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Antioxidant activity and physico-chemical analysis of *Campomanesia rufa* (O.Berg) Nied. fruits

Atividade antioxidante e análise físico-química de frutos de *Campomanesia rufa* (O.Berg) Nied.

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ABSTRACT

Campomanesia rufa (O. Berg) Nied. is a native Cerrado species that presents great edible potential. However, it is a species "in danger of extinction" as recommended by the International Union for the Conservation of Nature (IUCN). No technical and scientific information about the species exists, thus demonstrating the importance of its research. The present work aimed at the physical and chemical characterization of immature and mature *C. rufa* fruits. The fruits showed a change in coloration from green (b * = 25.11, h = 122.43) to yellowish-green (b * = 34.26 , h = 115.73), an increase in mass (6.54 g to 10.88 g), diameter (23.76 mm to 28.03 mm) and soluble solids (8.00 to 10.80%). The fruits presented high levels of total (1246.35 mg 100 g^{-1}) and soluble pectin (195.93 mg 100 g^{-1}), high water content (78.86 g 100 g^{-1}), low pH value (3.40), and high citric acid content (1.2%). However, the fruits had low protein (0.81 g 100 g^{-1}), lipid contents, and low caloric values (64.76 kcal 100 g^{-1}). The fruits presented significant values of carotenoids, phenolic compounds (312.47 mg 100 g^{-1}), vitamin C (263.60 mg 100 g^{-1}) as well as good *in vitro* antioxidant activity (1862.81 µM g^{-1}). The results obtained indicate that *C. rufa* fruits showed a similar composition to the fruits of other *Campomanesia* species, and their biological properties should be investigated additionally under *in vivo* conditions.

Index terms: Cerrado; Myrtaceae; casaqueira; endangered species; vitamin C.

RESUMO

Campomanesia rufa (O. Berg) Nied. é uma espécie nativa do Cerrado que apresenta grande potencial comestível. No entanto, é uma espécie "em perigo de extinção", segundo a União Internacional para a Conservação da Natureza (IUCN). Não existe informação técnica e científica sobre a espécie, demonstrando a importância das pesquisas. O presente trabalho teve como objetivo a caracterização físico-química de frutos imaturos e maduros de *C. rufa*. Os frutos apresentaram alteração na coloração de verde (b * = 25.11, h = 122.43) para verde amarelado (b * = 34.26, h = 115.73), aumento na massa (6.54 g para 10.88 g), diâmetro (23.76 mm a 28.03 mm) e sólidos solúveis (8.00 a 10.80%). Os frutos apresentaram altos teores de pectina total (1246.35 mg 100 g^{-1}) e solúvel (195.93 mg 100 g^{-1}), alto teor de água (78.86 g 100 g^{-1}), baixo valor de pH (3.40) e alto ácido cítrico conteúdo (1.2%). No entanto, os frutos apresentaram baixo teor de proteína (0.81 g 100 g^{-1}), teor de lipídios e baixo valor calórico (64.76 kcal 100 g^{-1}). Os frutos apresentaram valores significativos de carotenóides, compostos fenólicos (312.47 mg 100 g^{-1}), vitamina C (263.60 mg 100 g^{-1}) e boa atividade antioxidante *in vitro* (1862.81 µM g^{-1}). Os resultados obtidos indicam que os frutos de *C. rufa* apresentam composição semelhante aos frutos de outras espécies de Campomanesia, e suas propriedades biológicas devem ser investigadas adicionalmente em condições *in vivo*.

Termos para indexação: Cerrado; Myrtaceae; casaqueira; espécie ameaçada de extinção; vitamina C.

INTRODUCTION

The Cerrado biome occupies 22% of the Brazilian territory and houses a vast and diverse genetic heritage that is unique in the world (Reis; Schmiele, 2019). The Cerrado holds 40% of the endemic plant species and one-third of all Brazilian biodiversity (Luz et al., 2019). Many of the native Cerrado species produce fruits with commercial potential for natural consumption and the production of derivatives such as juices, jellies, and liquors (Neves et al., 2015). In addition, they can also be employed to obtain secondary metabolites such as phenolic compounds, antioxidants,

and antiproliferative agents of human carcinogenic cells (Araújo et al., 2018).

