

## Powdered leaves of ora-pro-nóbis (*Pereskia aculeata*): An emerging source of proteins, phytochemicals, bioactive compounds, and antioxidants

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### Abstract

Ora-pro-nóbis (*Pereskia aculeata*) is widely consumed in Brazil in the form of high-temperature cooked food, and its use in other forms is limited due to its high susceptibility, which restricts its potential for larger-scale use. The application of drying techniques aims to extend its shelf life and increase its applications. The objective of this research is to analyze the effect of cryo-drying on ora-pro-nóbis leaves on their nutritional composition, specialized metabolites, bioactive compounds, and antioxidant potential. Results show leaf powders with a high protein content (15.12%), carbohydrate content (60.0%), and a low lipid content (2.76%). In phytochemical terms, there is the presence of alkaloids, coumarins, and tannins, and the bioactive compounds, phenolic groups (55.47), antioxidant activity, ABTS 575.67 and DPPH 191.0 ( $\mu\text{molTE/g}$ ), and vitamin C content (160 mg/100 g). The functional groups expressed in the spectroscopy show the presence of amine groups, evidencing the high protein content: its morphology shows the maintenance of the stability of plant membranes and parenchyma in the cryo-drying process, highlighting the presence of fibers (cellulose, hemicellulose). The research shows that the use of lyophilization maintains the bioactive functional composition of ora-pro-nóbis, making it less perishable and with high potential for dietary and medicinal applications.

**Keywords:** ora-pro-nóbis; cryo-drying; antioxidant bioactivity.

## 1 INTRODUCTION

Despite the vast diversity of edible plants in the world, estimated at around 300,000 species, only a small number, between 150 and 200, are widely used in human food, notably rice, corn, and wheat, which together provide most of the calories and proteins consumed globally (FAO, 2014). However, economic factors can, in some cases, restrict access to various nutrients, compromising food and nutritional security, especially in vulnerable contexts (Pereira et al., 2023). In contrast, there is a growing appreciation for alternative plant species with medicinal, functional, and therapeutic properties, used by a considerable portion of the world's population (Ferreira et al., 2024).

In this scenario, research with these unconventional food plants (PANC) emerges as an important food resource as part of commercial agriculture, whose parts, such as leaves, flowers, stems, and seeds, are being introduced into usual diets with increased consumption. Relevant data show that in Brazil, a significant portion of the population already integrates them

into their daily diet and traditional health care practices (Ferreira et al., 2024; Kinupp, 2007; Kong et al., 2022).

Among the most popular and promising PANCs is *Pereskia aculeata*, popularly known as ora-pro-nóbis (OPN). Native to the Atlantic Forest, this species of the Cactaceae family is widely distributed in different regions of the country and is traditionally used in domestic cooking, especially in preparations such as broths, pies, flours, and stir-fries (Garcia et al., 2019; Silveira et al., 2020). Its dried leaves are also frequently used in capsule form for therapeutic purposes associated with the treatment of anemia, diabetes, and inflammation (Agostini-Costa, 2020).

From a nutritional standpoint, OPN has a high protein content between 14 and 26% on a dry basis in addition to mucilage that provides relevant technological properties, such as emulsifying capacity, making it attractive to the health food industry (Lise et al., 2021; Silva, L. R., et al., 2023). Additionally, it stands out for its richness in fiber, essential minerals (calcium, iron, potassium, among others), and fat-soluble and

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water-soluble vitamins, such as A, C, E, and folate (Barreira et al., 2020; Sato et al., 2018).

A large part of the beneficial effects attributed to *P. aculeata* is related to the presence of bioactive compounds, especially flavonoids, essential amino acids, and phenolic acids (Arnosso et al., 2019; Castro et al., 2022). However, its perishability—due to high moisture content—makes it difficult to preserve and fully utilize the plant, requiring the development of technological strategies that increase its durability without compromising its functional quality (Kumar et al., 2021).

In this context, freeze-drying, also known as cryo-drying, emerges as an effective technology for preserving foods with a high content of heat-sensitive compounds. The process consists of removing water by sublimation, under low temperatures and pressures, which minimizes nutritional and sensory losses (Bhatta et al., 2020). In studies with basil, jambu, and taro leaves, the technique has been shown to preserve phenolic compounds and flavonoids, although some more unstable micronutrients, such as vitamin C, may undergo slight degradation (Carvalho et al., 2024; Santos et al., 2022).

