Physicochemical and bioactive compounds evaluation of *Physalis pubescens Linnaeus*[1]

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ABSTRACT

Physalis pubescens L. is a UFP (unconventional food plant) that produces high quality fruits; however, in Rio Grande do Sul it is still considered a rare species. There is only few information regarding the characterization of these fruits in Brazil, and the main reports of this species are mainly focused on the pharmacology and agronomic conditions of the plant. The aim of this study was to analyze the physical and chemical composition, including bioactive compounds, of *Physalis* fruits obtained from a spontaneous culture of the southern region of Rio grande do sul. Soluble solids, total titratable acidity, vitamin C, moisture, ash, crude fiber, crude protein, lipids, carbohydrates, total caloric value, carotenoids, phenolic compounds, flavonoids and phenolic acids from pulp and seed were evaluated. It was observed that the fruit has acid characteristics (4.8%), in addition to a considerable presence of lipids (1.96%) and carbohydrates (10.85%), containing high content of carotenoids (171.36 μg β-carotene.g^{-1}), phenolic compounds (142.83 mg.EAG.100g^{-1}) and phenolic acids (38.55 mg EAC.100g^{-1}). It was possible to observe that the fruits of *Physalis pubescens* are nutritious, presenting light acidity and they are a rich source of bioactive compounds.

Keywords: proximal composition; phenolic compounds; carotenoids.

INTRODUCTION

Physalis pubescens L. is a tree found in subtropical regions that produce fruits that range color from green to yellow, containing many tiny seeds, sweet and slightly acidic juicy and a golden yellow skin (El Sheikha, 2004; Montet *et al.*, 2008). El Sheikha (2010), points out that the flavor of the *Physalis* fruits is bittersweet and comes close to the flavor of the tomato and pineapple mixture.

The fruits' physicochemical characteristics allow us to evaluate the degree of ripeness, to determine the harvest point, to define forms of postharvest handling and appropriate packaging, and to define storage and processing conditions to obtain its derivatives (Chitarra & Chitarra, 2005).

The main benefits associated with *Physalis* consumption are its nutritional composition (important source of vitamins and minerals) as well as the presence of bioactive compounds (carotenoids and phenolic compounds) (Salazar *et al*., 2008; Valdenegro *et al*., 2012). According to Kharchoufi *et al*. (2018) its consumption is linked to health promotion.

Studies with UFP, such as *Physalis pubescens L.*, have become increasingly attractive due to the continuous search for bioactive compounds of natural origin (Fernandes *et al*., 2019). In addition, the Brazilian biodiversity has great potential for expansion, as there are numerous native and exotic fruits still little economically explored. Due to its appearance, shape and different flavor, *Physalis pubescens L.* fruits have been investigated for better evaluation of their nutritional potential.

According to El Sheikha *et al.* (2008; 2009) e El Sheikha (2010), *Physalis* is considered an exotic fruit and it is included in the priority list of many government plans for horticulture and fruit exports, even though it is relatively

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unknown in import markets. However, in Egypt, Physalis has been known for a long time, and recently, its economic importance has increased due to its high acceptance for local consumption, mainly in the Arab and European market (El Sheikha, 2004).

By researching and defining the characteristics of these native and exotic fruits, it is possible to promote their commercialization and introduction into the population's food, both through *in natura* consumption and through the elaboration of derived products (de Almeida *et al*., 2013).

Even though we have witnessed a growth in the usage and an increase in the value attributed to these regional products, the information available about nutritional potential of *Physalis pubescens L* fruits is still limited (Souza *et al*., 2012). The present study had the objective of analyzing the physical and chemical composition, including bioactive compounds, of *Physalis* fruits obtained from a spontaneous culture of the southern region of Rio Grande do Sul.

MATERIALS AND METHODS

Physalis pubescens fruits were obtained in their maturity stage (straw-yellow chalice color) visually evaluated, and harvested (approximately 500g of fruits from each tree) from the countryside of Cerrito city (latitude 31º42'36" and longitude 52º45'39"), in the southern west region of Rio Grande do Sul (Figure 1), from 15 spontaneous culture in the period ranging from january to june 2017. Before the evaluation, all collected fruits were mixed and the analyzes were performed in triplicate.

