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Original Article

Comparative analysis of selected nutrients in *Pereskia* species

Análise comparativa de nutrientes selecionados em espécies de *Pereskia*

J. D. Lima[a], R. F. Menegazzo[a], M. F. Silva[b], M. P. S. B. Barbosa[c], A. R. Schuelter[d], E. Jacomassi[c] and G. J. Silva[a*]

[a]Universidade Paranaense - Unipar, Departamento de Biotecnologia, Programa de Pós-graduação em Biotecnologia Aplicada à Agricultura, Umuarama, PR, Brasil

[b]Universidade Tecnológica Federal do Paraná - UTFPR, Departamento de Engenharia de Bioprocessos e Biotecnologia, Toledo, PR, Brasil

[c] Universidade Paranaense - Unipar , Departamento de Plantas Medicinais e Fitoterápicos na Saúde Primária, Umuarama, PR, Brasil

[d]Universidade Estadual do Oeste do Paraná – Unieste, Programa de Pós-graduação em Engenharia Agrícola, Cascavel, PR, Brasil

Abstract

This investigation undertakes a comparative quantitative assessment of the macronutrient and micronutrient profiles of *Pereskia grandifolia* (Haworth) and *Pereskia aculeata* (Miller), commonly known as Ora-pro-nóbis, categorized as Non-Conventional Food Plants (NCFPs), juxtaposing these profiles against established food crops. Quantitative analysis revealed that both *Pereskia* species exhibit elevated concentrations of micronutrients such as iron and zinc, surpassing those of *Phaseolus vulgaris* (Linnaeus) and *Lens culinaris* (Linnaeus). Specifically, solubilized zinc levels in *P. aculeata* (13.6 mg/100g) and *P. grandifolia* (13.9 mg/100g) exceed those of lentils (7.5 mg/100g) and beets (3.7 mg/100g). Similarly, solubilized iron levels in *P. grandifolia* (12.4 mg/100g) and *P. aculeata* (10.5 mg/100g) surpass those of lentils (3.0 mg/100g) and beets (3.8 mg/100g). However, phytate levels are comparatively lower in the *Pereskia* species, with *P. grandifolia* demonstrating reduced concentrations at 1500 mg/kg, alongside significant preponderance in protein (20.8 g/100g) and nitrogen (14.6 g/100g). Principal Component Analysis (PCA) was applied, demonstrating a strong associative propensity of iron, zinc, and protein with the *Pereskia* genus. In contrast, Pearson's correlation coefficients elucidated a complex interdependency among the nutrient variables, except for an inverse correlation between phytate concentration and both iron and protein levels. In conclusion, this research sheds light on the enriched nutritional profiles of *P. grandifolia* and *P. aculeata*, emphasizing their potential as valuable dietary resources.

Keywords: micronutrients, non-conventional food plants (NCFPs), *Pereskia aculeata*, *Pereskia grandifolia*, protein, phytate.

Resumo

Esta investigação realiza uma avaliação comparativa quantitativa dos perfis de macronutrientes e micronutrientes de *Pereskia grandifolia* (Haworth) e *Pereskia aculeata* (Miller), comumente conhecida como Ora-pro-nóbis, categorized as Non-Conventional Food Plants (NCFPs), comparando esses perfis com os de culturas alimentares estabelecidas. A análise quantitativa revelou que ambas as espécies de *Pereskia* apresentam concentrações elevadas de micronutrientes como ferro e zinco, superando aquelas de *Phaseolus vulgaris* (Linnaeus) e *Lens culinaris* (Linnaeus). Especificamente, os níveis de zinco solubilizado em *P. aculeata* (13,6 mg/100g) e *P. grandifolia* (13,9 mg/100g) excedem os de lentilhas (7,5 mg/100g) e beterrabas (3,7 mg/100g). De forma semelhante, os níveis de ferro solubilizado em *P. grandifolia* (12,4 mg/100g) e *P. aculeata* (10,5 mg/100g) superam os de lentilhas (3,0 mg/100g) e beterrabas (3,8 mg/100g). No entanto, os níveis de fitato são comparativamente mais baixos nas espécies de *Pereskia*, com *P. grandifolia* demonstrando concentrações reduzidas de 1500 mg/kg, além de uma preponderância significativa em proteína (20,8 g/100g) e nitrogênio (14,6 g/100g). Foi aplicada a Análise de Componentes Principais (PCA), que demonstrou uma forte associação entre ferro, zinco e proteína com o gênero *Pereskia*. Em contraste, os coeficientes de correlação de Pearson elucidaram uma interdependência complexa entre as variáveis nutricionais, exceto por uma correlação inversa entre a concentração de fitato e os níveis de ferro e proteína. Em conclusão, esta pesquisa destaca os perfis nutricionais enriquecidos de *P. grandifolia* e *P. aculeata*, enfatizando seu potencial como valiosos recursos dietéticos.

