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ANTIOXIDANT ACTIVITY AND PHYSICOCHEMICAL CHARACTERISTICS OF LULO FRUIT CULTIVATED IN BRAZIL

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ABSTRACT - Underused crops have been expanding in the market due to their nutritional and antioxidant characteristics. Lulo, or naranjilla, can be considered an underused crop because it is grown on a small scale in Colombia and has high nutritional value. The edaphoclimatic conditions of cultivation of this fruit can influence its post-harvest characteristics. Thus, the objective of the work was to evaluate the antioxidant activity and the physicochemical characteristics of lulo fruit obtained from cultivation in Brazil. The post-harvest variables analyzed were antioxidant activity by ABTS, DPPH, FRAP methods, phenolic compounds, flavonoids, lycopene, β-carotene, ascorbic acid, and physicochemical characteristics of acidity, soluble solids, and ratio. Twenty replicates were used for each variable, including three replicates for antioxidant analyses and two replicates for carotenoids; each replicate consisted of one fruit. The mean and standard deviation were calculated. The FRAP antioxidant activity method showed 81.40 mg Ferrous Sulfate g^{-1} (dry mass), ABTS 57.00 mg Trolox g^{-1} (dry mass) and DPPH 14.40 mg Trolox g^{-1} (dry mass). The flavonoids contents were 1.00 mg Quercetin g^{-1} (dry mass), which is considered low. Lulo fruit showed to be a good source of β-carotene (7.00 mg 100 g^{-1}), lycopene (3.57 mg 100 g^{-1}) and ascorbic acid (57.71 mg 100 g^{-1}). The fruit is acidic (2.29 g Citric Acid 100 mL^{-1}), with low soluble solids content (5.12%) and low ratio (2.25).
Keywords: *Solanum quitoense* var. *septentrionale*, naranjilla, post-harvest.

ATIVIDADE ANTIOXIDANTE E CARACTERÍSTICAS FÍSICO-QUÍMICAS DE FRUTOS DE LULO CULTIVADOS NO BRASIL

RESUMO - As culturas subutilizadas têm conquistado o mercado devido suas características nutricionais e antioxidantes. O lulo pode ser considerado uma cultura subutilizada, pois é cultivado em pequena escala na Colômbia e possui elevado valor nutricional. As condições edafoclimáticas de cultivo desta frutífera podem influenciar suas características pós-colheita. Assim, o objetivo do trabalho foi avaliar a atividade antioxidante e as características físico-químicas de frutos de lulo obtidos do cultivo no Brasil. As variáveis pós-colheita analisadas foram atividade antioxidante pelos métodos ABTS, DPPH, FRAP, compostos fenólicos, flavonoides, licopeno, β- caroteno, ácido ascórbico, e características físico-químicas de acidez, sólidos solúveis e ratio. Foram utilizadas vinte repetições para cada variável, incluindo três replicatas para análises antioxidantes e duas replicatas para carotenoides; cada repetição consistiu em um fruto. A média e o desvio padrão foram calculados. As análises foram realizadas no mês de novembro de 2020. O método FRAP apresentou a maior atividade antioxidante (81,40 mg de sulfato ferroso g^{-1}, massa seca), seguido pelo ABTS (57,00 mg Trolox g^{-1}, massa seca), fenólicos (24,55 mg de ácido gálico g^{-1}, massa seca) e DPPH (14,40 mg Trolox g^{-1}, massa seca). A atividade dos flavonoides foi considerada baixa (1,00 mg de quercetina g^{-1}, massa seca). Mostraram ser uma boa fonte de β-caroteno (7,00 mg 100 g^{-1}) e licopeno (3,57 mg 100 g^{-1}), além de possuírem fontes boas de ácido ascórbico (57,71 mg 100 g^{-1}). Os frutos são ácidos (2,29 g de ácido cítrico 100 mL^{-1}), com baixo teor de sólidos solúveis (5,12°Brix) e baixo índice de maturação (2,25 ratio).
Palavras-chave: *Solanum quitoense* var. *septentrionale*, Naranjilla, pós-colheita.