Immediately after being collected, the fruits were washed with filtered water and stored under freezing (-18 °C). For the analysis of bioactive compounds, the fruit was lyophilized (Terroni, Enterprise II, Brazil), macerated to obtain a powder, following the seed separation by a 32 mesh size sieve (Bertel, 500 mm/ μ, Brazil).

The experiments were conducted at the Food Chromatography Laboratory of the Department of Agroindustrial Science and Technology (DCTA) located at FAEM (Eliseu Maciel College of Agronomy) at the Federal University of Pelotas, Capão do Leão, Campus / RS.

Physicochemical and proximal composition

The determination of soluble solids was performed using a digital refractometer (Palette Atago, A52444, Japan) and the results were expressed in °Brix according to the method of AOAC (2012). The hydrogen potential (pH) was determined according to the methodology described by Zambiazi (2010), using benchtop pH meter (Hanna, Hi 2221, Brazil).

Total acidity was determined according to the methodology described by AOAC (2012), by titration of the sample with 0.1N sodium hydroxide (NaOH) solution, expressing the results in percentage. Moisture was determined according to the methodology described by AOAC (2012), where a sample was placed in an oven at 105 °C until constant weight, expressing the results in percentage.

Ash content was determined in muffle oven according to the AOAC (2012) methodology, by incinerating the sample at 550 °C, and expressing the results in percentage. Crude fiber was obtained after acid and alkaline digestion of the dried and degreased sample, following the methodology described by AOAC (2012). The fiber content was calculated by the difference between the sample dried weight and the ash weight, expressing the results in percentage.

The total protein content determination was performed using the Micro-Kjeldhal method, according to the methodology described by AOAC (2012), expressing the results in percentage. Lipid determination followed the Bligh & Dyer (1959) methodology, using 2:1 (v / v) chloroform: methanol as the extraction mixture, expressing the results in percentage.

The total carbohydrate content was determined according to the Brazilian Legislation (2003), and the results were expressed in percentage. According to Brazilian Legislation (2003), the total caloric value was calculated by adding the calories generated by the major nutrients metabolism, and the results were expressed in Kcal.g^{-1}.

Bioactive composition

The determination of the samples' total carotenoid content was performed by the spectrophotometric method described by Rodriguez-Amaya *et al.* (2001). Readings were taken on a Uv-vis spectrophotometer (Jenway, 6705 Uv-vis, Spain), using a wavelength of 450 nm, expressing the results in μg of β-carotene.g^{-1} of lyophilized sample (equation 1).

$$\text{Carotenoid content } (\mu g\ \beta\text{-}carotene.g^{-1}) = \frac{A \text{x} B \text{x} 10^6}{2500 \text{x} 100 \text{x} P}$$

Equation 1.

Of which: A: wavelength absorbance of 450 nm; B: extract volume (mL); P: sample weight (g).

Initially, an extract was obtained using a 1: 20 hydroalcoholic solution (sample: 80% ethanol). The extracts were stored at 4 °C for 24 hours, and centrifuged (Sorvall, RC-5C, Alemanha) at 2800 xg for 20 minutes. The supernatant was removed, it was collected in an amber flask and frozen until the moment of the analysis (Repo & Encina, 2008).

The quantification of phenolic compounds was performed according to Singleton & Rossi (1965), by colorimetric reaction using Folin Ciocalteau reactive

solution, with the corresponding reading of the mixture in a spectrophotometer (Jenway, 6705 Uv-vis, Spain), at a wavelength of 725 nm. A standard curve of gallic acid (0.4 to 2.0 mg.L^{-1}) was used and the results were expressed in mg of gallic acid, equivalent per 100g sample (mg EAG.$100g^{-1}$ dry basis).

The flavonoid content was obtained according to the method proposed by the Chinese Pharmacopoeia Commission (2010), with few modifications. 1mL of hydroalcoholic extract was reacted with 100µL of 5% sodium nitrite solution under stirring for 6 minutes, and then 100µL of 10% aluminum chloride solution was also added under stirring and kept in rest for another 5 minutes. After this time, 1mL of 1M sodium hydroxide solution and 2mL of 80% ethanolic solution were added. Absorbance reading was performed using Uv-vis spectrophotometer (Jenway, 6705 Uv-vis, Spain) at a wavelength of 415nm. Quantification was performed by using a standard curve on quercetin (0 to 0.15 mg.L^{-1}). Results were expressed in µg of quercetin equivalent per gram of dry base sample (µg EQ.g^{-1}).