However, scientific literature on the effects of freeze-drying specifically on OPN is scarce. Investigating how this method affects the content of pigments, antioxidants, and other phytochemicals could expand the commercial use of the plant and strengthen its inclusion in functional and plant-based food products. Therefore, this study aims to characterize the profile of bioactive compounds in OPN leaves subjected to freeze-drying, contributing to the knowledge and appreciation of this species in human nutrition.

### 1.1 Relevance of the work

The results of this study show that cryo-drying is an efficient method for preserving the nutritional and functional aspects of ora-pro-nóbis leaves, maintaining high levels of protein, phytochemicals, and antioxidant compounds. Therefore, the application of cryo-drying expands the plant's technological potential, increasing its application in functional food matrices and nutraceutical formulations.

## 2 MATERIALS AND METHODS

### 2.1 Obtaining the raw material

Samples of fresh OPN were collected in the State of Pará, Brazil (latitude -1.127 and longitude 47.0627). These samples were packaged in low-density polyethylene (LDPE) plastic bags and transported to the Food Science Laboratory of the Faculty of Nutrition at the Universidade Federal do Pará (UFPA). The samples were selected, sanitized with a 2% sodium hypochlorite solution for 20 minutes, and then dried with paper towels. Subsequently, they were frozen and lyophilized (SOLAB-SL 404) for 48 hours, then macerated and stored in airtight containers protected from light until the respective analyses were performed. The plant specimen was prepared at the HF Professor Normélia Vasconcelos Herbarium (UFPA), under registration number HF00004659. This research is registered

with access activity A4C9B40 in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN).

### 2.2 Physicochemical analyses

The following physicochemical analyses were performed, in triplicate, on the lyophilized OPN leaf. Water activity (aw) was measured directly using the LabMaster instrument (Aw Neo Series 3TE, NOVASINA brand). Moisture, ash, protein (correction factor: 5.75 for vegetable proteins), and lipid analyses were performed (AOAC, 2016). Total carbohydrates were calculated by difference (AOAC, 2016); total energy value (TEV) expressed in kcal/100 g was calculated using Atwater's coefficients (Brasil, 2020).

### 2.3 Analysis of the concentration of bioactive compounds

#### 2.3.1 Total phenolic compounds

The total phenolic content was determined according to the Folin–Ciocalteu assay, as described by Aliakbarian et al. (2011), using a UV–Vis spectrophotometer, model IL-592 KASUAKI (São Paulo, Brazil), at a wavelength of 725 nm. Framed within the equation of the line generated by the standard curve ( $y = 0.0003x + 0.1931$ ;  $R^2 = 0.9916$ ).

#### 2.3.2 Flavonoids

The determination of flavonoids was carried out according to the methodology described by Lees and Francis (1972) with reading on a UV–Vis spectrophotometer, model IL-592 KASUAKI (São Paulo, Brazil) at a wavelength of 374 nm for flavonoids.

#### 2.3.3 Carotenoids

The determination of carotenoid content was performed according to the methodologies described by Rodriguez-Amaya (2001), with adaptations, using a UV–Vis spectrophotometer (KASUAKI, model IL-592, Brazil). Absorbance readings were taken at a wavelength of 450 nm for beta-carotene and 470 nm for lycopene.

#### 2.3.4 Chlorophyll a and b

It was quantified according to the methodology proposed by Nagata and Yamashita (1992) with absorbance readings at wavelengths of 663 nm for chlorophyll A and 647 nm for chlorophyll B in a UV–Vis spectrophotometer, model IL-592 KASUAKI. The results were expressed in mg/100 g fresh weight. The analysis of chlorophyll a and b content was obtained according to the Equations 1 and 2.

$$\text{Chlorophyll Contents a } (\mu\text{g/g}) = -0.999\text{A}663 + 0.989\text{A}645 \quad (1)$$

$$\text{Chlorophyll Contents b } (\mu\text{g/g}) = -0.328\text{A}663 + 1.77\text{A}645 \quad (2)$$

### 2.3.5 DPPH

The total antioxidant activity of the extracts was determined using the DPPH radical scavenging method, adapted from Blois (1958). Readings were taken using a Shimadzu model 1,800 UV-Vis spectrophotometer at a wavelength of 515 nm. A standard curve was constructed with different concentrations of trolox (50, 100, 200, 400, 600, 800, and 1,000  $\mu\text{M}$ ), with readings taken after 15 minutes of reaction in a dark environment. The equation of the line obtained for the standard curve was Equation 3:

$$Y = -0.0006X + 0.6499 \quad (R^2 = 0.9998) \quad (3)$$

The results were expressed in  $\mu\text{M}$  of trolox per gram of sample.

