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Physicochemical Composition and Antioxidant Activity of Fresh Leaves of *Pereskia aculeata* Miller (Ora-Pro-Nóbis)

Composição Físico-Química e Atividade Antioxidante de Folhas *in Natura* de *Pereskia aculeata* Miller (Ora-Pro-Nóbis)

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Abstract

Brazil has great plant biodiversity of plant resources and among these are unconventional food plants (PANCs) such as ora-pro-nóbis (*Pereskia aculeata* Miller). Considering its potential for food, this leafy vegetable represents an excellent option for enriching food products. The objective of this study was to evaluate the physicochemical and functional characteristics of the fresh leaves of P. *aculeata* Miller. The analyses showed a pH 5.13, titratable acidity 1.40 g/100 g, moisture 87%, proteins 2.60%, lipids 0.30%, ash 2% and total dietary fiber 2%. Regarding bioactive compounds, 98.09% of antioxidant capacity was observed, evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, and 190.68 mg/g of total phenolic compounds, quantified by ultraviolet-visible spectrophotometry (UV-Vis). These results demonstrate the high nutritional and functional value of the leaves, with emphasis on the significant levels of proteins, fibers, ashes and antioxidant compounds. In this way, its consumption can contribute to the reduction of nutritional deficiencies and oxidative stress, promoting health benefits. The functional potential of ora-pro-nóbis leaves reinforces their applicability in food formulations focused on innovation and nutritional enrichment.

Keywords: Ora-PRO-Nóbis. Physicochemical Characteristics. Functional Property.

Resumo
O Brasil possui grande diversidade de recursos vegetais e dentre esses destacam-se as plantas alimentícias não convencionais (PANCs) a exemplo de ora-pro-nóbis (*Pereskia aculeata* Miller). Considerando seu potencial alimentar, essa hortaliça é uma excelente opção para enriquecer alimentos. O objetivo deste trabalho foi avaliar as características físico-químicas e funcionais das folhas *in natura* de *P. aculeata* Miller. As análises indicaram pH 5,13, acidez titulável 1,40 g/100 g, umidade 87%, proteínas 2,60%, lipídeos 0,30%, cinzas 2% e fibra alimentar total 2%. Quanto aos compostos bioativos, observou-se 98,09% de capacidade antioxidante, avaliada pelo método do 2,2-difenil-1-picril-hidrazil (DPPH), e 190,68 mg/g de compostos fenólicos totais, quantificados por espectrofotometria no ultravioleta-visível (UV-Vis). Esses resultados evidenciam o alto valor nutricional e funcional das folhas, com destaque para os teores significativos de proteínas, fibras, cinzas e compostos antioxidantes. Dessa forma, seu consumo pode contribuir para a redução de deficiências nutricionais e do estresse oxidativo, promovendo benefícios à saúde. O potencial funcional das folhas de ora-pro-nóbis reforça sua aplicabilidade em formulações alimentícias voltadas à inovação e ao enriquecimento nutricional.

Palavras-chave: Ora-pro-Nóbis. Características Físico-Químicas. Propriedade Funcional.

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1 Introduction

The PANCs grow spontaneously under almost all climatic conditions in Brazil. Additionally, their agricultural management does not require the use of pesticides or fertilizers. From this perspective, PANCs are an option that can provide diversity, sustainability, and nutrients in a balanced diet (Padilha *et al.*, 2023).

Among the PANCs, *Pereskia aculeata* Miller stands out, also known as ora-pro-nóbis, which is a Non-Conventional Food Plant (PANC) native to the Neotropical region. This plant is well adapted to local cultivation conditions and is highly resistant to pests and diseases (Silva *et al*., 2023).

Several studies have already demonstrated its nutritional and functional value, including high levels of fiber, protein, phenolic compounds, and antioxidant capacity, in addition to containing substances belonging to the groups of phenolic acids, alkaloids, and flavonoids (Ciriaco *et al.*, 2023; Silva *et al.*, 2023; Teixeira *et al.*, 2023).

The present study aimed to determine the physicochemical and functional characteristics of ora-pro-nóbis leaves collected at the Universidade Federal de Viçosa, Minas Gerais.

2 Material and Methods

All experiments were conducted in duplicate. Leaves were pre-selected before collection and did not present stains, physical damage, or apparent dirt to minimize the risk of microbiological contamination. After collection, the leaves were transferred to low-density polyethylene bags and transported at room temperature to the laboratory for experiments. Initially, the leaves underwent sanitization by immersion of approximately 400 g of fresh leaves in 12 L of an aqueous sodium hypochlorite solution (200 mg/L) for 15 minutes, followed by rinsing with sterile distilled water. Subsequently, the leaves were allowed to drain and dry for 1 hour at room temperature, and drying was completed using paper towels, taking care not to damage them.