The phenolic acid content was obtained according to Mazza *et al.* (1999), with few modifications. Aliquots of 250µL of hydroalcoholic extract plus 250µL of the acidified ethanol solution (0.1% hydrochloric acid in ethanol 95%) and 4.55mL of 2% hydrochloric acid solution were used. Absorbance reading was performed at 320nm on a Uv-vis spectrophotometer (Jenway, 6705 Uv-vis, Spain). Quantification was performed by using a standard curve of caffeic acid (0 to 200 mg.L^{-1}) and the results were expressed in µg of caffeine acid equivalent per gram of dry sample (mg EAC.$100g^{-1}$).

Ascorbic acid (vitamin C) content was determined using the Lorenz-Steves titration method described by Zambiazi (2010). The determination is based on the reducing action of ascorbic acid by using 0.01N iodine standard solution, employing 0.01N sodium thiosulfate and 0.5% starch solution as an indicator. Results were expressed in mg of L-ascorbic acid per 100g of dry sample, according to equation 2.

$$\text{Vitamin C} = \frac{Y \text{x} 0.88}{P} \qquad \text{Equation 2.}$$

Where: Y= (total volume of iodine solution x solution factor) - (volume of thiosulfate solution x solution factor); P= sample weight (g); 1mL 0.01N iodine solution = 0.88mg ascorbic acid.

The analyzes were performed in triplicate and the results were evaluated using descriptive statistical analysis of the data by using analysis of variance (ANOVA) and Tukey's test or t test ($p < 0.05$) for samples with normal data distribution. The Mann Whitney nonparametric test ($p < 0.05$) was used for asymmetric data that did not show normality.

RESULTS AND DISCUSSIONS

The fruits differ in composition from each other, since intrinsic and extrinsic factors interfere in the concentration

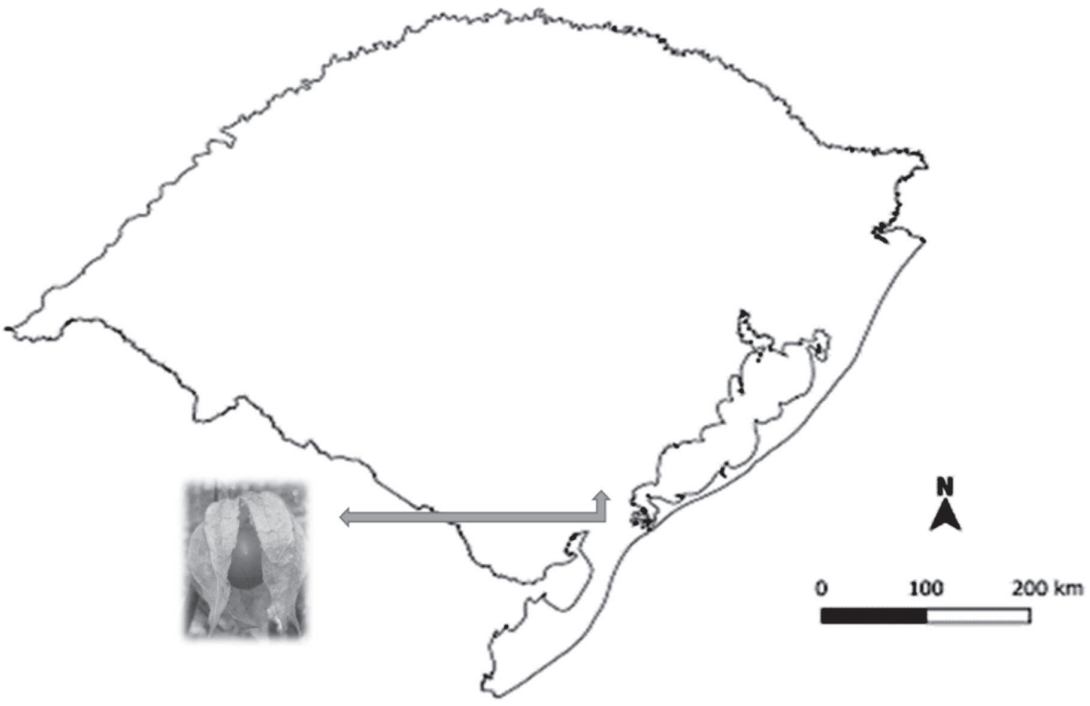

Figure 1: Map of Rio Grande do Sul indicating the place where the samples were collected.