### 2.3.6 Antioxidant activity assay

The ABTS radical scavenging activity of the extracts will follow the method of Rufino et al. (2010), with readings on a UV-Vis spectrophotometer, model IL-592 KASUAKI, at a wavelength of 734 nm. Framed within the equation of the line ( $Y = -0.3364X + 0.6239$  ( $R^2 = 0.997$ )), generated by the standard curve constructed with different concentrations of trolox 100, 500, 800, 1000, and 2000. The results were expressed in  $\mu\text{M}$  trolox/g.

## 2.4 Determination of phytochemicals

Specific tests were performed according to the methodology of Costa (1986), Matos (1997), and Simões et al. (2001) to identify the main classes of compounds present in the lyophilized leaves of OPN.

### 2.4.1 Alkaloids

In a test tube, 1 mL of a 92% hydroalcoholic extract at a concentration of 0.2 mg/mL, 6 mL of distilled water, 1 mL of hydrochloric acid (HCl), and four drops of Bouchardat's reagent (iodine and potassium iodide solution) were added. The expected result in case of a positive reaction is the presence of amorphous or crystalline precipitates, with color differentiation ranging from white to brownish-orange.

### 2.4.2 Tannins/phenols

In a test tube, 1 mL of the extract at a concentration of 0.2 mg/mL and 6 mL of distilled water were added. Subsequently, three drops of an alcoholic solution of ferric chloride were added, shaking vigorously to observe any color variation. A blue precipitate indicates the presence of hydrolyzable tannins, and green indicates the presence of condensed tannins.

### 2.4.3 Saponins

To 1 mL of the extract at a concentration of 0.2 mg/mL, 6 mL of water, 2 mL of distilled water, and three drops of hydrochloric acid were added. The solution was then stirred continuously for 3 minutes; the presence of persistent and abundant foam (collar) indicates the presence of saponins.

### 2.4.4 Coumarins

Notably, three drops of the extract were placed on filter paper, allowed to dry, and then three drops of a one molar aqueous solution of sodium hydroxide were added. The appearance of bright blue or green fluorescence under ultraviolet light (360 nm) is expected. Coumarins in alkaline solution develop a yellow color due to the rupture of the lactone ring.

### 2.4.5 Anthraquinones

For this determination, 0.5 mL of benzene was added to 1 mL of the extract at a concentration of 0.2 mg/mL and 6 mL of distilled water, followed by 10 drops of 10% ammonium hydroxide solution. The appearance of pink, red, or violet coloration in the aqueous phase will indicate the presence of anthraquinones.

## 2.5 Morphological analysis

Morphological analyses of the samples were performed using scanning electron microscopy (SEM) coupled with an X-ray energy spectroscopy system. Samples were mounted on supports and coated with Au/Pd (gold/palladium) to provide the necessary electrical conductivity for image formation, with an electron beam current of 85–90  $\mu\text{A}$ . The images or electron micrographs were obtained using a Tescan Vega3 scanning electron microscope.

## 2.6 Fourier transform infrared absorption spectroscopy

The absorption spectra of the samples were obtained by Fourier transform infrared absorption spectroscopy using a PerkinElmer spectrometer, model Frontier 98737 (Waltham, MA, USA), with recordings in the absorption spectral frequency range of 4,000 to 400  $\text{cm}^{-1}$ . The sample incorporation was performed in potassium bromide (KBr) pellets with Scan 100 and 4  $\text{cm}^{-1}$  resolution.

## 2.7 Statistical analysis

The analyses were performed in triplicate, and the results will be expressed as mean  $\pm$  standard deviation, compiled in Microsoft Excel®.

# 3 RESULTS AND DISCUSSION

## 3.1 Nutritional composition of freeze-dried OPN leaf

Table 1 presents the nutritional composition, bioactive compounds, phytochemicals, and antinutritional compounds present in the freeze-dried OPN leaf.