However, there is a large deficit of scientific studies on the native Cerrado species (Arruda; Pastore, 2019). This reduces the possibilities of developing new products and expanding the use of species already consumed by the local populations (Carvalho et al., 2019). For this reason, scientific studies were focused on properties and the uses of native plants (Arruda; Pastore, 2019).

The Myrtaceae family is second in terms of diversity in the phytogeographical domain of the Cerrado (Silvestre; Miranda; De-Carvalho, 2019). The *Campomanesia* genus belongs to the Myrtaceae family and possesses food, ornamental, and pharmaceutical potential (Zuninga et al., 2018; Carvalho et al., 2019). It has a limited distribution in the phytogeographical domains of the Atlantic and Cerrado Forest in Minas Gerais state, Brazil (Reflora, 2020).

Chemical studies with *Campomanesia* genera such as *Campomanesia adamantium*, *Campomanesia pubescens, Campomanesia cambessedeana, Campomanesia corimbosa, Campomanesia aurea*, *Campomanesia xanthocarpa, Campomanesia* guazumifolia, *Campomanesia* reitziana, and *Campomanesia lineatifolia* showed high content of phenolic compounds, antioxidants, and vitamin C (Sá et al., 2018; Cardozo et al., 2018; De Andrade Silva; Fonseca, 2016; Da Silva et al., 2016; Emer et al., 2018; Salmazzo et al., 2019; De Souza Duarte et al., 2020).

Campomanesia rufa is used by the local population. However, studies on *C. rufa* are scarce (Sant'Ana et al., 2018). It is classified as being 'vulnerable' by the Red List maintained by the International Union for Conservation of Nature – IUCN (1998), thus demonstrating the importance of exploratory studies with the species. Such studies will enable us to understand how these fruits can be utilized commercially in a better manner (Araújo et al., 2018).

Given the above arguments, this work aimed to characterize the physical and chemical properties of *C. rufa* during the immature and mature stages of development.

MATERIAL AND METHODS

Plant material

Immature and mature fruits of *C. rufa* (Figure 1) were collected from a natural population located at 21° 13'35.5" S latitude and 44° 59'00.7" W longitude in the region of Lavras, Minas Gerais state, Brazil. The climate in this region is characterized as a rainy season with dry winters and rainy summers with an average annual temperature of 19 ºC and an average annual rainfall of 1,530 mm.

Figure 1: Fruits of *Campomanesia rufa*.
Representation of immature fruits (A, B, and C) and mature fruits (D, E, and F). Bar = 2 cm.

The identification of the species was carried out by cross-checking with the plants maintaned in the ESAL Herbarium of the Federal University of Lavras with the collection number ESAL21198. Seeds with incomplete embryo development were considered immature fruits, and those with complete embryo development were considered mature fruits. For the analysis, both stages of fruits were used.

Diameter, mass, and pulp yield

The mean longitudinal and transverse diameters of the fruits were determined using a digital caliper. The average mass of the fruits was determined by the individual weighing of the fruits on a semianalytic scale.

To calculate the pulp yield, the initial weight of the ripe fruit, and the weight of the pulp was measured. The calculation was performed using the following equation: pulp weight/fruit weight * 100.

Color and firmness

The staining was determined using a Konica Minolta CR-400 colorimeter calibrated according to the CIE system with L*, a*, b*, h, and C* (Illuminant D65). The L* coordinate represented the brightness with values between 0 (totally black) and 100 (totally white). The coordinate a* assumed values between –80 to +100 in which the ends corresponded to green and red, respectively. The b* coordinate ranged from –50 to +70 with blue to yellow intensity. The hue angle (h) corresponded to the hue and identified the color between 0 ° and 360 ° and the Chroma (C*) was saturation or intensity of the color as outlined by McGuire (1992).

The firmness was determined in a Stable Micro System model TATX2i texturometer using the P/6N probe (2 mm in diameter). The penetration force of the fruits was measured at a speed of 5 mm/s and with a penetration distance of 30 mm with values previously determined. An HDP/90 platform was used as the base. The firmness of the fruits was expressed in Newton (N).