Palavras-chave: micronutrientes, plantas alimentícias não convencionais (PANCs), *Pereskia aculeata*, *Pereskia grandifolia*, proteína, fitato.

*e-mail: glacyjaqueline@prof.unipar.br
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1. Introduction

Adequate nutrition is fundamental to maintaining health and well-being, and the understanding of the nutritional elements of Non-Conventional Edible Plants (NCEPs) holds significant importance in this regard (Yan et al., 2023). In addition to a vast profile of vitamins and minerals, NCEPs provide essential nutrients like iron (Fe) and zinc (Zn), crucial for immune function and cognitive development (Pereira et al., 2023). NCEPs are esteemed for their nutrient richness, exhibiting a diverse array of vitamins, minerals, fibers, and bioactive compounds that can confer substantial health benefits (Milião et al., 2022). The bioavailability of Fe and Zn is particularly noteworthy, as these elements are vital for numerous physiological processes and are often less accessible in plant-based diets (Zhang et al., 2022).

Beyond their beneficial components, it is critical to acknowledge that substances previously classified as antinutrients within these plants are currently being reassessed for their antioxidant properties (Sharma et al., 2021). These compounds, once solely regarded as inhibitors of essential nutrient absorption, are now under investigation for their role in combating oxidative stress and contributing to the prevention of chronic diseases (Hoff et al., 2022). This evolving perspective underscores the intricate nutritional landscape of NCEPs and the necessity of ongoing research to fully elucidate their dietary impacts and therapeutic potentials.

Within the realm of NCEPs, the species *Pereskia grandifolia* and *Pereskia aculeata* are distinguished by their exceptional nutritional qualities. Popularly known in some regions of Brazil as 'Ora-pro-nóbis,' these plants have become subjects of study due to their high concentration of proteins, vitamins, minerals, and dietary fibers (Santos et al., 2022). Investigations into these species have revealed not only their rich content of minerals but also the presence of these minerals in solubilized forms, suggesting enhanced bioavailability and potential benefits for nutrient uptake (Aispuro-Hernández et al., 2022). Both species are also sources of phenolic compounds and flavonoids, acknowledged for their antioxidant properties and therapeutic potential (Maciel et al., 2023). The reassessment of so-called antinutrients in these plants, now recognized as antioxidants, broadens the understanding of their nutritional value and potential to enrich diets, contributing to food security and offering sustainable alternatives for cultivation and consumption.

Thus, the aim of this study was to quantify the nutritional components of interest — iron (Fe), zinc (Zn), tannins, phytates, proteins, and carbon — in the plant species *P. grandifolia* and *P. aculeata*, in comparison with common beans, lentils, and beets.

2. Material and Methods

2.1. Plant material

Pereskia aculeata and *Pereskia grandifolia* species were collected from the Medicinal Garden of the Paranaense University (UNIPAR), located at the geographical coordinates 23.765568 S, 53.276216 W. These specimens are part of the medicinal plant collection of the university (Figure 1). These plants are registered in the Brazilian National System of Management of Genetic Heritage (SisGen), under the registration number ACFB1A6. Bean, lentil, and beet samples were collected from supermarkets, sourced from local agricultural producers from the same city.