of nutritional and bioactive compounds. Among the main factors are the type of fruit and the variety, the soil, the climatic conditions, the degree of ripeness, each region and each cultivar will present different peculiarities and therefore the need for this study. The data of the proximal and physicochemical composition of *Physalis pubescens L*. fruits are shown in Table 1.

The fruit presented a pH of 3.76, thus being considered an acid fruit. This value was close to the values described in the literature for fruits of the same genus, which varies between 3.3 and 3.8 (El Sheikha *et al*., 2008; El Sheikha *et al*., 2010; Curi *et al*., 2018). Higher pH values (4.72) were reported by Мамедов *et al*. (2017). According to El Sheikha *et al*. (2008), the low pH value indicates strong fruit acidity, which may be linked to the presence of free-forms of organic acids.

The total titratable acidity of *Physalis* fruit was 4.80%, and the SS / TA ratio was 1.5%. It was observed that the high acidity of the fruits corresponded to the low pH value. The acidity content was higher than the one reported by Curi *et al*. (2018), 1.59%; by El Sheikha *et al*. (2008), 1.43%; and by Silva (2013), from 0.18 to 2.57%, all values reported for *Physalis pubescens L*. fruits. The ratio between total titratable acidity and soluble solids content (SST / ATT) is usually related to the ripeness and conservation status of the fruit (Chitarra & Chitarra, 2005).

The average of soluble solids content was 7.30 °Brix, being similar to those described for fruits of *Physalis pubescens* by Silva (2013) (6.52 °Brix) and by Curi *et al*. (2018) (8.33° Brix); however, it was lower than the values found by Мамедов *et al*. (2017) (9.65° Brix). According to Resende *et al*. (2010), soluble solids content is related to fruit quality and may vary between cultivars and environmental conditions, besides being an indicative of fresh fruit sweetness.

Table 1: Physicochemical composition of *Physalis pubescens L*. fruits

Evaluated parameters	Average content ± SD *
pH	3.76 ± 0.02
Soluble solids (°Brix)	7.30 ± 0.06
Total titratable acidity (%)	4.80 ± 0.07
SS/AT ratio (%)	1.5 ± 0.01
Moisture (%)	79.35 ± 1.10
Ashes (%)	2.18 ± 0.20
Crude fiber (%)	3.58 ± 0.30
Protein (%)	2.08 ± 0.14
Lipids (%)	1.96 ± 0.16
Carbohydrates (%)	10.85
Total caloric value (kcal.100g^{-1})	69.36

* Average values obtained from the analysis of 3 repetitions; SD = standard deviation; SS= 7.3% ; AT= 4.8%.

The moisture content of the *in natura* fruit (79.35%) is consistent with what is reported by El Sheikha *et al*. (2008), 81.34% in *Physalis pubescens*; and by Santos *et al*. (2017), 80.74% in *Physalis peruviana*. The high moisture content is found to be a characteristic of the family in question, besides being related to the stability, quality and composition of the fruits, where foods with high moisture could deteriorate faster during storage (Vallilo *et al*., 2008).

The average ash content of 2.18% was lower than the one reported by El Sheikha *et al*. (2008), 5.58% for fruit and 7.01% for juice, both from *Physalis pubescens*; and it was higher than those reported by Oliveira *et al*. (2011), 0.65% in *Physalis angulata*; and by Yıldız *et al*. (2015) of 2.98% in *Physalis peruviana*. In a food sample the ash content represents the total mineral content (Zambiazi, 2010).

The 2.08% of protein content of *Physalis* fruits is in accordance with the content of most native fruits. A varied content of protein for *Physalis* is reported by Oliveira *et al*. (2011), 0.85%; by Yıldız *et al*. (2015), 1.66%; and by Santos *et al*. (2017), 5.37%. Fruits and vegetables provide little protein, about 1 to 2% of their weight; however, they should not be disregarded (Monteiro, 2009).