Freeze-dried OPN leaves have low moisture and water activity ( $A_w$ ), characteristics influenced by the freeze-drying process. This process is seen as a preservation technique to extend the shelf life of food (Torres et al., 2021a).

The ash content observed in the freeze-dried OPN leaf was lower than that of conventional vegetables, which generally have about 10% fixed mineral residue on a dry basis (Cruz et al.,

2021). This difference may be related to genetic variability, climatic conditions, plant development stage, and soil characteristics where the plant is grown (Khandelwal & Dalai, 2024). Sato et al. (2018), for example, reported values similar to those of the present study when analyzing leaves harvested in the southeastern region of Brazil.

Lipids, as expected for this type of vegetable, showed reduced values (2.97% on a dry basis), corroborating findings by Moura et al. (2021) for other unconventional edible species, such as *Talinum paniculatum* (Major Gomes) and *Amaranthus deflexus* (Cariru). The low amount of fat reinforces the potential for using the leaf in diets with lipid control.

Regarding protein content, the freeze-dried OPN leaf presents a significant profile, with average values of 17.37% on a dry basis, using a conversion factor suitable for vegetable sources (5.61) (Silva, L. R., et al., 2023). This content surpasses several commonly consumed leafy greens, such as cassava (4.64 g/100 g) and taro (3.01 g/100 g) (Santos et al., 2024; Souza et al., 2024). The protein richness of *P. aculeata* also approaches that of legumes widely used in vegetarian diets, such as chickpeas (19 g/100 g) and lentils (9 g/100 g), according to data from Moura et al. (2022).

In addition to quantity, the quality of OPN proteins also stands out. Studies show good digestibility, close to 80%, and the presence of essential amino acids, especially lysine and tryptophan (Barreira et al., 2020; Neves et al., 2020; Torres et al.,

2021a). These characteristics highlight the plant's potential as an alternative and sustainable protein source.

Regarding carbohydrates, freeze-dried OPN leaf contains a significant amount, reaching 29.5% (Almeida & Corrêa, 2012; Barreira et al., 2020). A relevant part of this content can be attributed to the presence of mucilage, a non-toxic soluble polysaccharide, which can represent up to one-third of the plant's dry matter (Silva Porto et al., 2021). This substance has important functional properties, such as improving intestinal transit and reducing cholesterol absorption, contributing to lipid control in balanced diets (Lise et al., 2021).

Regarding energy value, the caloric content of freeze-dried OPN leaves was 325.6 kcal/100 g of dry matter. This value is associated with its carbohydrate and protein content, with fats accounting for a smaller fraction of the caloric intake. Thus, OPN leaves stand out as a nutritious food with moderate energy density.

Therefore, freeze-dried OPN leaves show promise as a functional food, with high protein content, the presence of mucilage with prebiotic properties, and a composition rich in vitamins, minerals, and phytochemicals. Despite the presence of some antinutritional compounds, its culinary use or processing with appropriate techniques can enhance health benefits and reinforce its role as a PANC (non-conventional food plant) of nutritional interest, and freeze-drying is a process that preserves nutrients when compared to other studies that analyzed the fresh leaf (Barreira et al., 2020; Silva Porto et al., 2021).

**Table 1.** Physicochemical analysis of freeze-dried leaves.

Parameters	Average/DP
Physicochemical analyses	
aW	0.6 ± 0.04
Moisture (g 100 g <sup>-1</sup> )	6.87 ± 0.16
Ash (g 100 g <sup>-1</sup> )	0.89 ± 0.19
Protein (g 100 g <sup>-1</sup> )	17.37 ± 0.69
Lipids (g 100 g <sup>-1</sup> )	2.76 ± 0.45
Carbohydrate (g 100 g <sup>-1</sup> )	72.11 ± 0.78
Total energy value (kcal/100 g)	382.76 ± 2.31
Vitamin C (mg/100 g)	160.75 ± 3.90
Bioactive compounds and antioxidant potential	
Total phenolics (mg EAG/g)	55.47 ± 0.94
Flavonoids (mg EQ/g)	1.43 ± 0.04
β-carotene (mg/100 g)	0.73 ± 0.06
Lycopene (mg/100 g)	0.59 ± 0.38
Chlorophyll a (μg/g)	2,717 ± 0.45
Chlorophyll b (μg/g)	1,902 ± 0.83
DPPH (μmol TE/g)	191 ± 0.76
ABTS (μmol TE/g)	575.67 ± 0.04
Phytochemicals	
Coumarins	+
Tannins	+
Saponins	-
Anthraquinones	-
Alkaloids	+

Data expressed on a dry basis. C\* and VET\* + (positive); - (negative).

