oranges from BRS 'Russas 02' variety also present a higher concentration of soluble solids and, consequently, a higher Ratio, being considered sweeter than the oranges from BRS 'Russas 01' variety.

### 3.2 Quantification of Citric Acid by High Performance Liquid Chromatography with Ultraviolet Detection (HPLC-UV)

The citric acid was also quantified by high-performance liquid chromatography with ultraviolet detection. The chromatogram of the standard is represented by one of the points obtained on the calibration curve, with a concentration of 1000 mg L<sup>-1</sup> of citric acid and a retention time (rt) of 5.46 minutes (Figure 4).

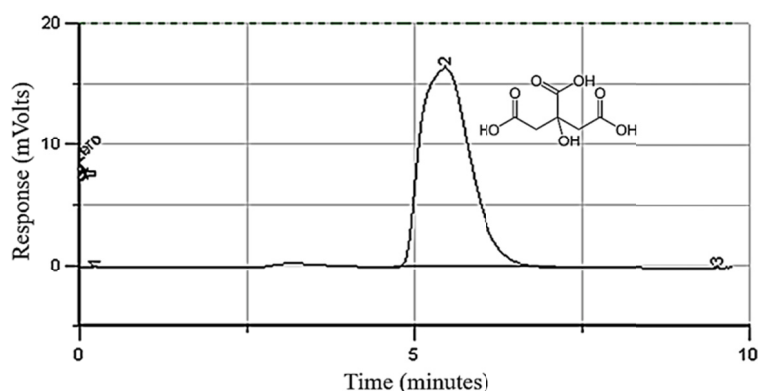


Figure 4. Chromatogram of the citric acid standard obtained by HPLC-UV

The coefficient of determination ( $R^2$ ) of the calibration curve was 0.9965, which is suitable for the quantification of the sample, following the established criteria (Brasil, 2017).

Table 2 shows the concentration of citric acid present in the whole juice from 'de Russas' orange varieties; it is quantified by high-performance liquid chromatography with ultraviolet detection. For the BRS 'Russas 01' variety, we observed average retention time (rt) has of 5.39 minutes, and for the BRS 'Russas 02' variety, has of 5.17 minutes, close to the retention time of the citric acid standard (5.46).

Table 2. Evaluation of citric acid in the whole orange juice 'de Russas' by HPLC-UV technique

Whole juice	Citric acid (g 100 mL <sup>-1</sup> )
'Russas 01'	0.90b±0.01
'Russas 02'	0.96a±0.01

*Note.* Means±standard deviation followed by the same letters in the column did not differ significantly ( $p \geq 0.05$ ) by Student's t test.

Source: Research data.

The results obtained in this study for the quantification of citric acid in the juices of the BRS 'Russas 01' and BRS 'Russas 02' varieties were similar to those reported by Navarro and Giraldo (2018). The authors aimed at analyzing the organic acids present in industrialized fruit juices marketed in Colombia, using HPLC and UV detector: in a total of ten samples, three were from orange juice (*C. × sinensis*). In all samples, citric acid was detected, with the lowest concentration in apple juice (0.04 g 100 mL<sup>-1</sup>) and the highest in one of the orange juices analyzed (0.51 g 100 mL<sup>-1</sup>).

In comparison with the study, a higher concentration of citric acid is observed in the analyzed 'de Russas' orange juices, in both varieties. This variation in the final concentration of citric acid may be related to the balance between the biosynthesis of the acid, its degradation, and its storage in vacuoles (Ma et al., 2018). Other variables interfere in the chemical composition of the fruit, such as the variety, the climate, the soil, the cultivation system, etc. (Marmitt et al., 2016), because they are fruits of different varieties, cultivated in different countries, which present a difference in the citric acid content.

Two other compounds were detected in the chromatogram of the citric acid quantification in the orange juice from the 'de Russas' orange varieties (Figure 5). The chromatographic peak corresponding to the citric acid in the orange juice samples from the BRS 'Russas 01' variety is the first and the one from the BRS 'Russas 02' variety is the second, according to the retention time obtained in the reading of the standard and by its intensity in relation to the other peaks present.

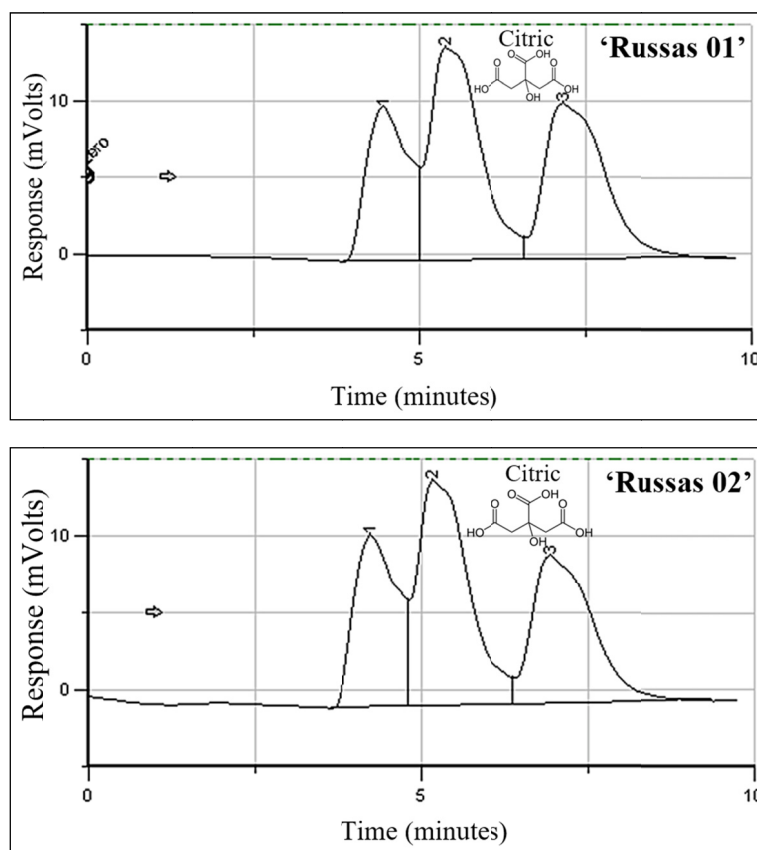


Figure 5. Chromatograms of the quantification of citric acid in the whole juice of 'de Russas' orange varieties obtained by HPLC-UV

This condition may be associated with the presence of other organic acids which are also detected in the same wavelength as citric acid, such as malic and tartaric acids. According to Carvalho et al. (2020), citric, ascorbic, and malic acids are the most abundant organic acids found in sweet oranges. Therefore, the presented situation may be associated to the presence of these compounds in significant concentration in the juice of the 'de Russas' orange varieties, being detected during the citric acid quantification by HPLC-UV.