Figure 1. Photographic depiction of *Pereskia grandifolia* (A) and *Pereskia aculeata* (B) species harvested from the Medicinal Garden at Paranense University (UNIPAR).

2.2. Sample preparation

The beans were initially washed and then subjected to maceration for 12 hours in deionized water at a ratio of 1:3 (bean to water), after which the water was discarded. Fresh deionized water was then added at a 1:2 (bean to water) ratio for subsequent cooking. The cooking process involved the use of an autoclave where the beans were subjected to a thermal treatment for 10 minutes at 121 ºC, adhering to the methodology described by Molina et al. (1975).

Beetroot samples were cubed to a uniform size of approximately 1 cm^3. Lentils, bens pre-processed and the cubed beetroots, were subjected to thermal processing in an uncovered stainless-steel cookware over a medium flame for a duration of 30 minutes, employing a 1:2 biomass-to-deionized water ratio with incremental water addition during the simmering phase. Post-cooking, the specimens and residual liquid were desiccated in a convection oven at 60 ºC for a period of 48 hours, pulverized using a Marconi-brand knife mill (special model, 60 cycles), sifted through a 30-mesh screen, encased in polyethylene containers, and refrigerated at an approximate temperature of 11 ºC pending analytical assessment. Ora-pro-nobis leaf material was air-dried in a convection oven at 35 ºC over a span of 28 days and subsequently underwent the meshing procedures.

2.3. Concentration of ash, moisture, protein and nitrogen

Using the Kjeldahl technique (Instituto Adolfo Lutz, 2008), the nitrogen content was assessed. Three replicates of a 0.5 g ground specimen were dried to a stable weight at 65°C and then stored on parchment. Following the preparation of the sulfuric acid (H_2SO_4), boric acid (H_3BO_3) indicator, hydrochloric acid (HCl), and sodium hydroxide (NaOH) solution, the sample underwent digestion in a Marconi MA 4025 apparatus with an addition of 5 mL of Aldrich® sulfuric acid (PA) to the tubes. These tubes were then subjected to a gradual temperature increase over 3 hours, culminating at 450 °C. The processed sample was then distilled using a Marconi MA 036 apparatus with the inclusion of 25 mL of 50% sodium hydroxide. Capturing the distillate at a steam temperature of 100 °C, it was transferred to an Erlenmeyer flask already containing 25 mL of a 4% boric acid solution. This distilled solution underwent titration with 0.1 M HCl, marking completion upon a color change from green to pink. Finally, the nitrogen content was quantified based on the obtained data. The results were expressed in g/100g.

2.4. Fe and Zn concentration

The concentrations of iron and zinc were assessed following the standard analytical procedure (AOAC, 1984), albeit with modifications. A 1:12 ratio of 1M HNO_3 was introduced to the sample and maintained at 32 ºC for a period of 48 hours. Subsequently, the mixture was heated to 100 º C until complete evaporation took place. After which, a 1:6 ratio of H_2O_2 was incorporated, the solution was filtered, and its volume was adjusted to 10 ml. Measurements were then undertaken using the Flame Atomic Absorption Spectrophotometer, specifically the GBC model 932. The results were expressed in mg/100g.