### 3.2 Bioactive compounds and antioxidant potential of lyophilized OPN leaf

The composition of bioactive compounds in lyophilized *P. aculeata* (OPN) leaves is presented in Table 1. The concentration of total phenolic compounds in the leaves was estimated at 55.47 ± 0.94 mg gallic acid equivalents (GAE) per 100 g, a significant value indicating a substantial capacity for neutralizing free radicals. Antioxidant activity was evaluated using ABTS and DPPH assays, with results of 575.67 ± 0.04 μM trolox/g and 191 ± 0.76 μM trolox/g, respectively. The ABTS method was more effective, which is consistent with previous findings in the literature, such as the study by Garcia et al. (2019), which found similar results in fresh OPN leaves. Analyzing other plant species, such as hibiscus (*Hibiscus sabdariffa* L.), which showed even higher levels, with 429.84 ± 5.16 mg GAE/100 g and antioxidant activity of 626.32 ± 8.03 μmol trolox/L, it was possible to detect that the effects of OPN leaves are lower; however, they also present beneficial effects for health (Abreu et al., 2020).

A study conducted by Souza et al. (2024) with taro leaves (*Xanthosoma sagittifolium*) showed not only a high content of phenolic compounds (122.3 ± 4.6 mg GAE/g), but also a significant hypocholesterolemic effect in dyslipidemic mice, associated with a reduction in serum LDL levels and an increase in HDL. In addition, the antioxidant activity measured by the DPPH and ORAC methods reinforced the species' potential as a functional food. With values similar to those found in OPN.

The cryo-drying technique has also been shown to be effective in preserving bioactive compounds. In basil leaves (*Ocimum basilicum*), Carvalho et al. (2024) observed that lyophilization maintained high levels of phenolic compounds ( $7.52 \pm 0.24$  mg GAE/g) and total flavonoids ( $5.04 \pm 0.14$  mg QE/g), in addition to significant antioxidant activity (ABTS: 71.53% and DPPH: 65.28%), surpassing methods such as oven drying. These results corroborate the application of lyophilization as a technology for retaining sensitive compounds in species with functional potential. Therefore, it can be said that the current work also managed to preserve the chlorophyll and flavonoid levels of lyophilized OPN leaves. This is because, when compared with Maciel et al. (2018), who used fresh leaves, the levels in the lyophilized leaf were higher.

Freeze-dried OPN leaves exhibit high levels of photosynthetic pigments, with a predominance of chlorophyll a ( $2.717 \pm 0.45$  mg/100 g) over chlorophyll b ( $1.902 \pm 0.83$  mg/100 g). Chlorophylls, which give the plant its typical green color—an important aspect for the sensory acceptance of vegetables and condiments—play relevant bioactive roles. In the case of OPN, its antioxidant properties, cellular detoxification potential, modulation of inflammatory processes, and promotion of tissue regeneration stand out (Silva Porto et al., 2021).

In comparison, in species such as *O. basilicum*, an inverse trend was observed, with higher levels of chlorophyll b relative to chlorophyll a. This inversion suggests that chlorophyll b may exhibit greater thermal stability, being less degraded during processing, such as in heat treatments. Although this is relevant for technological applications, the composition of OPN stands out for its balance between biological functionality and nutritional value (Garcia et al., 2019).

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In addition to chlorophylls, freeze-dried OPN also contains carotenoids in significant quantities, notably  $\beta$ -carotene ( $0.73 \pm 0.06$  mg/100 g) and lycopene ( $0.586 \pm 0.38$  mg/100 g). Although considered moderate levels, these compounds have high biological relevance. They act as natural antioxidants and as precursors of vitamin A, being essential for maintaining ocular and immune health and epithelial integrity (Moura et al., 2021). The combination of chlorophylls and carotenoids reinforces

the functional value of OPN, consolidating it as a non-conventional food plant (PANC) with high nutritional potential and application in formulations aimed at promoting health (Moura et al., 2021).