INTRODUCTION

The growing demand for nutraceutically beneficial foods is creating a market for underused crops. These crops are grown on small scales, recognized for their traditional uses in indigenous areas, and have high nutritional and antioxidant factors (CHANG et al., 2018). One of the underused crops is the fruit called lulo (*Solanum quitoense*); thus, knowledge of this fruit's composition and nutritional value is essential. Its perennial plant belongs to the *Solanaceae* family and is also known as naranjilla, native to South America, being widely consumed in Colombia (FLÓREZ-VELASCO et al., 2015). Its geographical distribution extends from Venezuela to Peru, cultivated at an altitude between 1000 and 1900 m above sea level (IGUAL et al., 2014).

In 2015, lulo production in Colombia reached 82,000 tons, occupying an area of approximately

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10,000 ha (MADR, 2015). Two geographical varieties of *Solanum quitoense* are known: var. *quitoense*, found in southern Colombia and Ecuador, which has no thorns; and var. *septentrionale*, which contains thorns, found in central Colombia, Panama, and Costa Rica (HEISER, 1972). Its fruit is spherical, with a yellow-orange skin at physiological maturity, covered with short hairs. The pulp is green, juicy, with acidic flavor and tiny seeds, consumed mainly in juices and jellies (HINESTROZA-CÓRDOBA et al., 2020).

Lulo has high nutritional value, containing minerals (such as phosphorus, calcium, and iron), vitamins (such as niacin, thiamin, riboflavin, and vitamins A and C), and antioxidants (GANCEL et al., 2008). Mertz et al. (2009) identified phenolic compounds as the main antioxidant contributors in lulo and also described that the primary carotenoid present in the fruit is β-carotene. The authors concluded that the antioxidant potential of lulo is higher than in most fruit.

The growth and development of this tropical fruit are dependent on environmental conditions and can cite factors such as temperature, altitude, and precipitation. Ramírez et al. (2018) cited that the differences in the growth of lulo fruit observed in different studies can be attributed mainly to temperature range; higher average temperatures accelerate the growth rate of the fruit, while lower average temperatures tend to cause increased time for growth and maturation.

Many studies present the chemical, physical, physicochemical (ANDRADE-CUVI et al., 2016; MATARAZZO et al., 2013) and antioxidant (CONTRERAS-CALDERÓN et al., 2011; MERTZ et al., 2009) characterization of lulo fruit, however, few studies of these compounds are found for the fruit cultivated in Brazil. Therefore, this work aimed to evaluate the antioxidant activity and the post-harvest physicochemical characteristics of lulo fruit obtained from cultivation in Brazil.

MATERIAL AND METHODS

Lulo samples were obtained from a commercial orchard in the municipality of Socorro, São Paulo State, Brazil (latitude 22º35'29" South, longitude 46º31'44" West, and altitude of 752 m) in September 2020. The region's climate is characterized as humid subtropical (ROLIM and APARECIDO, 2016).

Ripe fruit (totally orange), with homogeneous size, without defects, and healthy were selected. The fruit were washed with water and sanitized by immersion in 0.2 mL L^{-1} sodium hypochlorite solution at room temperature for one minute and dried in air. Afterward, they remained frozen (-18°C) until the moment of analysis. The samples had an average equatorial diameter of 4 cm and an average dry mass of 13.72%.

The ethanolic extracts were prepared for antioxidant activity (ABTS, DPPH, and FRAP), phenolic compounds, and flavonoids analyses. The fruit pulp samples were weighed and macerated with ethanol in a 1:10 (m/v) ratio. Subsequently, they were placed in an ultrasonic bath (UNIQUE, USC-2850A) for 15 min. and centrifuged at 20.000 *g* in a centrifuge (MPW 350-350R) at 4ºC for 20 min. After centrifugation, the extracts were filtered with qualitative filter paper and transferred to test tubes. The extracts were stored at -18°C until analysis.

For determining lycopene and β-carotene levels, samples of the fruit peel were weighed and macerated in acetone for analysis (NELLIS et al., 2017), in proportion of 0.5:10 (m/v). Subsequently, they followed the same steps described above of ultrasonic bath, centrifugation, and filtration.