Despite the detection of other acids during the quantification of citric acid, the effectiveness of the HPLC-UV method for the analysis of organic acids is not depreciated, since, in recent years, it has been one of the most used techniques, due to its speed, selectivity, sensitivity and reliability (Rodrigues et al., 2021).

The determination of citric acid by titratable acidity (Table 1) showed lower concentrations than the quantification by liquid chromatography (Table 2). Due to the presence of other acids detected in the citric acid quantification, the stability of the citric acid present in the juice samples of the 'de Russas' orange varieties were

not carried out, following only with the quantification and evaluation of the maintenance of the ascorbic acid content during three days under refrigeration (4 °C) after obtainment.

### 3.3 Validation of the Ascorbic Acid Method by High Performance Liquid Chromatography With Ultraviolet Detection (HPLC-UV)

The chromatogram of the ascorbic acid standard is represented by one of the points obtained on the calibration curve, with a concentration of 250 mg L<sup>-1</sup> of ascorbic acid, and a retention time (rt) of 4.20 minutes (Figure 6).

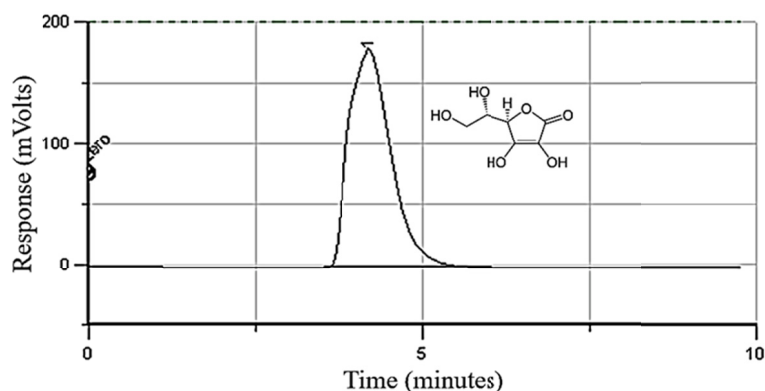


Figure 6. Chromatogram of the ascorbic acid standard obtained by HPLC-UV

The selectivity parameter, or the specificity of the method, was confirmed by the absence of interfering peaks in the retention time of ascorbic acid, by the proximity of the retention time of ascorbic acid for the standard and for the samples analyzed, and by the proportional increase in peak areas when the standard was added.

Table 3 shows the data from the validation of the method for quantification of ascorbic acid by high-performance liquid chromatography with ultraviolet detection.

Table 3. Results of the validation of ascorbic acid through the HPLC-UV technique in whole orange juice from 'de Russas' oranges

Validation parameters		
Linearity (R <sup>2</sup> )	Recuperation (%)	Repeatability (%CV)
0.9983	91.10	2.86
Validation parameters		
Intermediate precision (%CV)	Detection limit (mg L <sup>-1</sup> )	Limit of quantification (mg L <sup>-1</sup> )
3.14	20	50

Note. Abbreviations: CV, coefficient of variation.

Source: Research data.

The coefficient of determination (R<sup>2</sup>) of the calibration curve of ascorbic acid was greater than 0.990 (Table 3), which is appropriate for analysis by the external standard method (Brasil, 2017). The percentage recovery (accuracy) of the method (Table 3) was considered acceptable by the criteria of AOAC (2016a), which recommends average recovery of 80 to 110%.

For precision, the results were evaluated in terms of repeatability and of intermediate precision. According to the manual of analytical quality assurance of MAPA, for the areas of identity and quality of food and inputs, the criterion of acceptability adopted for the repeatability and intermediate precision is the one in which the coefficient of variation is less than or equal to 20% (CV ≤ 20%). Therefore, it is evidenced the efficiency of the validated method for the quantification of ascorbic acid in the elaborated orange juices (Brasil, 2015).

The limit of detection (LOD) corresponds to the lowest concentration of the analyte that can be detected, but not necessarily quantified, and the limit of quantification (LOQ) indicates the lowest concentration capable of being measured, under the defined experimental conditions, with acceptable precision and accuracy (Brasil, 2017). The

limits of detection and quantification demonstrate high sensitivity of the method (Table 3), considering the experimental conditions adopted.

The results obtained in this study for the validation of the method for the determination of ascorbic acid in the juices of the BRS ‘Russas 01’ and BRS ‘Russas 02’ varieties were similar to those presented by Klimczak and Gliszczynska-Świgło (2015), with emphasis on the repeatability and intermediate precision of the present study (2.86 and 3.14%). The authors validated two chromatographic methods (ultra-performance liquid chromatography—UPLC and HPLC) for the determination of ascorbic acid contents in fruit juices and beverages and in pharmaceutical preparations. The methods were validated in terms of linearity (0.9990 for both methods), limits of detection (0.049 and 0.024 mg L<sup>-1</sup>) and quantification (0.149 and 0.073 mg L<sup>-1</sup>), repeatability (2.20 and 1.70%), intermediate precision (2.40% and 1.90%), and recovery (99.8 and 99.9%), for HPLC and UPLC, respectively.

The results found in this study also are similar to those described by Hasan et al. (2021). A method was validated for the quantification of ascorbic acid through the coefficient of determination of the calibration curve (0.9988), recovery (95.71%), and limits of detection (1.032 mg L<sup>-1</sup>) and quantification (3.103 mg L<sup>-1</sup>), in fruit pulp juice of *Citrus macroptera* Montrouz. (Rutaceae) (according to the GBIF), a semi-wild citrus species native to Malaysia, Melanesia, and Bangladesh, by high-performance liquid chromatography with variable wavelength detector, in Sylhet (Bangladesh).