Another relevant compound found was vitamin C, with values of  $160.75 \pm 3.9$  mg of ascorbic acid/100 g of leaves. This value is higher than the recommended daily intake (RDA), which is 75 mg for adult women and 90 mg for adult men, according to international guidelines (Brasil, 2021). Vitamin C plays a fundamental role as a water-soluble antioxidant, neutralizing reactive oxygen species (ROS) and preventing oxidative damage to DNA, lipids, and proteins (Silva, N. N., et al., 2023). Consuming 100 g of fresh leaves is unlikely in practice, justifying the use of technological processes such as freeze-drying, which preserves bioactive compounds and allows the development of supplements in capsules or concentrated powders.

Discrepancies in reported vitamin C levels between different studies, such as that of Barreira et al. (2020), may be related to the analytical methodology used. High-performance liquid chromatography (HPLC), although more specific and sensitive, can present losses in the detection of ascorbic acid if samples are not properly preserved (Torres et al., 2021b). Conversely, the use of lyophilization and appropriate colorimetric methodologies can result in higher values, as observed in the studies discussed here.

Flavonoids, with a concentration of  $1.43 \pm 0.04$  mg/100 g, were also identified in lyophilized OPN leaves. These compounds exhibit multiple mechanisms of action, including the inhibition of pro-inflammatory enzymes, the modulation of cell signaling pathways, and protection against oxidative damage. Dala-Paula et al. (2024) highlighted that flavonoids are related to cardiovascular protection, anticarcinogenic, antiviral, and antidiabetic effects, and modulation of inflammatory processes. The structure of these compounds favors their interaction with enzymes and proteins that regulate oxidative metabolism, in addition to contributing to the protection of cellular DNA against mutations induced by external agents.

Thus, the data available in the scientific literature, combined with recent laboratory results, show that *P. aculeata* has high nutritional and phytochemical value, comparable to other species rich in bioactive compounds, with promising applications in the development of functional foods, nutraceuticals, and biomaterials with bioactive properties.

### 3.3 Phytochemicals from the lyophilized OPN leaf

Phytochemical analysis performed on lyophilized leaves of *P. aculeata* revealed the presence of alkaloids, coumarins, tannins, and flavonoids. These secondary metabolites play an essential role in protecting the plant against external agents and are widely recognized for their therapeutic properties, such as antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective activity, among others (Maciel et al., 2021; Silva Porto et al., 2021).

The presence of alkaloids was confirmed through specific reactions, consistent with previous studies that identified

compounds such as abrin, tryptamine, and mescaline in different parts of the plant (Moraes et al., 2021; Silva, N. N., et al., 2023). These substances exhibit a wide range of pharmacological activities, potentially acting as analgesics, antitumor agents, antiparasitics, bronchodilators, and neuroprotective agents. According to Maciel et al. (2021), alkaloids also demonstrate an effect on the modulation of the central nervous system, making them promising in phytotherapeutic formulations.

Coumarins were also identified, with strong evidence of their antioxidant activity. These compounds are recognized for their ability to eliminate free radicals and prevent cellular oxidative stress, in addition to exerting anticoagulant, antimicrobial, anti-inflammatory, and photoprotective action (Agostini-Costa, 2020; Cruz et al., 2021). The presence of coumarins in lyophilized leaves confirms previous findings and reinforces their potential use in dermocosmetic and pharmaceutical formulations. However, their concentration may vary according to genetic factors, the phenological stage of the plant, and environmental conditions (Souza et al., 2021).

These metabolites are of great scientific interest because they participate in several biochemical processes with therapeutic relevance. In addition, coumarins have stood out as antioxidant agents with fluorescence properties, used as probes in pharmacological studies (Franco et al., 2020). Together with other phenolic compounds, coumarins contribute to the redox stability of the cell and may modulate cell signaling pathways related to apoptosis and tissue regeneration.

Tannins were confirmed through reaction with ferric chloride, with dark blue coloration and precipitation observed, indicative of the presence of hydrolyzable and condensed tannins. Studies such as those by Garcia et al. (2019) and Torres et al. (2021b) also detected tannins in leaves and extracts of OPN, reinforcing their action as natural antioxidants. These compounds exert an astringent effect, have a chelating capacity for heavy metals, and have potential as inhibitors of digestive enzymes, in addition to acting as prebiotics in the regulation of the intestinal microbiota.