2.1 pH determination

The pH was determined using a Kasvi benchtop pH meter (K39-1420A), Curitiba, Brazil, calibrated with pH 4.0 and 7.0 buffer solutions (AOAC, 2016).

2.2 Determination of titratable acidity

Titratable acidity was determined using potentiometric volumetry with a standard sodium hydroxide solution (0.1 mol/L) until the mixture's pH reached 8.2–8.4, at which point titration was stopped (AOAC, 2016). Results were expressed as % total titratable acidity (g/100 g of product).

2.3 Determination of moisture, ash, protein, fat, and crude fiber content

The moisture, ash, protein, fat, and crude fiber contents were determined according to the methodology of the Adolf Lutz Institute (IAL, 2005).

2.4 Determination of the functional characteristics

Samples of approximately 50 mg of washed and naturally dried ora-pro-nóbis leaves were weighed on a Shimadzu analytical balance (AUW220, capacity 10/220 g, precision 1 mg/0.1 mg) and ground with a mortar and pestle while gradually adding an ethanol:water mixture (1:1) until reaching a final volume of 50 mL. Extractions were performed in duplicate, and the antioxidant capacity and total phenolic compounds analyses were conducted on the same day.

The free radical scavenging activity (FRSA) using 2,2-diphenyl-1-picrylhydrazyl

(DPPH) of the leaf extracts was determined according to the methodology described by Hatano *et al*. (1988). To 4 mL of the sample, 1 mL of DPPH (0.5 mmol/L) diluted in ethanol was added. The mixture was placed in an amber test tube and stirred. After 30 minutes, absorbance was measured at 517 nm using a Shimadzu spectrophotometer (UV-1601), São Paulo, Brazil.

The sample absorbance was measured with DPPH and sample; the blank absorbance used methanol; and the control absorbance was DPPH solution without the sample, with an absorbance between 0.9 and 1.0. Analyses were performed in duplicate, and the free radical scavenging capacity (%FRSA) was expressed as a percentage according to Equation 1:

$$\%FRSA = 100 - \left[\frac{(\text{Sample Absorbance}-\text{Blank Absorbance}) \times 100}{\text{Control Absorbance}}\right]$$

For the calculation of IC_{50}, corresponding to the concentration required to inhibit 50% of the DPPH radical, the straight-line equation obtained from the %FRSA graph was used by replacing the y-value with 50 to obtain the sample concentration capable of reducing 50% of DPPH.

For the determination of total phenolic content, quercetin was used as the standard. A calibration curve was constructed with quercetin concentrations ranging from 0 to 30 mg/L (n=7 points) prepared from a quercetin stock solution (100 mg/L) in water. The total phenolic content was determined using UV-Vis spectrophotometry. For the determination, 2.0 mL of the prepared ora-pro-nóbis extract was added to 70 µL of 1% (w/v) ferric chloride solution. Absorbance was measured using a Shimadzu spectrophotometer (UV-1601), São Paulo, Brazil, at 294 nm (Bazani *et al*., 2021).

3 Results and Discussion

The average pH value (5.13) found in ora-pro-nóbis leaves (Table 1) was similar to that reported by Martinevski (2011) and Cazagranda *et al.* (2022) for ora-pro-nóbis flour. The average titratable acidity (1.40) of the evaluated leaves (Table 1) was similar to those found by Silveira (2016) and Reinert *et al*. (2023) in fresh ora-pro-nóbis leaves.

Table 1 - Average values of the physicochemical characterization, antioxidant capacity, and total phenolic compounds of fresh ora-pro-nóbis leaves

Physicochemical Characteristics of Fresh Leaves	Values
pH	5.13 ± 0.00
Titratable acidity (g/100 g)	1.40 ± 0.01
Moisture content (g/100 g)	87.00 ± 1.00
Protein (g/100 g)	2.60 ± 0.10
Lipids (g/100 g) Ash (g/100 g)	0.30 ± 0.04 2.00 ± 0.10
Total dietary fiber (g/100 g)	2.00 ± 0.10
Functional characteristics of fresh leaves	**Values**
Antioxidant capacity DPPH (%)	98.09 ± 0.34
IC_{50} (mg/g)	1.87 ± 0.36
Total phenolic compounds (mg/g)	190.68 ± 0.17

pH: Hydrogen ion potential; IC_{50}: concentration required to inhibit 50% of the DPPH radical; Values expressed as mean ± standard deviation (n=2).
Source: research data.

There is no established regulatory standard for the acidity of ora-pro-nóbis; however, the analysis of fresh leaves is important because higher acidity levels provide greater protection against spoilage microorganisms, thus maintaining the vegetable suitable for consumption (Dias; Leonel, 2006). The high acidity of the leaves is likely due to the presence of acids such as folic acid, ascorbic acid (vitamin C), uronic acid, caftaric acid, caffeic acid, among others, which are found in high concentrations in this plant (Garcia *et al.*, 2019).