Titratable Acidity (AT), pH, Soluble Solids (SS), and SS/AT ratio

The determination of titratable acidity (AT) was performed by titration with a 0.01 M sodium hydroxide solution (NaOH) using phenolphthalein as an indicator according to methods from the (Adolfo Lutz Institute - IAL, 2005). The results were expressed as percent citric acid. The pH of the pulp was determined using a TEC-3 MP Tecnal® pH meter according to the technique of the Adolfo Lutz Institute (IAL, 2005). The soluble solids content (SS) was determined using a digital refractometer, and the results were expressed in percentage. The soluble solids/titratable acidity ratio (SS/AT ratio) was determined by dividing the first variable by the second variable (IAL, 2005).

Proximate analysis

Proximate analysis (g 100 g^{-1}) was performed according to the methods proposed by (Association of Official Analytical Chemistry - AOAC, 2012). The moisture content was determined by oven drying at 65 °C until a consistent weight was reached. The lipid content was determined in a Soxhlet apparatus. The protein content was determined by the Kjeldahl method considering a conversion factor of 6.25. The crude fiber was determined by the gravimetric method. Ash determination was performed by the gravimetric method of incineration in a muffle furnace at 550 ºC. Nitrogen-free extract (NFE) was determined from the formula: NFE = 100 - (moisture + lipids + proteins + fiber + ash). The results of the proximate analyses were expressed in g 100 g^{-1}. The caloric value was estimated using Atwater's conversion values: 9 kcal per g of lipids, 4 kcal per g of protein, and 4 kcal per g of carbohydrates (De Angelis, 1977).

Total and Soluble Pectin

The extraction of pectic substances was performed according to the technique described by McCready and McComb (1952). Here, 5 g of fresh fruit was homogenized with 45 mL of 70% ethyl alcohol and the homogenate was left to stand for 18 h to remove the total sugars. Subsequently, the homogenate was filtered and washed thrice with 30 mL of 70% ethyl alcohol. The filtrate was used to determine the total sugars, and the alcohol-insoluble residue (AIR) was homogenized with 50 mL of distilled water for 1 h and again filtered to determine the soluble pectin.

For the determination of total pectin, 50 mL of versene solution (0.5% EDTA) was added to the homogenate (after the removal of sugars), and the pH was increased to 11.5 with NaOH solution. The mixture was allowed to stand for 30 min, and the pH was reduced to 5.5 using acetic acid. Further, 0.1 g of pectinase was added and stirred for 1 h. The mixture was filtered, and a total volume of 100 mL was obtained with versene solution.

For the determination of soluble pectin (after the extraction of sugars), the filtered residue (AIR) was placed in Erlenmeyer flasks, and 50 mL of distilled water was added. It was then homogenized on a shaker for 1 h and filtered with a quantitative filter paper.

The colorimetric quantification was performed by the technique of Bitter and Muir (1962). The results were expressed in mg of galacturonic acid 100 g^{-1} of pulp.

Total Sugars

The filtrate obtained from pectin extraction (AIR) was heated for evaporation of the alcohol until the volume was reduced to 10 mL. Distilled water was added to make a final volume of 50 mL. This solution was used for the quantification of sugars by the method of Somogyi adapted by Nelson (1944), and results were expressed in g of glucose 100 g^{-1} of pulp.

Vitamin C

For vitamin C determination, 2 g of the sample was homogenized in 20 mL of 0.5% oxalic acid using the Polytron crusher. Subsequently, the homogenate was transferred to the stirring table for 30 min and then filtered (qualitative filter paper, 15 cm in diameter, Unifil®). The extract obtained was used to determine vitamin C. The ascorbic acid content (after oxidation to dehydroascorbic acid) was determined by the colorimetric method using 2,4 dinitrophenylhydrazine according to the method of Strohecker and Henning (1967). The results were expressed as mg of ascorbic acid 100 g^{-1} of pulp.