These results reinforce the functional value of *P. aculeata* and the importance of its phytochemical study, especially under post-processing conditions such as lyophilization, which can preserve these properties and enhance its application in nutraceutical and pharmaceutical products aimed at preventive health.

### 3.4 Microscopy of OPN leaves

SEM micrographs of lyophilized OPN leaves are illustrated in Figure 1.

As observed in Figure 1 (100 kx resolution), the morphology shows fibrous leaf structures marked by deep folds and collapsed regions, indicative of cell wall shrinkage and loss of turgor resulting from the previous cryo-drying process. It also presents a rough surface with residues of plant parenchyma and sclerenchyma; the veins indicate the presence of fibers in the OPN powders. The maintenance of the leaf structure indicates that cryo-drying at low temperatures < 100°C and low pressure

has a small effect on the microstructure of the OPN powder (Figure 1).

The enlarged image (Figure 1, 500 kx resolution) shows a possible effect of the temperature and low-pressure combination on the degradation of plant cell wall polysaccharides, breaking the structures of cellulose, hemicellulose, and lignin bundles. These changes may affect the ability of OPN powders to absorb water and oil, key technological properties for industrial use (Giombelli et al., 2023).

Torres et al. (2021b) found that the application of compressed fluids to *P. aculeata* leaves exhibited an increase in fissures and microfractures on the surface, expanding the contact area and favoring the release of phenolic and protein compounds. Thus, the observed morphology reflects the combination of the drying method and extraction treatments, resulting in a surface with greater roughness and technofunctional potential, adjustable according to the desired application, whether for thickening, gelation, or controlled release of bioactives.

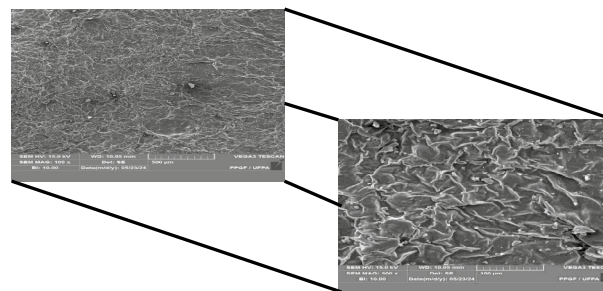
Comparing these effects of cryo-drying with conventional drying in research with maple leaves (*Acer pseudoplatanus*), as shown in the research by Yeasmen and Orsat (2024), the strength of the combined interaction of temperature and mechanical grinding on the morphological structure of the leaves, which was caused by the breaking of hydrogen bonds.

In research applying cryo-drying to freeze-dried leaves of taioba (*Xanthosoma taioba*) and jambu (*Acmella oleracea*), these showed similar modifications in the structures of the cell wall (plant parenchyma) with fiber bundles, possibly cellulose and hemicellulose constituents similar to those in this research (Santos et al., 2021; Santos et al., 2022).

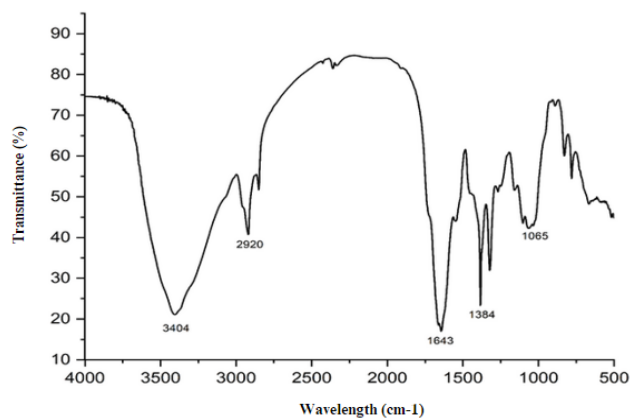
### 3.5 FTIR spectroscopy of the freeze-dried OPN leaf

In the analysis of the freeze-dried *P. aculeata* leaf, it was possible to observe several bands in the spectrum (Figure 2) that indicate the presence of relevant organic compounds.