The fiber content of 3.58% was lower than what was reported by El Sheikha *et al*. (2008), 5.8% in *Physalis pubescens*, and by Ramadan (2011), 4.9% for *Physalis peruviana;* and the values were higher than the content in *Physalis angulata*, reported by Oliveira *et al*. (2011) with only 0.84%.

The average of lipid content in these fruits, 1.96%, was higher than what was found by El Sheikha *et al*. (2010): 1% in *Physalis pubescens*; by Yıldız *et al*. (2015), 0.18% in *Physalis peruviana*; by Santos *et al*. (2017), 0.99% in *Physalis peruviana*; and by Oliveira *et al*. (2011), 0.59% in *Physalis angulata*. According to Ramadan & Mörsel (2003), *Physalis* fruit has 2% oil, with 1.8% present in the seed and the other 0.2% in the skin and pulp.

The fruits' carbohydrate content was 10.85%. This content was also consistent with what is reported by Santos *et al*. (2017), 10.92% in *Physalis peruviana*; and lower than what was reported by Yýldýz *et al*. (2015), 13.86% also in *Physalis peruviana*. These compounds stand out for being an important source of energy (Sapata *et al*., 2006).

The caloric content of the *Physalis* was 69.36 kcal.100g^{-1} of fruit. This value was higher than the one reported in the review by Rufato *et al*. (2013), 49 kcal.100g^{-1} of *Physalis* (Flóres *et al*., 2000); it was similar to the values found in *Physalis peruviana L*. by the National Research Council (1989), 73 kcal.100g^{-1}, and by Repo & Encina (2008), 76.8 kcal.100g^{-1}; and it was higher than the ones reported by Flóres *et al*. (2000) and Osorio & Roldan (2003), 49 kcal.100g^{-1}, as shown in the review of Puente *et al*. (2011); by Joshi & Joshi (2016), 44.29 Kcal.100g^{-1} of *Physalis peruviana;* and by El Sheikha *et al*. (2008), 49 kcal.100g^{-1}

in *Physalis pubescens L*. According to Vallilo *et al*. (2008), that characterized fruits of *Campomanesia xanthocarpa* O. Berg, the low caloric value (57.3 kcal .100 g^{-1}) is mainly due to the high moisture content.

Smaller fruits, such as *Physalis,* have a range of macro and micronutrients, as well as bioactive compounds that can bring benefits to human health when consumed regularly (Vizzotto, 2012). Bioactive compounds are considered micronutrients, which usually occur in small amounts in foods and vary widely in structure and chemical function. Among the main bioactive compounds are carotenoids, phenolic compounds and ascorbic acid. The main bioactive compounds of *Physalis pubescens L.* are shown in Table 2.

Significant difference was observed on the content of bioactive compounds between the pulp and seed variables. For all parameters analyzed, the pulp presented the highest values.

The carotenoid content in the pulp was 171.37 µg β-carotene.g^{-1}. Wen *et al.* (2017) report lower levels of carotenoids and carotenoid esters in *Physalis pubescens*, which ranged from 12.8 µg β-carotene.g^{-1} to 13.8 µg β-carotene.g^{-1} of fruits, beeing β-carotene the predominant carotenoid, comprising 36-40% of the total carotenoids present in the fruit. β-carotene was the predominant carotenoid also reported in other *Physalis* species, especially yellow ones (Wen *et al.*, 2017).

The average content of phenolic compounds in *Physalis pubescens* pulp was 142.83 mg EAG.100g^{-1}, it was higher than the values reported by Silva *et al.* (2016), 112.37 mg EAG.100 g^{-1} of *Physalis pubescens* fresh fruits, and by Curi *et al*. (2018), 25.54 mg EAG.100g^{-1} of *Physalis pubescens*. Мамедов *et al*. (2017) reported 318 mg EAG.100g^{-1} dry weight content in *Physalis pubescens*. According to the authors, this species is the richest in polyphenols, followed by *Physalis peruviana.* The authors emphasize that the polyphenol content is specific to each species of the subtropical climate.