Carotenoids

A combination of solvents was used for the extraction of carotenoids: acetone/petroleum ether. The analysis consisted of sampling and sample preparation, extraction and partition with a solvent, saponification and washing, solvent concentration, chromatographic separation, and identification and quantification with a spectrophotometer. The extraction of carotenoids was performed according to Rodriguez-Amaya (2001). The sample (5 g) was kept in a flask in the dark, and 20 mL of cold acetone (P.A.) was added. The contents were stirred for 20 min and filtered in an Erlenmeyer Flask with the aid of a filter paper. The sample was then washed with acetone until the residue left on the filter paper turned transparent (washed thrice with 20 mL, 5 mL, and 15 mL of acetone (P.A.)). The filtrate was transferred to a separating funnel, and the funnel was covered with aluminum foil. Next, 30 mL of petroleum ether and 70 mL of distilled water were added to it. The denser liquid was discarded. This procedure was repeated thrice to remove acetone.

The extract was transferred to a 100 mL volumetric flask, and petroleum ether was added to make the final volume to 100 mL. The contents were filtered again and stored in a dark bottle until their absorbance was recorded. The "white" to reset the equipment was P.A.

Carotenoids were quantified using a spectrophotometric method (Rodriguez-Amaya, 2001). Carotenoids like α-carotene, β-carotene, δ-carotene, γ-carotene, and lycopene were measured at 444 nm, 450 nm, 456 nm, 462 nm, and 470 nm, respectively. The content of each carotenoid was calculated according to the formula: $\mu g\ g^{-1} = A \times V \times 106/A1\ cm\ -1\% \times M \times 100$. A was the absorbance of the solution at a specific wavelength, V was the final solution volume, A1 cm –1% was the coefficient of molar absorptivity of the pigment in a given specific solvent (petroleum ether), and M was the mass of the sample taken for analysis in g. Results were expressed in µg 100 g^{-1} fruit.

Total phenolic content and antioxidant activity

Extraction

The extraction was performed according to the methodology adopted by Rufino et al. (2010). For this, 1 g of the sample was weighed in a 50 mL beaker, and 10 mL of 50% methanol was added to it; the solution was homogenized and left to stand for 20 min in the dark. Later, the solution was incubated in an ultrasound bath for 15 min and filtered (paper qualitative filter, 15 cm in diameter, Unifil®); the supernatant was transferred to a dark flask. To the residue of the first extraction, 10 mL of 70% acetone was added. The solution was homogenized and left to stand for 20 min in the dark. Subsequently, it was incubated in an ultrasound bath for 15 min and then filtered (qualitative filter paper, 15 cm of diameter, Unifil®). The supernatant was transferred to the dark flask containing the first supernatant, where they were homogenized again. The obtained extract was used to determine the total phenolic content and the antioxidant capacity by the ABTS and FRAP methods.

Total phenolic content

The total phenolic content was determined by the Folin-Ciocalteu method (Waterhouse, 2002). The blue color produced by the reduction of the Folin-Ciocalteu reagent by the phenols was measured spectrophotometrically at the absorption wavelength of 750 nm. The phenolic content was calculated by the equation of the straight line obtained from the standard curve of gallic acid. The results were expressed in milligrams of gallic acid equivalent (GAE) 100 g^{-1} pulp.

Antioxidant activity

For the determination of the antioxidant activity, the ABTS radical capture method and the iron reduction method (FRAP) were used.

The antioxidant activity by the ABTS °+ radical capture method was performed according to Rufino et al. (2007). The ABTS °+ radical capture solution was prepared by mixing a 5 mL stock solution of 7.0 mM ABTS with 88 mL of 140 mM potassium persulfate solution. The mixture was kept in the dark at room temperature for 16 h. Then, 1 mL of this mixture was diluted in ethyl alcohol until an absorbance of 0.70 nm ±0.05 nm was obtained at 734 nm.

The absorbance (734 nm) of the samples was determined at room temperature after 6 min of reaction time. Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the reference antioxidant. The results were expressed in μM of Trolox g^{-1}.

The total antioxidant activity by the FRAP method was performed according to Rufino et al. (2006). The FRAP reagent was obtained from the combination of 25 mL of 0.3 M acetate buffer, 2.5 mL of a 10 mM TPTZ solution, and 2.5 mL of a 20 mM aqueous solution of ferric chloride.

The absorbance (595 nm) of the samples was determined after 30 min of reaction time in a water bath at 37 °C. Ferrous sulfate was used as a reference. The results were expressed as μM ferrous sulfate g^{-1}.