Among the most significant intervals are the absorption bands between 3,404 and 1,065  $\text{cm}^{-1}$ . The band near 3,404  $\text{cm}^{-1}$ , broad in shape and low in intensity, is associated with the presence of hydroxyl groups (-OH), common in compounds with antioxidant activity and a striking characteristic of plant structures (Paiva, 2010; Pinheiro et al., 2024). The peaks around



**Figure 1.** Scanning Electron Microscopy of lyophilized OPN, 100 kx and 500 kx resolution.



**Figure 2.** Fourier transform infrared absorption spectroscopy in OPN powder.

2,920 and 1,384  $\text{cm}^{-1}$  suggest the existence of methyl ( $\text{CH}_3$ ) and methylene ( $\text{CH}_2$ ) groups, which are frequently associated with molecules such as lipids and polysaccharides.

In the 1,643  $\text{cm}^{-1}$  region, a band related to the vibration of the carbon–oxygen double bond ( $\text{C}=\text{O}$ ) was identified, characteristic of compounds such as carboxylic acids and proteins. Furthermore, the band around 1,065  $\text{cm}^{-1}$  is attributed to the presence of  $\text{C}-\text{O}$  bonds, found in structures such as esters, ethers, and other oxygenated compounds of plant origin (Macedo et al., 2023).

Another noteworthy point was the presence of a band around 890  $\text{cm}^{-1}$ , which is indicative of polysaccharide structures, probably associated with the leaf cell wall. This signal may be related to the plant's mucilage, a functional polysaccharide that plays an important role in its nutritional composition.

Similar results were reported by Macedo et al. (2023), who analyzed freeze-dried leaves of the same species, reinforcing the consistency of the findings regarding the presence of these chemical groups in the fresh leaf. It is observed, therefore, that the chemical composition of the leaf is complex and rich in compounds that go beyond proteins, including structures such as polyphenols, mucilages, and other bioactive substances.

Finally, the presence of nitrogenous compounds was also detected, indicating that a significant portion of the leaf proteins is associated with stable secondary structures, such as sheets and helices, as suggested by Neves et al. (2020). These findings reinforce the importance of further investigations into the distribution and concentration of these nutrients directly in the fresh leaves of *P. aculeata*, considering its potential as a functional food and plant source of bioactive compounds.

## 4 CONCLUSION

The research shows that the use of freeze-drying maintains the bioactive functional composition of OPN, making it less perishable, helping to reduce moisture and water activity, and with high potential for dietary and medicinal application, prolonging its shelf life and increasing its application on an industrial scale.

The physicochemical analyses revealed a significant protein content, reinforcing its applicability in nutritional strategies aimed at healthy eating, especially in diets with restrictions on foods of animal origin. Furthermore, the vitamin C content contributes to its antioxidant action and can act as a cofactor in various metabolic functions.

Phytochemical screenings confirmed the presence of compounds such as alkaloids, tannins, and coumarins, which have recognized antioxidant, anti-inflammatory, and antimicrobial activity, expanding the functional value of this plant. The antioxidant activity measured by the DPPH and ABTS methods reinforces the potential of freeze-dried OPN in neutralizing free radicals, indicating its contribution to the prevention of oxidative stress and associated diseases.

Spectroscopic analyses using FTIR made it possible to identify functional groups related to the presence of proteins, polysaccharides, and phenolic compounds, while images obtained by SEM revealed important structural aspects of the leaf, suggesting the preservation of structures that favor the release and action of bioactive compounds.

Therefore, this article reinforces the relevance of freeze-dried OPN leaves as a functional and accessible ingredient, with strong appeal for applications in industrial-scale production strategies and the use of its bioactive compounds to promote health. By gathering consistent nutritional and functional data, this work contributes to the scientific and popular appreciation of this unconventional food plant (PANC), encouraging its use in daily diets and its recognition as a strategic functional food in the construction of more diversified, sustainable diets rich in bioactive compounds.

## AUTHORS' CONTRIBUTIONS

JG: conceptualization, methodology, investigation, data curation, formal analysis, original draft preparation, writing—reviewing. LMCF: investigation, methodology, investigation, data curation, formal analysis, data curation. OVS: investigation, methodology, data curation, writing—reviewing, investigation, data curation, writing—reviewing and editing, funding acquisition, supervision, project administration.

## DATA AVAILABILITY STATEMENT

The authors declare that the data supporting the findings of this study are available within the paper. Should any raw data files be needed in another format, they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

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