#### 3.4 Evaluation of Ascorbic Acid Stability by High Performance Liquid Chromatography With Ultraviolet Detection (HPLC-UV)

The whole orange juice from the BRS ‘Russas 01’ variety presented an average ascorbic acid content varying from 55.16 to 43.76 mg 100 mL<sup>-1</sup> during the four days of analysis, with a significant difference between the storage times. The juice from the BRS ‘Russas 02’ variety showed a decrease in ascorbic acid content from 57.07 to 49.31 mg 100 mL<sup>-1</sup>, differing significantly between the days of analysis, except at T1 and T2, which were statistically equal ( $p \geq 0.05$ ). The two varieties studied showed a decrease in ascorbic acid contents during storage (Table 4).

Table 4. Stability of ascorbic acid in whole orange juice from ‘de Russas’ oranges stored under refrigeration (4°C) for three days after elaboration using HPLC-UV technique

Whole juice	Ascorbic acid stability (mg 100 mL <sup>-1</sup> )			
	Storage time (Days)			
	T0	T1	T2	T3
‘Russas 01’	55.16aA±0.22	51.60bB±0.21	45.61cB±0.09	43.76dB±0.12
‘Russas 02’	57.07aA±0.67	54.45bA±0.08	53.24bA±0.09	49.31cA±0.14
Legislation*	Minimum 25			

*Note.* Means±standard deviation followed by the same lowercase letters on the same line did not differ significantly between days of storage ( $p \geq 0.05$ ) by Student’s t test.

Means±standard deviation followed by equal capital letters in the same column did not differ significantly between varieties ( $p \geq 0.05$ ) by Student’s t test.

\*Normative Instruction n° 37 (Brasil, 2018).

T0: day of the juice preparation; T1: first day of refrigerated storage (4 °C); T2: second day of refrigerated storage (4 °C); T3: third day of refrigerated storage (4 °C).

Source: Research data.

While evaluating the vitamin C stability in the orange juices between the varieties BRS ‘Russas 01’ and BRS ‘Russas 02’, it was observed that only on the day of preparation (T0) there was not significant difference between them ( $p \geq 0.05$ ). From the first day of storage (T1), a significant difference between the varieties was observed, showing that the ascorbic acid maintenance in the samples was influenced by the ‘de Russas’ orange variety (Table 4).

The percentage of ascorbic acid degradation in the orange juices from the BRS ‘Russas 01’ and BRS ‘Russas 02’ varieties is presented in Figure 7.

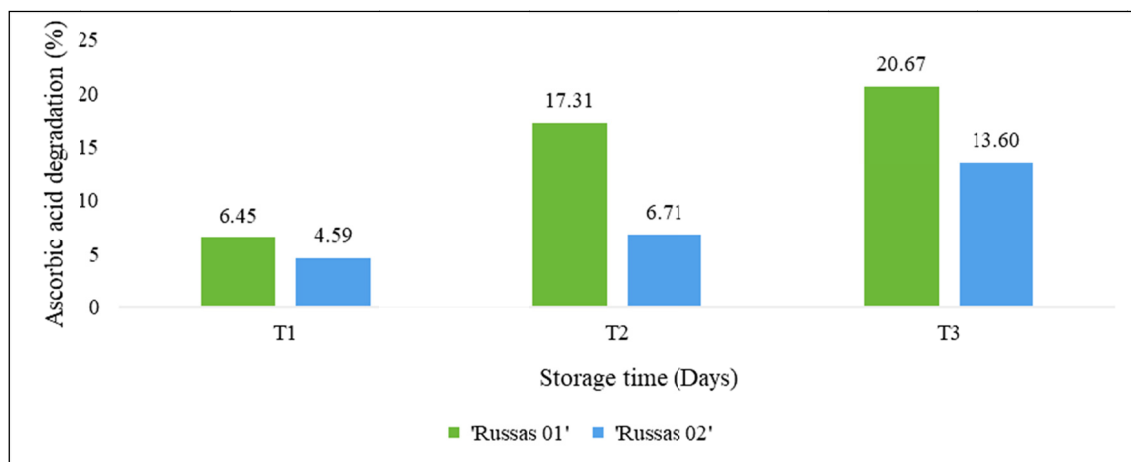


Figure 7. Percentage of ascorbic acid degradation in 'de Russas' orange juice during storage

In other words, the vitamin C concentration of the orange juices was maintained at 79.33% for the BRS 'Russas 01' and 86.40% for the BRS 'Russas 02', which indicates that the juice from the BRS 'Russas 02' variety has a greater capacity to preserve nutritional characteristics during refrigerated storage than the juice from the BRS 'Russas 01' variety.

The results obtained in this study for the quantification of ascorbic acid in the juice of the BRS 'Russas 01' and BRS 'Russas 02' varieties were similar to those reported by Scherer et al. (2012). The authors validated a methodology for simultaneous determination of organic acids by HPLC-DAD in ready-to-drink fruit juices obtained in Campinas (São Paulo/Brazil), and they evaluated the ascorbic acid stability every 2 days in the orange juice samples (*C. × sinensis*) during 14 days. The results showed an initial average concentration of 30 to 38 mg 100 mL<sup>-1</sup> of ascorbic acid in the analyzed orange juices, with a decrease of 27% in the 10th day of storage (5 °C), which means that 73% of vitamin C content was maintained.

Sombra et al. (2018b) obtained an average content of 77.95 mg 100 mL<sup>-1</sup> for the juice of the BRS 'Russas 02' variety in their study, higher than the obtained (55.16 and 57.07 mg 100 mL<sup>-1</sup>) for the '01' and '02' varieties, respectively. This difference refers to the different rootstocks used, which may influence the composition of the fruits, even if they are of the same variety.

Furthermore, it is known that ascorbic acid degrades when exposed to light, causing a rapid oxidation of the molecules. Therefore, it is recommended that juices produced for home consumption, or in a homemade way, should be produced and subsequently consumed, since they will not have added preservative additives (Dallago et al., 2020).

After opening the package, the food prepared and conserved under refrigeration at 4 °C or below should be consumed within a maximum of five days, and if conservation is between 4 and 5 °C, the maximum period for consumption should be reduced to ensure the hygiene and sanitary conditions of the product (Brasil, 2004).