Statistical analysis

For the analyses of longitudinal and transverse diameter, fresh mass, pulp yield, and staining and firmness, 30 replications per stage of maturation were used. For other analyses, ten replicates per stage of maturation were used. The design was completely randomized, the data were analyzed using the R software v. 3.5.2 (R Core Team, 2019), and the means were compared by the Student's t-test considering $p < 0.05$ (Ferreira; Cavalcanti; Nogueira, 2014) as significant.

RESULTS AND DISCUSSION

The fruits of *C. rufa* presented increased longitudinal and transverse diameters after ripening, and this was accompanied by an increase in their mass (Table 1). The pulp yield in the mature fruits was 51.35%.

It was possible to identify the color development associated with maturation (Table 1). In the mature fruits of *Campomanesia cambesedeana,* the coloration was darker (L* = 26.09) while *Campomanesia pubescens* changed from a darker (L* = 4.65) to a lighter color (L* = 36.80) during development (Morzelle et al., 2015; Silva et al., 2009). No representative changes were observed in the a* coordinate between the immature and mature fruits, and the fruits remained in the green region. However, the b* coordinate showed an increased intensity in the yellow region (Table 1, Figure 1).

The values of °h (hue angle) indicated that the coloration of the fruits was between pure yellow and pure green. The color changed from green to yellowish-green with maturation, thus demonstrating an increased chromaticity with maturation (Table 1).

Table 1: Longitudinal and transverse diameters, fresh mass, L*, a*, b*, h, C*, firmness, total and soluble pectin in the immature and mature fresh *C. rufa* fruits.

	Immature fruit	Mature fruit	p-value
Longitudinal diameter (mm)	23.76	28.03	5.08×10^{-10} *
Transverse diameter (mm)	24.39	27.14	2.36×10^{-07}*
Fresh mass (g)	6.54	10.88	1.02×10^{-14}*
L*	42.94	49.14	6.31×10^{-05}*
a*	–15.73	–16.06	0.58
b*	25.11	34.26	8.59×10^{-09}*
h	122.43	115.73	4.22×10^{-11}*
C*	29.39	37.90	1.08×10^{-07}*
Firmness (N)	12.65 a	1.27	3.34×10^{-12}*
Total pectin (mg 100 g^{-1})	860.05	1246.35	1.11×10^{-04}*
Soluble pectin (mg 100 g^{-1})	93.79	195.93	4.67×10^{-05}*

* Means differ significantly from each other, as determined by the Student t-test with a 5% probability.

C. pubescens fruits also showed increased yellowing during maturation that was different from the reddish color of mature *C. cambessedeana* fruits. For *C. phaea* fruits, the green color prevailed with decreased gloss and increased opacity with ripening (Silva et al., 2009; Morzelle et al., 2015; Bianchini et al., 2016).

The firmness values of the *C. rufa* fruits decreased during ripening; the values were 12.65 N and 1.27 N in the immature and mature fruits, respectively (Table 1, Figure 1). Firmness value is an important attribute of fruits for the industry chain because it directly affects the fruit quality, consumer preference, transportability, and shelf life. Moreover, it also affects the ability of the cultivars to be machine harvested and in reducing the financial and labor costs (Li et al., 2018; Cappai et al., 2018).

Pectic substances are the main components responsible for the change in the texture of fruits and vegetables (Huang et al., 2019). The immature and mature fruits showed a high total pectin content (860.05 and 1246.35 mg 100 g^{-1}, respectively) (Table 1) compared to the mature fruits of *C. cambessedeana* (258.54 mg 100 g^{-1}) and *Byrsonima crassifolia* (746.81 mg 100 g^{-1}) (Morzelle et al., 2015). Similarly, the soluble pectin content in the mature fruits of *C. rufa* was 195.93 mg 100 g^{-1}, whereas it was 131.15 mg 100 g^{-1} in *C. cambessedeana* and 72.18 mg 100 g^{-1} in *B. crassifolia* mature fruits (Morzelle et al., 2015). Pectin gets solubilized with ripening and makes the fruit softer. This explains the increase in the soluble pectin concentration in the mature fruit of *C. rufa* compared to that in the immature fruits (Morzelle et al., 2015) (Table 1).