2.5. In vitro availability of Fe and Zn

The *in vitro* analysis for iron and zinc availability was conducted based on the guidelines provided by AOAC (1984), with certain modifications. A quantity of 250 mg of the specimen was blended with 15 ml of ultrapure water, maintained at 37 ºC, and stirred at 200 rpm for an hour. During the preparation, 5M HCl was integrated to achieve a pH of 2, followed by the addition of 0.75 mL of pepsin that had been pre-mixed with 0.1 M HCl. For the hydrolysis phase, the pH was brought up to 6 using 1 M sodium bicarbonate, after which a pancreatin mixture was added and the solution was centrifuged at 250 rpm and 4 ºC. Next, a combination of 5 ml NaCl and 5 ml of 5 M KCl, both at 37 ºC, was introduced, and the mixture was agitated at 200 rpm for another hour. Both the sediment and the clear liquid above were then subjected to drying in an air-circulating oven at 60 ºC until a stable weight was achieved. Post-drying, a 1:12 ratio of 1M HNO_3 was incorporated and kept at 32 ºC for two days. The mixture was then heated to 100 ºC until it evaporated completely. This was followed by the addition of a 1:6 ratio of H_2O_2, after which the solution was filtered and its volume brought up to 10 ml. Measurements were taken using the Flame Atomic Absorption Spectrophotometer, specifically the GBC 932 model. The results were expressed in mg/100g.

2.6. Determination of phytic acid and tannin

The assessment of Phytic Acid was conducted based on the procedure outlined by Haug and Lantzsch (1983), though with modifications. Measurements were taken at 515 nm using the SPECTRAMAX PLUS 384 microplate reader (ELISA). Tannin quantities are denoted in terms of mg of catechin per kg of tissue. The findings were presented as mg kg^{-1} of phytic acid equivalents for every g^{-1} of the analyzed specimen, using the reference catechin curve. The quantification of tannin was based on the guidelines provided by Price et al. (1980), albeit with certain changes. The specimen's reading was done on an ELISA spectrophotometer at a wavelength of 500 nm. A reference curve was constructed to derive the equation. The results were expressed in mg/kg.

Furthermore, calibration curves were developed to estimate the concentrations of tannin and phytic acid. The equations obtained from the calibration curves were as follows: tannin = $0.3255x + 0.0026$ ($R^2 = 0.989$) and phytic acid=$0.2541x+0.0038$ ($R^2 = 0.975$). These equations establish the relationship between the concentration of analytes and the response of the analytical method, allowing for precise quantification.

2.7. Statistical analysis

All measurements were conducted in triplicate. For enhanced clarity, the data were represented using a box plot graph. We utilized Principal Component Analysis (PCA) to decode the inherent variability in the dataset. Before running PCA, the dataset was normalized to ensure a mean of zero and a standard deviation of one. The dimensionality was then condensed to the first two principal components, capturing the bulk of the data's variance. A Factor Analysis was also performed to unearth underlying patterns and understand the interconnections among the different treatments. This analysis yielded two main factors, which highlighted prevalent trends in the treatments. The outcomes from both the PCA and Factor Analysis were integrated and showcased on a two-dimensional graph. On this graph, distinct plant species were color-coded and positioned accordingly, while the treatments were superimposed to illustrate their impact on the extracted factors. To better understand the direct relationships between the studied variables, a correlation study using the Pearson correlation coefficient was conducted. Correlations are categorized into high (values ranging from 1 to 0.7 or -1 to -0.7), medium (values from 0.69 to 0.4 or -0.69 to -0.4), and low (values closer to 0), both in positive and negative directions.

The data processing and visualization tasks were accomplished using the Python language, leveraging tools from the pandas, seaborn, and scikit-learn libraries.

2.8. Chemicals and reagents

Chemicals and reagents used in this study were of analytical grade and sourced from reputable suppliers. Sulfuric acid (H_2SO_4) was employed for digestion purposes, while boric acid (H_3BO_3) served as an indicator in the Kjeldahl method. Hydrochloric acid (HCl) was utilized for pH adjustment and as a component in the pepsin solution. Sodium hydroxide (NaOH) was used for sample digestion and pH adjustment, particularly in the Kjeldahl and in vitro availability assays. Pepsin and pancreatin were enzymatic reagents involved in the in vitro availability experiments. Sodium bicarbonate ($NaHCO_3$) was used to maintain pH during the in vitro availability assessment. Hydrogen peroxide (H_2O_2) was employed for sample digestion in the determination of Fe and Zn concentrations. Additionally, 1M nitric acid (HNO_3) was utilized for digestion purposes, and ultrapure water was used throughout the experimental procedures for sample preparation and dilution.