Considering this information, the present study followed the stability of the fresh whole orange juice for 3 days after its elaboration. The juice was protected from light and under refrigeration (4 °C) since it did not contain preservatives and it was not thermally treated.

The orange juice must have 25 mg 100 mL<sup>-1</sup> (equivalent to 100 mg) of ascorbic acid in its composition (Brasil, 2018). Due to this determination, it was observed that the orange juices produced, independently of the variety, complied with the legislation, even in the last day of storage (T3), presenting initially (T0) the double of the required concentration (Table 4).

The Recommended Daily Intake (RDI) of vitamin C for adults is 45 mg (Brasil, 2005). Thus, the consumption of only 100 mL of the orange juice from the BRS 'Russas 01' variety protected from light at a temperature of 4 °C for up to 3 days already provides the RDI of vitamin C for adult men and women.

For a food to be considered a source of vitamins and minerals, it must meet at least 15% of the RDI in a 100 mL or 100 g serving and it can be attributed as high content if it contains 30% of the RDI in 100 mL or 100 g of the

food (Brasil, 2012). Therefore, the variety juices from the 'de Russas' oranges can be considered high in vitamin C because they have concentrations higher than 100% of the RDI of this nutrient in a 100 mL serving.

It is noteworthy that the high-performance liquid chromatography method with ultraviolet detection was able to identify and quantify ascorbic acid, since all the samples analyzed were above the detection and quantification limit (Tables 3 and 4).

The chromatograms of ascorbic acid stability in whole orange juice varieties from BRS 'Russas 01' and BRS 'Russas 02' are shown in Figures 8 and 9, respectively. For the BRS 'Russas 01' variety, we observed average retention time (rt) has of 4.17 minutes, and for the BRS 'Russas 02' variety, has of 4.19 minutes, close to the retention time of the ascorbic acid standard (4.20).

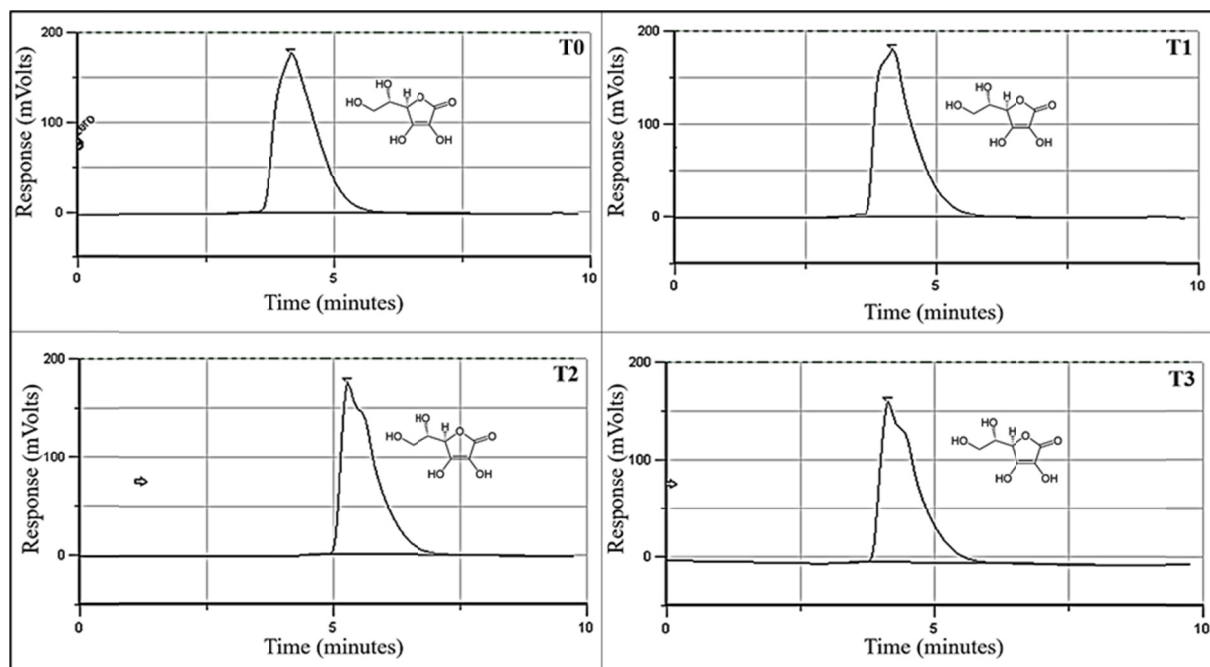


Figure 8. Chromatograms of the stability of ascorbic acid in the whole orange juice from the variety BRS 'Russas 01' obtained by HPLC-UV. *Note.* T0: day of the juice preparation; T1: first day of refrigerated storage (4 °C); T2: second day of refrigerated storage (4 °C); T3: third day of refrigerated storage (4 °C)