The pH values decreased from 3.60 to 3.40 with the maturation of *C. rufa* fruits (Table 2). The mature fruits had higher levels of acidity than those of *C. cambessedeana* (4.25) and similar acidity values to that of the *C. lineatifolia* (3.47) fruits (Table 2) (Morzelle et al., 2015; Lima et al., 2016). The *C. phaea* fruits displayed greater acidity (2.80) (Sanches et al., 2016). Thus, there existed a wide variation in the pH values of the *Campomanesia* species. It is influenced by environmental factors and maturation stages (Goldoni et al., 2019).

In white-fleshed pitaya (*Hylocereus undatus*), a linear pH increase occurred that reached values between 3.06 and 4.5 (Magalhães et al., 2019) with maturation. According to Vallilo et al. (2005), fruits with high acidity are used in the industry to produce sweets. Fruit acidity is due to the presence of organic acids, such as malic and citric acids present in most mature fruits (Batista-Silva et al., 2018).

Despite the variation in the pH between the two stages, no difference was observed in the titratable acidity (1.22 and 1.21% citric acid in immature and mature fruits, respectively) (Table 2). These values were lower compared to the 3.0% value of *C. phaea* but were close to that of *C. pubescens* (approximately 1.5%) and higher than that of *C. cambessedeana* (0.19%) (Valillo et al., 2005; Silva et al., 2009; Morzelle et al., 2015).

Table 2: Mean values of pH, titratable acidity (AT) expressed as citric acid, soluble solids (SS), SS/AT ratio, proximate composition, total sugars, and calorific value of fresh *C. rufa* fruits at two maturation stages.

	Immature fruit	Mature fruit	p-value
pH	3.60	3.40	9.44×10^{-05}*
AT (%)	1.22	1.21	0.69
SS (%)	8.00	10.80	1.41×10^{-06}*
SS/AT	6.58	8.98	1.36×10^{-06}*
Moisture (g 100 g^{-1})	79.92	78.86	0.45
Ethereal extract (g 100 g^{-1}) †	0.18	0.18	0.95
Protein (g 100 g^{-1})	0.72	0.81	0.47
Fiber (g 100 g^{-1})	4.28	4.56	0.32
Mineral residue (g 100 g^{-1})	0.52	0.61	0.03107
NFE (g 100 g^{-1})	14.38	14.98	0.57
Total sugars (g 100 g^{-1})	1.22	6.88	2.20×10^{-16}*
Calorific value (kcal 100 g^{-1})	62.01	64.76	0.54

* Means differ significantly from each other, as determined by the Student's t-test with a 5% probability. † Dry weight.

The content of soluble solids increased as the green fruits ripened into the mature fruits (from 8.0 to 10.8%, respectively) (Table 2). These values were lower than those found in the accessions of *C. phaea* (12.50 to 13.30%) and *C. lineatifolia* (11.55%) (Bianchini et al., 2016; Lima et al., 2016).

The SS/AT ratio is one of the best ways to evaluate sugar content (Teerachaichayut; Ho, 2017). The SS/AT ratio increased in the mature fruits as compared with the green fruits (Table 2). SS increased gradually with the development of the fruit, whereas AT decreased (Sanches et al., 2016).

The high moisture value seen in *C. rufa* was closer to that observed in *C. cambessedeana* (77.02 g 100 g^{-1}) and *C. pubescens* (81.4 g 100 g^{-1}) and lower than that in *C. phaea* (88.80 g 100 g^{-1}) (Sanches et al., 2016) (Table 2). The *C. rufa* stages did not show differences in their moisture content (Table 2). Thus, in general, the fruits of the *Campomanesia* genera displayed a high moisture value (De Paulo et al., 2020). Factors such as plant age, water availability, fertilization, and climatic conditions may influence the moisture content (Sanches et al., 2016).

Considering the levels of ethereal extract and protein, the values obtained for *C. rufa* were lower than 1.0% and did not differ between the immature and mature fruits (Table 2). *C. phaea* presented low protein content (0.44%) and higher lipid content (1.53%). *C. xanthocarpa* presented higher levels of protein (1.1%) and lipids (1.9%) similar to the values of *C. cambessedeana* for proteins (1.43%) and lipids (1.32%) (Vallilo et al., 2005; Vallilo et al., 2008; Morzelle et al., 2015).