The antioxidant activities were determined by the ABTS, DPPH, and FRAP methods. The ABTS method estimates the ability of the sample to scavenge the ABTS [2,2'-AZINO-BIS(3-ethylbenzothiazoline-6-sulfonic acid)] radical and was performed according to Rufino et al. (2007). In 30 µL of ethanolic extract, 3 mL of ABTS reagent was added (5 mL of ABTS stock solution 7 mM added 88 µL of potassium persulfate solution 140 mM, kept for 16 h in the dark and diluted in ethanol until absorbance of 0.700 ± 0.05 nm at 734 nm). After 6 min in a dark environment, readings were taken at 734 nm in a spectrophotometer (Shimadzu, UV-1800, Japan). The results were expressed in mg g^{-1} fresh and dry mass, in Trolox equivalent (T), by means of the calibration curve for Trolox at concentrations from 0.025 to 0.325 mg ($y = -0.6747x + 0.4654$, $R^2 = 0.99$).

The DPPH method measures the ability to scavenge the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and was performed according to Ancos et al. (2002). In 3 mL of ethanol, 0.5 mL of ethanolic extract and 0.3 mL of DPPH solution (0.5 mmol L^{-1}) were added, reacting for 60 min. in the dark. Later, the absorbance was measured in a spectrophotometer at 517 nm. The results were expressed in mg g^{-1} of fresh and dry mass, in Trolox equivalent (T), by means of a calibration curve for Trolox at concentrations 0.005 to 0.035 mg ($y = -0.0809x + 0.0417$, $R^2 = 0.99$).

The FRAP (Ferric Reducing Antioxidant Power) method determines the reduction of iron ions in a sample and is performed according to Rufino et al. (2006). In 90 µL of ethanolic extract, 270 µL of distilled water and 2.7 mL of the FRAP reagent (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 ferric chloride aqueous solution) were added. After 30 min in a dark environment at 37°C, readings were taken at 595 nm in a spectrophotometer. The results were expressed in mg g^{-1} fresh and dry mass, in Ferrous Sulfate (FS) equivalent, through the calibration curve for Ferrous Sulfate at concentrations 0.05 to 0.35 mg ($y = 0.676x - 0.18$, $R^2 = 0.99$).

Total phenolic compounds were determined according to Georgé et al. (2005). A 0.5 mL aliquot of ethanolic extract was added to 2.5 mL of Folin-Ciocalteu solution: water (1:10 v/v) and 2.0 mL of 7.5% (m/v) sodium carbonate solution. After 15 min at 50°C,

the absorbance was measured in a spectrophotometer at 760 nm. The results were expressed in mg g^{-1} fresh and dry mass, in gallic acid equivalent, through the calibration curve for gallic acid at concentrations from 0.005 to 0.08 mg mL^{-1} ($y = 0.186x - 0.0152$, $R^2 = 0.99$).

Total flavonoids were determined according to Chang et al. (2002). In 0.5 mL of the ethanolic extract, 4.3 mL of 80% ethanol in water (v/v), 0.1 mL of 10% (m/v) aluminum chloride, and 0.1 mL of 1 M potassium acetate were added. After 40 min. in the dark at room temperature, absorbance was measured at 415 nm. The results were expressed in mg g^{-1} fresh and dry mass, in Quercetin equivalent (Q), through the calibration curve for Quercetin at concentrations from 0.01 to 0.07 mg ($y = 0.2787x + 0.0018$, $R^2 = 0.99$).

The extracts obtained from the acetone extraction were measured in a spectrophotometer at 470 nm for lycopene determination and 450 nm for β-carotene (RODRIGUEZ-AMAYA, 2001; RODRIGUEZ-AMAYA and KIMURA, 2004). Thus, the carotenoids were determined according to Equation 1.

$$\text{Carotenoid levels (mg 100 g}^{-1}) = \frac{\text{A x V x 1,000,000}}{\text{A}\frac{1\%}{1\text{cm}} \text{ x M x 100}} \qquad \text{(Equation 1)}$$

Where:

A = absorbance of the solution at a wavelength of 470 nm for lycopene and 450 nm for β-carotene,

V = final volume of the solution,

$A\frac{1\%}{1\ cm}$ = molar extinction coefficient (3450 for lycopene and 2592 for β-carotene) and

M = fresh mass of the sample.