The average flavonoid content was 0.14µg EQ.g^{-1} and for phenolic acids an average content of 38.55 µg EAC. g^{-1}. When evaluating the flavonoid content in *Physalis peruviana*, Santos *et al*. (2017) reported the average content of 8.23mg.100g^{-1} on wet basis; Antunes *et al*. (2016) reported 0.032mg.g^{-1} on wet basis; and Correia *et al*. (2017) presented 0.340 mg QE. 100g^{-1} in wet weight. Hassanien (2011) reports much higher values in *Physalis peruviana,* in extracts obtained by supercritical carbon dioxide extraction, 234 mg.g^{-1} of *in natura* fruit.

Flavonoids are comprised of a group of phenolic compounds that contribute to the sensory quality of fruits, including astringency and bitterness (Vendramini & Trugo, 2004; Vasco *et al*., 2008). Phenolic acids are characterized by having a benzene ring, a carboxylic group and one or more hydroxyl and / or methoxyl groups in the molecule, granting antioxidant properties on fruits and vegetables (Soares, 2002) as well as on the consumer's organism.

The phenolic acid content found in the present study was 38.55 mg EAC. 100g^{-1}, which is lower than the one reported by Rockenbach *et al*. (2009), 346.1 mg. 100g^{-1} on dry basis in *Physalis peruviana L*; and by Zavaleta *et al*. (2005), 3.85 mg EAC.g^{-1} dry weight *Physalis pubescens*. Deng *et al*. (2016) reported that flavonoids and caffeic acid derivatives are the main compounds of *Physalis pubescens L,* which are responsible for contributing to health benefit.

Through the analysis carried out in this study, the fruits' content of vitamin C was 6.07 mg of acid. L-ascorbic.100g^{-1} on a dry basis, which is very similar to that found by Мамедов *et al*. (2017), 9.9 mg of ascorbic acid.100g^{-1} of fruit on a dry basis. Lower contents were reported by El Sheikha *et al*. (2010), 0.4g.100g^{-1} of ascorbic acid in dry matter in *Physalis pubescens.*

Puente *et al*. (2011) point out that ascorbic acid is a water-soluble vitamin, so in fruits with water content higher than 50%, it is largely present. According to Мамедов *et al*. (2017), *Physalis* fruits that are native from regions with lower temperatures presents a lower content of ascorbic acid than the ones grown in warmer temperatures. This could partially explain the vitamin C content found in the *Physalis* fruits evaluated in this study.

Table 2: Content of pulp and seed of *Physalis pubescens L* bioactive compounds

Evaluated Parameters*	**Average content ± standard deviation**	
	Pulp *	**Seed ***
Carotenoids (µg β-caroteno. g^{-1})	171.36^{a} ± 24.5	22.53^{b} ± 5.2
Phenolic compounds (mg EAG.100g^{-1})	142.83^{a} ± 0.6	50.03^{b} ± 2.2
Flavonoids (µg EQ. g^{-1})	0.14^{a} ± 0.002	0.04^{b} ± 0.002
Phenolic acids (mg EAC.100g^{-1})	38.55^{a} ± 3.6	11.91^{b} ± 2.7
Vitamin C (mg of L-ascorbic acid.100g^{-1})	6.07^{a} ± 0.45	0.70^{b} ± 0.1

ANOVA test of variance and the Tukey test ($p < 0.05$) were used for phenolic compounds, flavonoids and vitamin C data; the Mann Whitney test ($p < 0.05$), for p values of 0.049535, was used for carotenoids and phenolic acids data; Means followed by the same letter on the line do not differ statistically; * dry basis.

CONCLUSIONS

In view of the results found for the fruits of *Physalis pubescens L.*, it was observed that these fruits are a significant source of bioactive compounds such as carotenoids and phenolic compounds, mainly in the pulp fraction, and therefore can be used by the food and pharmaceutical industries.

Furthermore, after proximal analysis, the fruits showed nutritional potential, being sources of carbohydrates and lipids, with high potential for insertion in human food.

As it is an acidic fruit, its ingestion in its natural form may be limited, but the insertion of these fruits in preparations or through derived foods is an excellent alternative for consumption.

Physalis pubescens L. is an unconventional food plant, being this species still little explored in Brazil, but with the results of this study we can emphasize the health benefits of these fruits so that they may become part of our diet.

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