The crude fiber content in *C. rufa* did not differ between the immature and mature stages (Table 2). The values were close to those found in *C. phaea* (4.00 g 100 g^{-1}) and lower than the *C. xanthocarpa* values (6.3 g 100 g^{-1}) (Vallilo et al., 2005; Vallilo et al., 2008). According to the Collegiate Board Resolution (RDC) No. 54/12 of the National Health Surveillance Agency (ANVISA), a good source of crude fiber must contain at least 1.5 g 100 mL^{-1} of fiber (Brasil, 2012).

The mineral residue content did not differ between the immature and the mature fruit (Table 2) and presented similar values in *C. cambessedeana* (0.41 g 100 g^{-1}) and *C. pubescens* (0.5 g 100 g^{-1}) (Morzelle et al., 2015; Silva et al., 2009). Fruits are important sources of essential elements and minerals consumed in the human diet (Marles, 2017).

The nitrogen-free extract (NFE) differed approximately by 15 g 100 g^{-1} between the immature and mature fruits of *C. rufa* (Table 2). In *C. cambessedeana*, the carbohydrate value was 15.68 g 100 g^{-1} whereas it was 8.9 g 100 g^{-1} in *C. xanthocarpa* (Morzelle et al., 2015; Vallilo et al., 2008). The NFE, represented by glycols like sugars and starch, can include soluble fibers in the case of fruits. Since the sugar content in the *C. rufa* fruits ranged from 1.22 to 6.88, it was suggested that these fruits also contained soluble fiber that was not determined as crude fiber. Therefore, the presence of starch could not be ruled out.

The caloric value of *C. rufa* (Table 2) was higher than that of *C. xanthocarpa* (57.3 kcal 100 g^{-1}) in the mature fruits (Vallilo et al., 2008). The low caloric value of *C. xanthocarpa* was due to the high moisture content and consequent decrease in the concentration of sugars, lipids, and proteins (Vallilo et al., 2008); this applies to the *C. rufa* fruits as well.

There was a decrease in the content of α and β-carotenes and an increase in γ-carotene content with the ripening of the housekeeper fruits. However, no difference was detected for δ-carotene and lycopene between the immature and mature fruits (Table 3). α-carotene was most abundant in the green fruits, and β-carotene was most abundant in the mature fruits (Table 3). β-carotene deserves attention because it has a higher pro-vitamin A activity (Reksamunandar et al., 2017).

In *Psidium guajava* var. Paluma, the contents of β-carotene (366.3 µg 100 g^{-1}), and lycopene (6999.3 µg 100 g^{-1}) were higher than those in the *C. rufa* fruits (Table 3). *Mangifera indica* L. var. Tommy Atkins presented higher β-carotene content (1557.1 µg 100 g^{-1}) and lower lycopene content (77.2 µg 100 g^{-1}) (Oliveira et al., 2011) than that of the *C. rufa* fruits (Table 3). Carotenoids have antioxidant properties with functional potential for the prevention of non-transmissible chronic diseases such as cancer and cardiovascular diseases. Lycopene is one of the most studied carotenoids in this sense (Müller et al., 2016). However, the fruits of *C. rufa* generally do not stand out as sources of carotenoids compared to the fruits known to be rich in carotenoids.

The total phenolic value of *C. rufa* was higher than that of the *C. lineatifolia* fruits (229.37 mg 100 g^{-1}) (Lima et al., 2016) (Table 3). The phenolic content was comparable to that of fruits traditionally considered rich in these compounds, such as strawberry and grape. Considering seven varieties of strawberry and grape, the phenolic content ranged from 205 to 318 mg 100 g^{-1} and 65 to 391 mg 100 g^{-1} (Pinto et al., 2008) (Abe et al., 2007), respectively. Phenolic compounds are mainly

responsible for the color, smell, and protection of fruits and flowers (Martins; Barros; Ferreira, 2016). Moreover, the role of phenolic compounds lies in the stabilization and oxidation of lipids. Thus, they are directly related to antioxidant activity (Araújo; Souza, 2018). Despite each class of phenolic compounds being mainly responsible for specific bioactivity, they commonly evidence polyvalent reactions (Martins; Barros; Ferreira, 2016).