The moisture content of the ora-pro-nóbis leaves evaluated in this study was high (Table 1) and close to the values reported by Silveira (2016) and Trennepohl (2016) in fresh weight, which observed values of 86.5 g per 100 g and 87.25 g per 100 g, respectively. Low moisture values indicate that the leaves may have undergone cell rupture during cleaning or preparation for analysis, which could have caused water leakage from the cells. Both high and low moisture values can also be related to abiotic factors (temperature, humidity, gravity, wind speed, and irrigation management adopted during field cultivation). With dry air, transpiration of plants occurs more rapidly due to the establishment of a higher gradient. Relative humidity is greatly influenced by temperature, meaning that at the same relative humidity, the water potential difference can increase with rising temperatures, as described by Pimentel (2004), Monteiro (2009), and Silva *et al.* (2021).

The protein content observed in the ora-pro-nóbis leaves was lower than the values reported by Tennepohl (2016), who found 3.4 g of protein per 100 g of fresh leaves, which may be related to differences in cultivation conditions or genetic variation of the plant since, the higher the light intensity, the lower the protein content (Queiroz *et al*., 2015). According to the Collegiate Board Resolution RDC No. 54 of November 12, 2012 (Brasil, 2012), a food is only considered a source of protein if it contains at least 6 g of protein per 100 g, which is not observed in fresh ora-pro-nóbis leaves.

The lipid content of the leaves (Table 1) was similar to that observed in other PANCs, as verified by Botrel *et al.* (2020) in nasturtium (0.11 g per 100 g). Thus, like nasturtium, which presents 0.11 g/100 g according to Botrel *et al.* (2020), ora-pro-nóbis, like most leaves, is not a significant source of lipids, making it an appropriate option for calorie-restricted diets.

Ora-pro-nóbis is widely recognized for its high mineral content (Oliveira *et al*., 2013), which justifies the higher ash content in the fresh leaves. The observed value was close to that reported by Takeiti *et al.* (2009), who detected 1.7 g/100 g. High ash values are related to inorganic matter present after incineration and are influenced by plant management practices such as fertilization, irrigation, exposure to sunlight, as well as the mineral content of the soil (Junior; Soares, 2020).

On the other hand, this PANC can contribute to increased intake of dietary fibers, which are of great importance in nutrition, improving bowel function, preventing constipation and the formation of polyps, and potentially preventing intestinal tumors, as well as ovarian cancer (Paschoal; Souza, 2019). The fiber content observed in fresh leaves (Table 1) was close to that found in leafy vegetables, such as raw collard greens and white cabbage (Pimenta, 2020).

The extracts and leaves of ora-pro-nóbis are also considered rich in carotenoids and phenolic compounds, phytochemicals that provide healing, anti-inflammatory, antifungal, antioxidant activities and also have analgesic potential (Macedo *et al*., 2023). It is important to note that the quantity and profile of these bioactive compounds can vary significantly depending on several factors, such as the plant cultivar, edaphoclimatic conditions, harvest time, degree of maturation, and extraction methods used, as demonstrated in recent studies (Moomin *et al*., 2023). However, when in excess, these free radicals can compromise cellular integrity by degrading proteins and lipids. Due to this, the plant organism has developed specific metabolic pathways to ensure its integrity and protection, making care during cultivation important (Gill; Tuteja, 2010).

High antioxidant capacity values in the ora-pro-nóbis leaves, as well as phenolic compounds, indicate that its use in food may contribute to reducing oxidative stress, promoting a protective effect against various diseases (Table 1). The lower the IC50, the better the antioxidant activity (Olugbami; Gbadegesin; Odunola, 2014). The IC50 result obtained (Table

1) was considered low, suggesting that ora-pro-nóbis may act as a reducing agent, directly neutralizing free radicals.

Regarding the human body, the generation of free radicals is a normal physiological process involved in various biological functions and biochemical reactions. Controlled production of these compounds enables several essential functions of life. However, their excessive production can compromise cellular integrity and favor the development of chronic diseases. Therefore, the consumption of fruits and vegetables is essential as it provides reducing agents that neutralize free radicals, as well as increasing endogenous antioxidant capacity and modulating cells, as demonstrated by recent studies (Paschoal; Souza, 2019, Zhang *et al.*, 2023).

4 Conclusion

Fresh ora-pro-nóbis leaves present significant levels of ash, protein, and total dietary fiber, which can help reduce deficiencies in macronutrients and minerals. Fresh ora-pro-nóbis leaves have high antioxidant capacity, related to their total phenolic compounds and antioxidant capacity.

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