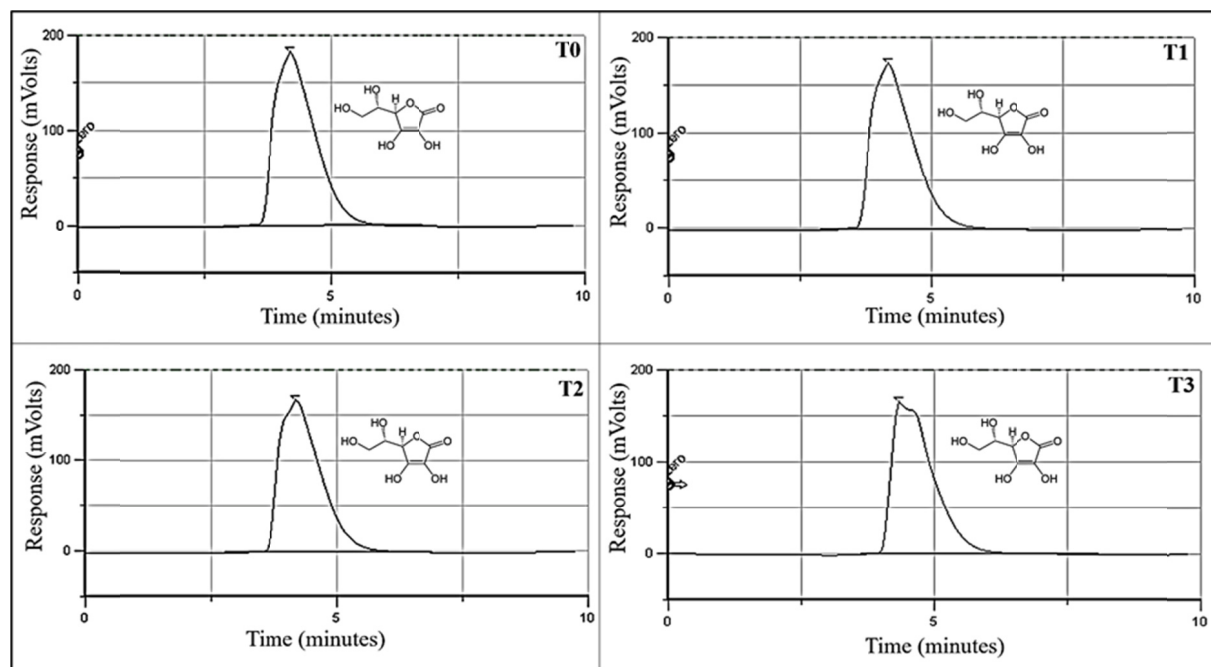


Figure 9. Chromatograms of the stability of ascorbic acid in the whole orange juice from the variety BRS 'Russas 02' obtained by HPLC-UV. Note. T0: day of the juice preparation; T1: first day of refrigerated storage (4 °C); T2: second day of refrigerated storage (4 °C); T3: third day of refrigerated storage (4 °C)

The results obtained in this study resemble those reported by Antakli et al. (2016). The ascorbic acid content present in the pulp and skin of nine citrus fruits in Syria was evaluated using HPLC-UV-VIS. In the pulp of the fruits, it was obtained for sweet orange (*C. × sinensis*) 30.53 to 76.16 mg 100 g<sup>-1</sup> of vitamin C; for lemon (*Citrus × limon* (L.) Burm.fil.) (Rutaceae) (according to the GBIF), the contents ranged from 15.57 to 52.10 mg 100 g<sup>-1</sup>; for tangerine (*C. reticulata*) from 32.90 to 57.98 mg 100 g<sup>-1</sup>; from 21.92 to 51.99 mg 100 g<sup>-1</sup> ranged the values for grapefruit (*Citrus × paradisi* Macfad.) (Rutaceae) (according to the GBIF); and 49.56 to 60.42 mg 100 g<sup>-1</sup> is the range of ascorbic acid for sour orange (*Citrus × aurantium* L.) (Rutaceae) (according to the GBIF).

The results obtained in this study for the quantification of ascorbic acid in the juice of the BRS 'Russas 01' and BRS 'Russas 02' varieties were also similar to those reported by Romeo et al. (2020). They studied the effect of phenolic enrichment in vitamin C and in the antioxidant activity in industrialized orange juice (*C. × sinensis*) produced in Valencia (Spain), (through HPLC coupled with DAD detector, analyzing it for 60 days (interval of 15 days) conserved in temperature of 6 °C. For the control sample, an initial content of 47.76 mg 100 mL<sup>-1</sup> was found. After 60 days of storage, 32.34 mg 100 mL<sup>-1</sup> of ascorbic acid was found in the fresh juice, representing a decrease of 32%.

The results found in this study are similar to those reported by Soceanu et al. (2021). The quantification of Vitamin C in orange juices (*C. × sinensis*) commercialized in Constanta (Romania), without and with added sugar, was determined by spectrometric and voltammetric methods. The results obtained ranged from 29.98 to 42.27 34 mg 100 mL<sup>-1</sup> of ascorbic acid. In the juices with added sugar, there was a lower loss of ascorbic acid.

In compliance with what has been mentioned, the samples of whole juice obtained from varieties of the orange tree 'de Russas' present higher contents of ascorbic acid, and less decline during storage. These disparities in vitamin C concentrations between the aforementioned studies and the present study are associated with the fruit production site, variety differences, ripening stage, storage conditions and additives used in the processing of ready-to-drink juices (Fonseca & Petean, 2018).

### 3.5 Analysis of Volatile Organic Compounds by Gas Chromatography Coupled to Mass Spectrometry (GC-MS)

Thirty volatile organic compounds were identified in the volatile fraction of the orange juice from the BRS 'Russas 01' variety. From these, 8 peaks were presented as majorities, subdivided in chemical classes, with 7 of them being terpenes and 1 of them being aldehyde, as described in Table 5, with retention time, chemical class, aroma, molecular formula, molecular weight, and similarity percentage.



Table 5. Majority volatile organic compounds identified in whole orange juice from variety BRS ‘Russas 01’ extracted by HS-SPME and analyzed by GC-MS

ID	rt (min)	Compound	Chemical class	Flavor	Formula	MW (g/mol)	Similar. (%)
A	6.24	$\alpha$ -Phellandrene	Terpene	Eucalyptus	C <sub>10</sub> H <sub>16</sub>	136.23	22.27
B	6.62	$\alpha$ -Pinene	Terpene	Pine	C <sub>10</sub> H <sub>16</sub>	136.23	36.89
C	6.95	Octanal	Aldehyde	Lemon, fruity	C <sub>8</sub> H <sub>16</sub> O	128.21	66.42
D	7.13	3-Carene	Terpene	Sweet, poignant	C <sub>10</sub> H <sub>16</sub>	136.24	15.21
E	7.98	D-Limonene	Terpene	Citric, freshness	C <sub>10</sub> H <sub>16</sub>	136.24	70.00
F	9.58	$\alpha$ -Linalool	Terpenoid	Floral, citric	C <sub>10</sub> H <sub>18</sub> O	154.25	79.73
G	12.53	Decanal	Terpenoid	Orange skin	C <sub>10</sub> H <sub>20</sub> O	156.27	52.70
H	14.21	Citral	Terpenoid	Lemon, sweet, citric	C <sub>10</sub> H <sub>16</sub> O	152.23	65.22

Note. Abbreviations: ID, identification; rt, retention time; MW: Molecular weight; Similar., Similarity.

Source: Research data.

In the chromatogram of the volatile compounds analysis in the orange juice from the BRS ‘Russas 01’ variety, the majority peaks can be visualized, which are highlighted by the letters A-H. The compounds E and B presented higher relative abundance rates, with emphasis on the peak E (D-Limonene), which presents the largest area, inferring that it is the compound of higher predominance in the analyzed sample (Figure 10).