The ascorbic acid of the fruit was determined by titration with 2,6-dichlorophenol-indophenol, according to Benassi and Antunes (1998), with modifications. A 5 g sample was weighed and added to 50 mL of 2% oxalic acid solution. It was then titrated with the 0.01% 2,6-dichlorophenol-indophenol solution until persistent pink coloration. An ascorbic acid standard was used. The results were expressed in mg 100 g^{-1}.

Titratable acidity was obtained by titrating 5 g of fruit pulp diluted in 95 mL of water with a standardized 0.1 N sodium hydroxide solution, using 1% phenolphthalein as an indicator. The results were expressed in g of citric acid per 100 mL^{-1} (IAL, 2008).

The soluble solids content was determined by reading the pulp in a digital refractometer, with results expressed in %, and the ratio was obtained by the soluble solids quotient and titratable acidity.

The Shapiro-Wilk test (95% reliability) was applied using the SISVAR statistical system to verify the normality of the data (FERREIRA, 2014). Twenty replicates were used for each variable, including three replicates for antioxidant analyses (ABTS, DPPH, FRAP, phenolics, flavonoids) and two replicates for carotenoids; each replicate consisted of one fruit. The mean and standard deviation were calculated using Excel software.

RESULTS AND DISCUSSION

The ABTS, DPPH, FRAP, phenolic compounds, flavonoids, carotenoids (lycopene and β-carotene), and ascorbic acid methods were measured to estimate the antioxidant activity of the lulo fruit (Table 1). All studied variables showed average data using the Shapiro-Wilk test ($p<0.05$).

TABLE 1 - Antioxidant activity by the ABTS, DPPH, FRAP methods, phenolic compounds, flavonoids, carotenoids (lycopene and β-carotene), and ascorbic acid of lulo fruit.

Analysis	Fresh mass	Dry mass	CV(%)
ABTS (mg T g^{-1})	7.82 ± 0.43	57.00 ± 3.12	5.47
DPPH (mg T g^{-1})	1.98 ± 0.28	14.40 ± 2.01	13.94
FRAP (mg FS g^{-1})	11.17 ± 1.05	81.40 ± 7.66	9.41
Phenolics (mg GA g^{-1})	3.37 ± 0.35	24.55 ± 2.54	10.34
Flavonoids (mg Q g^{-1})	0.14 ± 0.02	1.00 ± 0.13	13.08
Lycopene (mg 100 g^{-1})	3.57 ± 0.37	-	10.36
β-carotene (mg 100 g^{-1})	7.00 ± 0.54	-	7.77
Ascorbic acid (mg 100 g^{-1})	57.71 ± 4.50	-	7.80

Notes: Mean ± Standard Deviation (n=20), T = trolox equivalent, FS = ferrous sulfate equivalent, GA = gallic acid equivalent, Q = quercetin equivalent, CV = coefficient of variation.

More than one method is recommended to estimate the antioxidant activity of the sample complex. Among the methods used, FRAP showed the highest average, followed by ABTS and DPPH. FRAP is based on the iron-reducing capacity and not on free radical scavenging, as in the ABTS and DPPH methods. In addition, it measures the antioxidant activity of samples with hydrophilic nature, while ABTS and DPPH measure hydrophilic and lipophilic compounds. Therefore, the most hydrophilic samples may have influenced these differences due to the different sensitivities of the methods to the compounds that may

be present. According to Mertz et al. (2009), the antioxidant and pro-oxidant activity of lulos results from the interaction of their water-soluble free components (organic acids), some water-soluble phenolic compounds, and carotenoids.