The ascorbic acid value of *C. rufa* exceeded that of *C. phaea* (33 mg 100 g^{-1}) and *C. lineatifolia* (74.44 mg 100 g^{-1}) but was lesser than that of *C. cambessedeana* (383.33 mg 100 g^{-1}) (Vallilo et al., 2005; Lima et al., 2016; Morzelle et al., 2015). Ramful et al. (2011) classified the fruits into three categories according to their ascorbic acid content: low (<30 mg 100 g^{-1}), medium (30–50 mg 100 g^{-1}), and high (>50 mg 100 g^{-1}).

The vitamin C content of *C. rufa* was higher than that of several traditional fruits such as *Citrus aurantium* L. (125.76 mg 100 g^{-1}) (Silva Júnior et al., 2010) and seven cultivars of strawberries that ranged from 65.0 to 112.0 mg 100 g^{-1} (Table 3) (Da Silva Pinto; Laojolo; Genovese, 2008). However, the vitamin C content was similar to that of the *Hancornia speciose* fruit (260 mg 100 g^{-1}) (Silva et al., 2017).

The values of the antioxidant activity detected by the ABTS radical capture method were higher than those found in the natural fruits of *Malpighia emarginata* (1336.42 μM g^{-1}), *Anacardium occidentale* (254.34 μM g^{-1}), and *Psidium guajava* (130.77 μM g^{-1}) (Freire et al., 2013). These values also surpassed those found in the epicarp (543.18 μM g^{-1}) and in the mesocarp (1,230.00 μM g^{-1}) of *Caryocar brasiliense* (Morais et al., 2013).

The antioxidant activity did not differ between the green and the mature fruits (Table 3) when detected by the iron reduction method (FRAP). The values found were lower than those found in the mesocarp (2085.70 μM g^{-1}) of *C. brasiliense* and higher than those in the epicarp (14.99 μM g^{-1}) of *C. brasiliense* and the pulp of *Byrsonima verbascifolia* (148.42 μM g^{-1}) (Morais et al., 2013).

The *C. rufa* fruit can be considered an important source of bioactive compounds since it presents high phenolic compounds, ascorbic acid levels, and antioxidant capacity (De Paulo et al., 2020). The consumption of high antioxidant foods is necessary to prevent damage caused by free radicals as these antioxidants help in neutralizing the free radicals (Alkadi, 2020). However, the overload of free radicals over time may become irreversible and lead to certain diseases (Schiassi et al., 2018).

Table 3: Mean values of carotenoids, total phenolics, vitamin C (ascorbic acid), and antioxidants, as determined by the method of ABTS ° + radical capture expressed in Trolox and by the iron reduction method (FRAP) expressed in ferrous sulfate of fresh weight in *C. rufa* fruits.

	Immature fruit	Mature fruit	p-value
α- carotene (μg 100 g^{-1})	279.84a	240.32b	2.31×10^{-15} *
β- carotene (μg 100 g^{-1})	257.74a	247.94b	8.44×10^{-08} *
δ- carotene (μg 100 g^{-1})	139.40a	143.89a	0.1267
γ- carotene (μg 100 g^{-1})	142.50b	152.81a	9.73×10^{-11}*
Lycopene (μg 100 g^{-1})	136.38a	128.04a	9.28×10^{-11}*
Total polyphenols (mg 100 g^{-1})	311.40a	312.47a	0.9305
Vitamin C (mg 100 g^{-1})	279.08a	263.60a	0.5606
ABTS (μM trolox g^{-1})	1985.54a	1862.81a	0.07427
FRAP (μM ferrous sulfate g^{-1})	1397.40a	1443.47a	0.141

* Means differ significantly from each other, as determined by the Student's t-test with a 5% probability.

CONCLUSIONS

C. rufa fruits are succulent, and their maturation is marked by a color change from green to yellowish-green and an increase in pectin solubilization and soluble solids content. The fruits have a low caloric value, high levels of crude fiber, vitamin C, polyphenols, and high antioxidant activity under *in vitro* conditions. Moreover, the presence of high pectin levels and low pH indicates a potential for industrial use in the manufacturing of gels and jellies. These results suggest that *C. rufa* fruits with good nutritional value have the potential for additional studies on bioactive compounds such as polyphenols, and their biological properties need to be investigated under *in vivo* conditions.

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