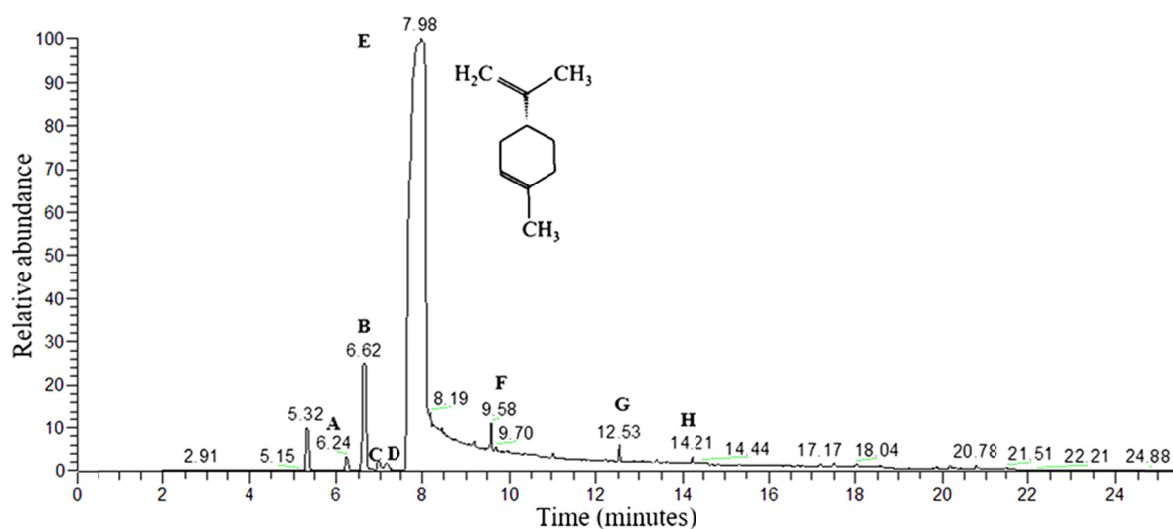


Figure 10. Chromatogram of the major volatile organic compounds identified in whole orange juice from the BRS ‘Russas 01’ variety extracted by HS-SPME and analyzed by GC-MS

Note. A:  $\alpha$ -Phellandrene; B:  $\alpha$ -Pinene; C: Octanal; D: 3-Carene; E: D-Limonene; F:  $\alpha$ -Linalool; G: Decanal; H: Citral.

Sixty-one volatile organic compounds were identified in the volatile fraction of the orange juice from the BRS ‘Russas 02’ variety. From these, 10 peaks were presented as majorities, subdivided in chemical classes, with 8 of them being terpenes and 2 of them being aldehydes, as described in Table 6, with retention time, chemical class, aroma, molecular formula, molecular weight, and similarity percentage.

Table 6. Majority volatile organic compounds identified in whole orange juice from variety BRS ‘Russas 02’ extracted by HS-SPME and analyzed by GC-MS

ID	rt (min)	Compound	Chemical class	Flavor	Formula	MW (g/mol)	Similar. (%)
A	6.22	$\alpha$ -Phellandrene	Terpene	Eucalyptus	$C_{10}H_{16}$	136.23	22.27
B	6.62	$\alpha$ -Pinene	Terpene	Pinhe	$C_{10}H_{16}$	136.23	36.89
C	6.95	Octanal	Aldehyde	Lemon, fruity	$C_8H_{16}O$	128.21	66.42
D	7.13	3-Carene	Terpene	Sweet, poignant	$C_{10}H_{16}$	136.24	15.21
E	7.98	D-Limonene	Terpene	Citric, freshness	$C_{10}H_{16}$	136.24	70.00
F	9.56	$\alpha$ -Linalool	Terpenoid	Floral, citric	$C_{10}H_{18}O$	154.25	79.73
G	11.00	Citronellal	Terpenoid	Floral, citric	$C_{10}H_{18}O$	154.25	86.00
H	12.53	Decanal	Terpenoid	Orange skin	$C_{10}H_{20}O$	156.27	52.70
I	14.21	Citral	Terpenoid	Lemon, sweet, citric	$C_{10}H_{16}O$	152.23	65.22
J	18.04	Dodecanal	Aldehyde	Citric	$C_{12}H_{24}O$	184.32	33.68

Note. Abbreviations: ID, identification; rt, retention time; MW: Molecular weight; Similar., Similarity.

Source: Research data.

In the chromatogram of the volatile compounds analysis in the orange juice from the BRS ‘Russas 2’ variety, it can be observed the major peaks highlighted by the letters A-J. The compounds E and B presented higher relative abundance, as well as in the chromatogram of the juice from the BRS ‘Russas 01’ variety, where peak E (D-Limonene) also stood out (Figure 11).

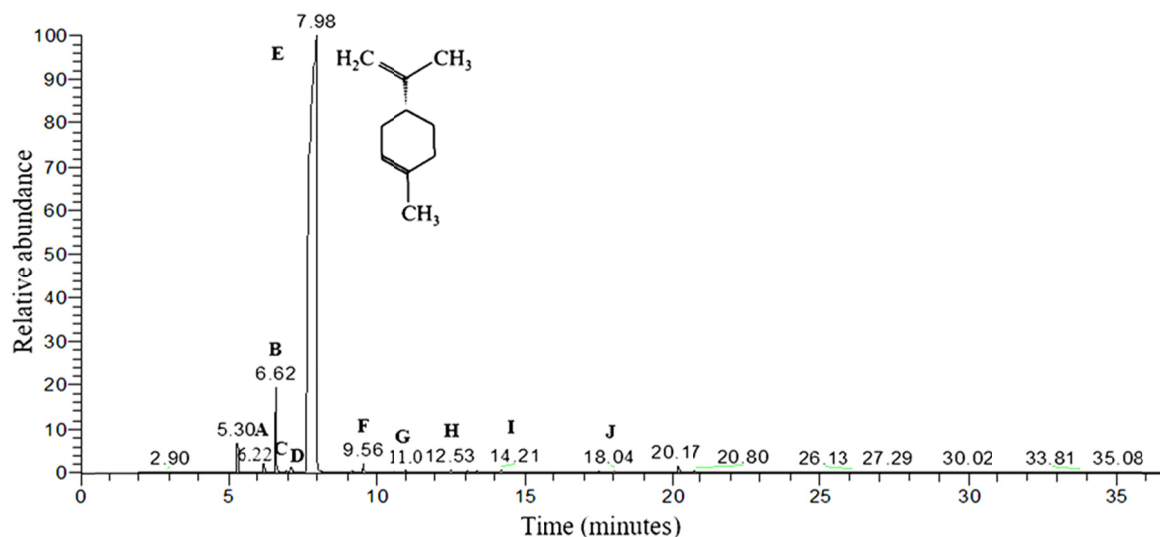


Figure 11. Chromatogram of the major volatile organic compounds identified in whole orange juice from the BRS ‘Russas 02’ variety extracted by HS-SPME and analyzed by GC-MS