Hinestroza-Córdoba et al. (2020), in work with dried lulo pomace, reported that the ABTS method was more sensitive when compared with DPPH, which corroborates our results. Llerena et al. (2020) found higher antioxidant activity in Ecuadorianulo fruit by the ABTS method (19.12 mg Trolox g^{-1}), followed by phenolic compounds (7.75 mg Gallic Acid g^{-1}) and DPPH (5.32 mg Trolox g^{-1}), expressed in dry mass. However, the results obtained in our study are higher, which may be associated with the different edaphoclimatic conditions in the cultivation of this fruit. Compared to the results found here, lower ABTS findings (3.05 mg Trolox g^{-1}, fresh mass) were also observed by Contreras-Calderón et al. (2011) in lulo fruit from Colombia. The methods indicated the presence of different antioxidant compounds, and the fruit are considered potential sources of natural antioxidants, with results higher than those cited for cupuaçu, papaya, and pear (CONTRERAS-CALDERÓN et al., 2011).

Phenolic compounds showed higher mean antioxidant activity compared to flavonoids (Table 1). Phenolics are secondary metabolites with antioxidant properties, with the flavonoids in their group (VUOLO et al., 2019). Gancel et al. (2008) found values for phenolics of 10.08 mg Gallic Acid g^{-1} (dry mass) in lulo fruit obtained in Ecuador, classifying them with intermediate antioxidant activity. Our results were higher (24.55 mg g^{-1}), which can be explained by the biosynthesis of phenolic compounds, coming mainly from the phenylpropanoid pathway, which directs aromatic compounds from the shikimic acid pathway. The triggering of the metabolism is associated with the exposure of the plant or plant organ to environmental stresses, resulting in oxidative stresses (LIU et al., 2015).

Chang et al. (2018) report that through high phenolic content, between 1.13 and 16.20 mg Gallic Acid g^{-1} (fresh mass), it is possible to classify a fruit into a superfruit. Thus, lulos (3.37 mg Gallic Acid g^{-1}) can be considered a superfruit. According to Gancel et al. (2008), the phenolics of lulos are due to the presence of chlorogenic acids, their hexosides, and dihydrocaffeoyl spermidines.

The fruit showed low antioxidant activity measured by flavonoids (Table 1). Its action occurs by scavenging free radicals by chelating metal ions or suppressing reactive oxygen species' formation reactions and may also regulate endogenous antioxidant defenses, showing potential health benefits (TAO et al., 2023). The main factor that alters its presence and distribution in plants is luminosity because its formation is accelerated by light (ALARA et al., 2021). Therefore, edaphoclimatic factors can influence the composition of these compounds. The main flavonoid reported in lulo fruit is dicaffeoylquinic (MERTZ et al., 2009).

Regarding carotenoids, Ramón-Valderrama and Galeano-García (2020) described that β-carotene is the primary carotenoid found in lulos, possessing provitamin A activity, and can be converted in the body into retinol. The carotenoid contents found in the fruit peels showed higher averages for β-carotene, followed by lycopene. Gancel et al. (2008) reported that the peel of the lulo has higher amounts of carotenoids than the pulp and placental tissue. In addition, the authors averaged 7.45 mg 100 g^{-1} for β-carotene in the fruit's peel, a result similar to our study. Although the peel is not consumed, its use in product processing is recommended for its good source of these compounds.

No studies were found that reported the lycopene content in lulo. However, it was observed that this carotenoid presented half of the β-carotene content found in the peel. Dias et al. (2017) observed lycopene content of 3.54 mg 100 g^{-1} in tomatoes grown in Brazil, indicating it as the main carotenoid in the fruit. According to Liu et al. (2015), the antioxidant action of carotenoids is a fundamental characteristic of lycopene that inhibits the oxidative potential of free radicals, indicating it to be a considerable carotenoid in lulo peels.

Other compounds may contribute to fruit's antioxidant capacity, such as ascorbic acid; thus, its content was evaluated. According to Franco et al. (2002), fruit can be classified as high sources of ascorbic acid (100 to 300 mg 100 g^{-1}), medium (50 to 100 mg 100 g^{-1}), low (25 to 50 mg 100 g^{-1}), and extremely low (less than 25 mg 100 g^{-1}). Hence, the samples evaluated can be classified as medium sources for ascorbic acid (57.71 mg 100 g^{-1}). The levels of this compound can vary as a result of several factors such as varieties, soil and climate conditions, maturation, solar incidence, and others. Wood et al. (2022) reported that the incidence of sunlight seems to stimulate the synthesis of ascorbic acid because L-ascorbic acid (the main active form of vitamin C), in the photosynthetic mechanism, dissipates the excess light energy absorbed in the form of heat, in addition to the elimination of ROS (Reactive Oxygen Species). Acosta et al. (2009) cite an average of 12.5 mg 100 g^{-1} for ascorbic acid, below the result of this study. The authors report that the value found is lower than that observed by other researchers.