Note. A:  $\alpha$ -Phellandrene; B:  $\alpha$ -Pinene; C: Octanal; D: 3-Carene; E: D-Limonene; F:  $\alpha$ -Linalool; G: Citronellal; H: Decanal; I: Citral; J: Dodecanal.

The commercial polyacrylate fiber is among the most used, it is recommended for the extraction of polar semi volatile compounds (Nascimento et al., 2018). The volatile compounds identified in the orange juice have polarity between the extraction range of the fiber (80-300 g/mol), with molar mass ranging from 128 to 184 g/mol, justifying the efficiency of the polyacrylate coating in the extraction of volatiles (Tables 5 and 6).

The results obtained in this study for the analysis of volatile compounds in the juices of the BRS ‘Russas 01’ and BRS ‘Russas 02’ varieties were similar to those published by Mastello et al. (2015), when they performed the extraction of volatiles by HS-SPME in samples of Brazilian orange juice frozen concentrate (*C. × sinensis*) which was supplied by a citrus farm in Araraquara (São Paulo/Brazil); they then analyzed by gas chromatography with a flame ionization detector, quantifying the maximum possible compounds by GC-MS.

They identified 62 compounds in the volatile fraction, as well as in the present study: the D-Limonene presented peak with higher intensity in the chromatogram, indicating to be present in higher concentrations in the sample. Other compounds such as ethyl butanoate, ethyl octanoate, nonanal and decanal were considered of great relevance to the juice odor.

The results observed in this study are similar to those listed by Zhou et al. (2020). The authors determined the volatile organic compounds present in orange (*C. × sinensis*) and tangerine (*C. reticulata*) juices by HS-SPME and GC-MS. Ripe fruits were purchased from orchards and from supermarkets in China, including 27 sweet orange and 19 mandarin cultivars. In the orange juice samples, most of the identified compounds also belonged to the terpene class, highlighting that D-Limonene was present in almost all varieties, evidencing the highest concentration.

With the identification of the volatile compounds present in the juices of varieties from 'de Russas' oranges, in comparison with the aforementioned studies, it can be noticed that, even though they are fruits of different varieties, cultivated in different places, the monoterpenes compose the largest volatile fraction of citrus fruits derived, especially the D-Limonene, responsible for its characteristic aroma. It is the majority component of the essential oil of citrus peels, in concentrations ranging from 70 to 95%, and because it has pharmacological properties, it is safe for human use (Anandakumar et al., 2021; Madji et al., 2019).

In relation to the major compounds, which are the compounds with greater significance in the volatile fraction of the elaborated juices, a similarity between the two varieties was observed, which is demonstrated by the superposition of the chromatograms obtained in the identification of the volatile compounds of the whole orange juice from the BRS 'Russas 01' and BRS 'Russas 02' varieties (Figure 12).

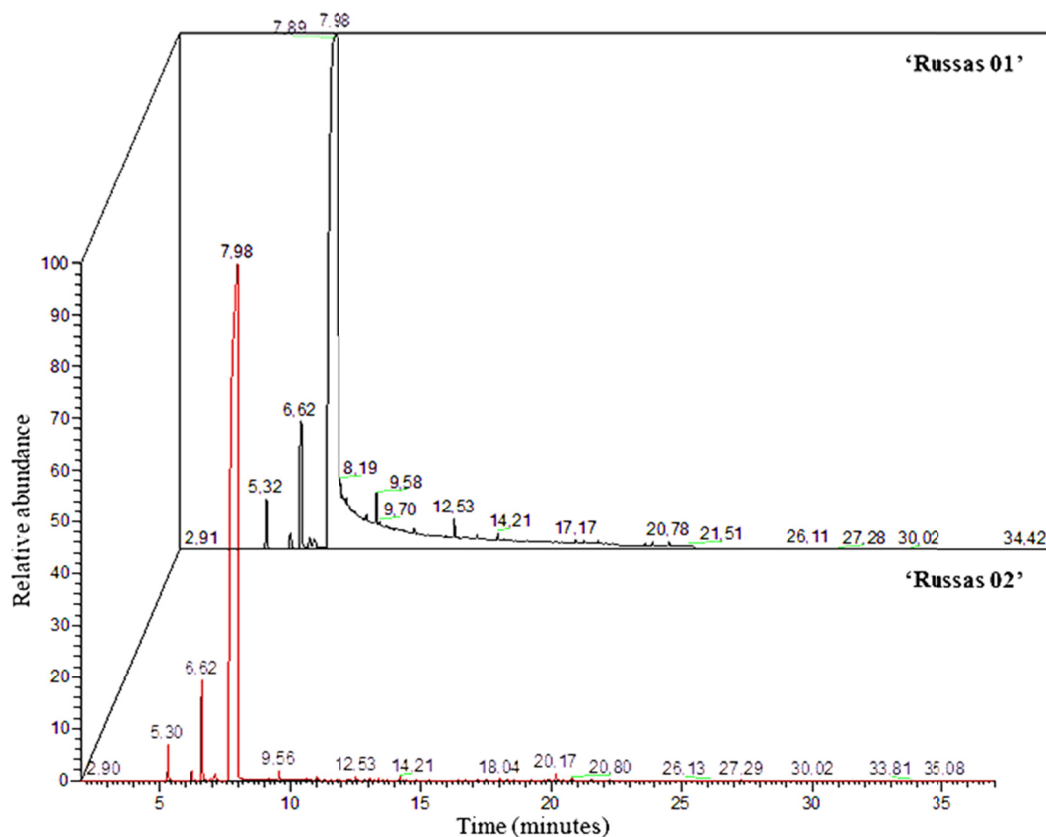


Figure 12. Overlapping chromatograms of the major volatile organic compounds identified in whole orange juice from varieties BRS 'Russas 01' and BRS 'Russas 02' extracted by HS-SPME and analyzed by GC-MS

The juice from the BRS 'Russas 02' variety also presented two more compounds, citronellal and dodecanal. Citronellal is a terpenic compound, classified as a monoterpene, which participates mostly in the composition of essential oils of aromatic plants (Bezerra et al., 2019). The dodecanal is an aldehyde, which as well as the octanal

and the terpenes identified in the juices of varieties of the 'de Russas' orange, are the compounds that confer citric, fruity, sweet, and floral aroma to the orange juice obtained manually (Perez-Cacho & Rouseff, 2008).

In the light of the data above, it was observed that eight major compounds are related among varieties, allowing the performance of principal component analysis (PCA). Two other major compounds belonging only to the BRS 'Russas 02' variety were not correlated (citronellal and dodecanal).

The scatter plot generated by the main component analysis of the majority compounds of the juice from the BRS 'Russas 01' and BRS 'Russas 02' varieties showed that the principal component 1 obtained represented 99.9% of the total variance (Figure 13).

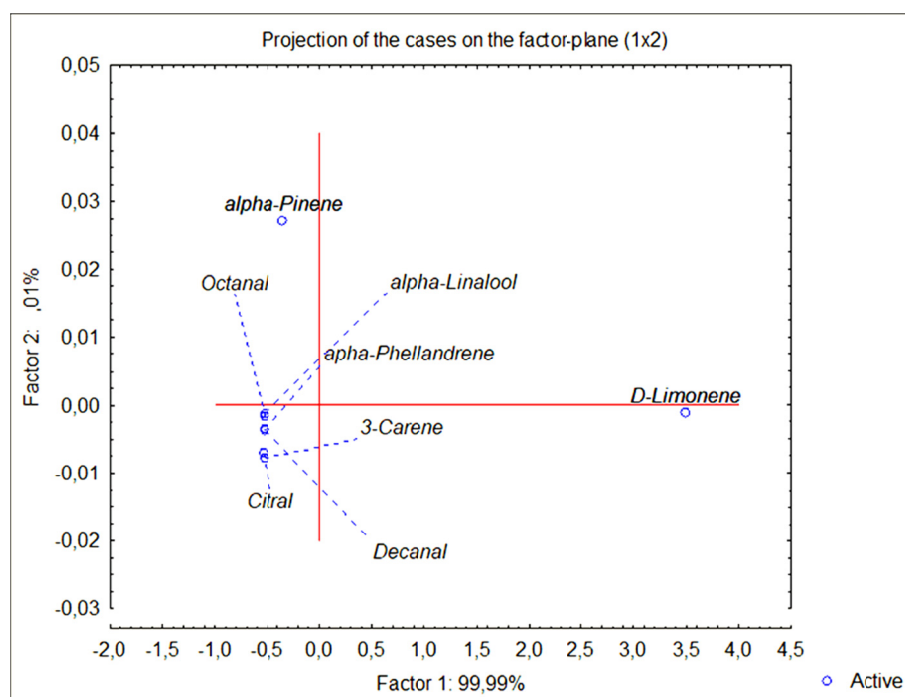


Figure 13. Analysis of the main components of the major volatile organic compounds identified in whole orange juice from varieties BRS 'Russas 01' and BRS 'Russas 02' extracted by HS-SPME and analyzed by GC-MS

Component 1 was positively correlated with  $\alpha$ -Linalool,  $\alpha$ -Pinene,  $\alpha$ -Phellandrene, citral, decanal, octanal, 3-Carene and D-Limonene, which were highly detected in the juice of BRS 'Russas 01' variety. Component 2 was positively correlated with D-limonene, the most prevalent compound in the juice of BRS 'Russas 02' variety (Figure 13).

The results of this study for the analysis of volatile compounds in the juice of the BRS 'Russas 01' and BRS 'Russas 02' varieties were close to those described by Perestrelo et al. (2019). The authors extracted the juice from ripe fruits, including orange (*C. × sinensis*), grown in Madeira Island (Portugal). Part of the juice was subjected to pasteurization, and the volatile compounds of the fresh and pasteurized juices were analyzed. The orange juices projected in the first two positive main components were mostly associated with limonene, as observed in this study.

Therefore, the area of volatile compounds in the orange juice of the BRS 'Russas 01' variety infers a higher concentration in comparison to the BRS 'Russas 02' variety. And BRS 'Russas 02' orange juice has a more numerous volatile matrix, comprising more than the double of identified volatile organic compounds when compared to the BRS 'Russas 01' orange juice.

#### 4. Conclusion

The whole fruit juices from varieties of the orange tree 'de Russas' meet the physicochemical parameters established by the Brazilian legislation and are able to supply the Recommended Daily Intake of vitamin C for adults. The validation of the high-performance liquid chromatography method with ultraviolet detection for ascorbic acid analysis confirmed its performance.

The orange juice obtained from the BRS 'Russas 02' variety has a higher Ratio, proving a higher degree of sweetness; moreover, it keeps a higher vitamin C content during storage and presents a higher number of major volatile compounds, compared to the BRS 'Russas 01' variety. Thus, among the studied varieties, the BRS 'Russas 02' variety presents a higher potential for the production of whole fruit juice.

For future approaches, it is suggested to evaluate the microbiological quality of juices, observing the compliance with the current legislation and the hygienic-sanitary quality of the processing, and identify quantitatively the volatile compounds present in each variety. Other proposals would analyze the nutritional and sensorial profile of the juices in order to identify the nutritional quality and the consumer's behavior towards the product, and to verify the potential of commercialization of a derivative of the orange 'de Russas' in the market and extract the essential oil from 'de Russas' orange peel, as a way to use the residues.

For a more comprehensive study on the possible use of this orange cultivar, it is recommended that the parameters are assessed for a longer storage period, besides the consumption *in natura* (proposal of this study) and that the refrigeration technique may be compared with other non-conventional conservation methods.

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