Table 2 shows the physicochemical characterization of the fruit. The acidity found was 2.29 g of citric acid 100 mL^{-1}, similar to the works of Rotili et al. (2018) for dovyalis fruit (2.35 g of citric acid 100 mL^{-1}) and Nakayama and Matsuda (2022) (mean 2.5% citric acid) for passion fruit. Its content classifies this fruit with medium acidity. Gancel et al. (2008) described that citric acid represents the main organic acid in lulos (97% of the total).

Acosta et al. (2009) observed acidity of 2.63 g citric acid 100 g^{-1} for Costa Rican lulo fruit and 3.65 g citric acid 100 g^{-1} for Colombian lulo fruit, indicating that Colombian fruit, compared to the Costa Rican one,

have higher acidity. Our acidity results are lower; the production season, the cultivars used, and the environment interfere in the concentration of acids (FERREIRA, 2017).

The lulo fruit presented low soluble solids content. Other authors showed higher averages for the variable in lulo fruit from different localities, being 7.30% in the fruit from Ecuador (GANCEL et al., 2008) and 10.30% in the fruit from Colombia (MEJÍA et al., 2012). These differences are dependent on the variety, the carbon dioxide (CO_2) assimilation rate of the leaves, the number of leaves and fruit, and the climatic conditions during fruit growth and maturation (LLERENA et al., 2020).

During the ripening process, there is an increase in soluble solids, which possibly occurs due to the translocation of sucrose from the leaves by hydrolysis of polysaccharides in the cell walls, producing soluble sugars that increase during ripening of climacteric fruit as an enzymatic result, such as sucrose-phosphate synthase (FERREIRA, 2017). According to Gancel et al. (2008), organic acids contribute 40% of this value in lulos.

TABLE 2 - Physicochemical characteristics of lulo fruit.

Analysis	Mean ± Standard Deviation (n= 20)	CV (%)
Titratable acidity (g CA 100 mL^{-1})	2.29 ± 0.17	7.47
Soluble solids (%)	5.12 ± 0.32	6.35
Ratio	2.25 ± 0.23	10.43

Notes: CA = citric acid, CV = coefficient of variation.

The ratio indicates the balance of sugars and organic acids, which are related to the flavor and ripening of the fruit, reflecting increase of sugars and reduction of acids (PEREIRA et al., 2019). The results indicate that the fruit is acidic since the ratio is higher in sweet fruit and has a low ripening index.

Lulo is mainly consumed processed (HINESTROZA-CÓRDOBA et al., 2020) because they are acidic fruit with low soluble solids content, according to the results presented. Thus, it can be interesting for the Brazilian fruit market, presenting the potential for product diversification (PEREIRA et al., 2019). Finally, lulo cultivated in Brazil presented medium acidity, low soluble solids content, and low ripeness, making them suitable for processing.

Therefore, studies focused on the processing of this fruit and the inclusion of its peel in the production of different products are suggested. Future work may seek acceptance in the Brazilian consumer market.

CONCLUSIONS

The FRAP antioxidant activity method showed 81.40 mg Ferrous Sulfate g^{-1} (dry mass), ABTS 57.00 mg Trolox g^{-1} (dry mass) and DPPH 14.40 mg Trolox g^{-1} (dry mass).

The flavonoids contents were 1.00 mg Quercetin g^{-1} (dry mass), which is considered low.

Lulo fruit showed to be a good source of β-carotene (7.00 mg 100 g^{-1}), lycopene (3.57 mg 100 g^{-1}) and ascorbic acid (57.71 mg 100 g^{-1}).

The fruit is acidic (2.29 g Citric Acid 100 mL^{-1}), with low soluble solids content (5.12%) and low ratio (2.25).

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