3. Results

Based on the Boxplot analysis (Figure 2), species *P. grandifolia* and *P. aculeata* show elevated concentrations of Fe and Zn in both total and solubilized forms. For solubilized Zn, bean and lentil samples have higher values compared to beet. In terms of tannins, *P. grandifolia* has the highest concentration, while bean and lentil exhibit lower levels, followed by *P. aculeata* and beet. Regarding phytates, species from the *Pereskia* genus display reduced values, contrasting with the higher concentrations found in bean and lentil. In relation to protein and nitrogen content, *P. grandifolia* has the highest concentration, with *P. aculeata* having the next highest protein concentration. For carbon, lentil and bean have the highest concentrations, while *Pereskia* species, along with beet, show lower values.

Considering the complexity of the dataset, we utilized Principal Component Analysis (PCA) for dimensionality reduction. The results from PCA (Figure 3), depicted through a Biplot, are influenced by two main components that capture 96.9% of the total variance. This substantial percentage highlights the importance of these components in elucidating the primary trends within our dataset. The analysis conducted via PCA reveals that the variables under investigation, pertaining to the nutrients, predominantly reside in the lower quadrants of the biplot. Notably, phytate and carbon are positioned proximate to the bean and lentil samples, while iron, zinc (both total and solubilized), protein, and nitrogen are associated with the 'Ora-pro-nóbis' species (*P. aculeata* and *P. grandifolia*). In contrast, tannin is distinctly located in the upper quadrant, aligned with the *P. aculeata* and beet species.

To assess the degree of interrelation among the evaluated variables, a Pearson correlation matrix was executed (Figure 4). Within this matrix, it was discerned that variables such as iron, solubilized Fe, total Zn, and protein exhibited strong positive correlations with solubilized Zn. Regarding to the total Zn variable, significant correlations were observed with total Fe, solubilized Fe, tannin, protein, and nitrogen. The variables solubilized Fe and total Fe not only showed great correlations with each other but also with tannin and protein. Both exhibited a pronounced negative correlation with phytate. Tannin displayed a potent positive correlation with protein and negative associations with carbon and phytate. The variable phytate manifested a strong correlation with carbon and a negative interrelation with protein. Furthermore, protein and nitrogen were found to be highly intercorrelated.

4. Discussion

The primary objective of this study was to delve into the understanding of *Pereskia* species, which are recognized as NCFPs, and to assess their nutritional potential in comparison with other species that are already established for their alimentary benefits. NCFPs, such as *P. grandifolia* and *P. aculeata*, have been garnering attention due to their traditional uses and potential nutritional and health benefits (Silveira et al., 2020; dos Santos et al., 2022) which have not been extensively explored scientifically.

The data delineated through the Boxplot and PCA, when correlated with the Pearson correlation matrix, provided a comprehensive and nuanced overview of the chemical composition of the species under study. The species *P. grandifolia* and *P. aculeata* were particularly notable for their high concentrations of Fe and Zn, in both total and solubilized forms. These findings are consistent with the outcomes reported in the literature (Silva et al., 2023) and bear significant nutritional importance considering the essential role of iron in oxygen transport and zinc in immune function, among other metabolic processes (Islam et al., 2023). Nevertheless, there is a substantial void in the literature regarding studies on the bioavailability of these minerals, highlighting a pressing research avenue for future explorations and dietary applications.

The bioavailability of the micronutrients Fe and Zn in NCFPs is a critical variable in assessing their nutritional value. In the case of the species *P. grandifolia* and P. *aculeata*, an elevated *in vitro* bioavailability of these minerals has been observed, which heightens interest in their nutritional properties. However, the presence of tannins, particularly

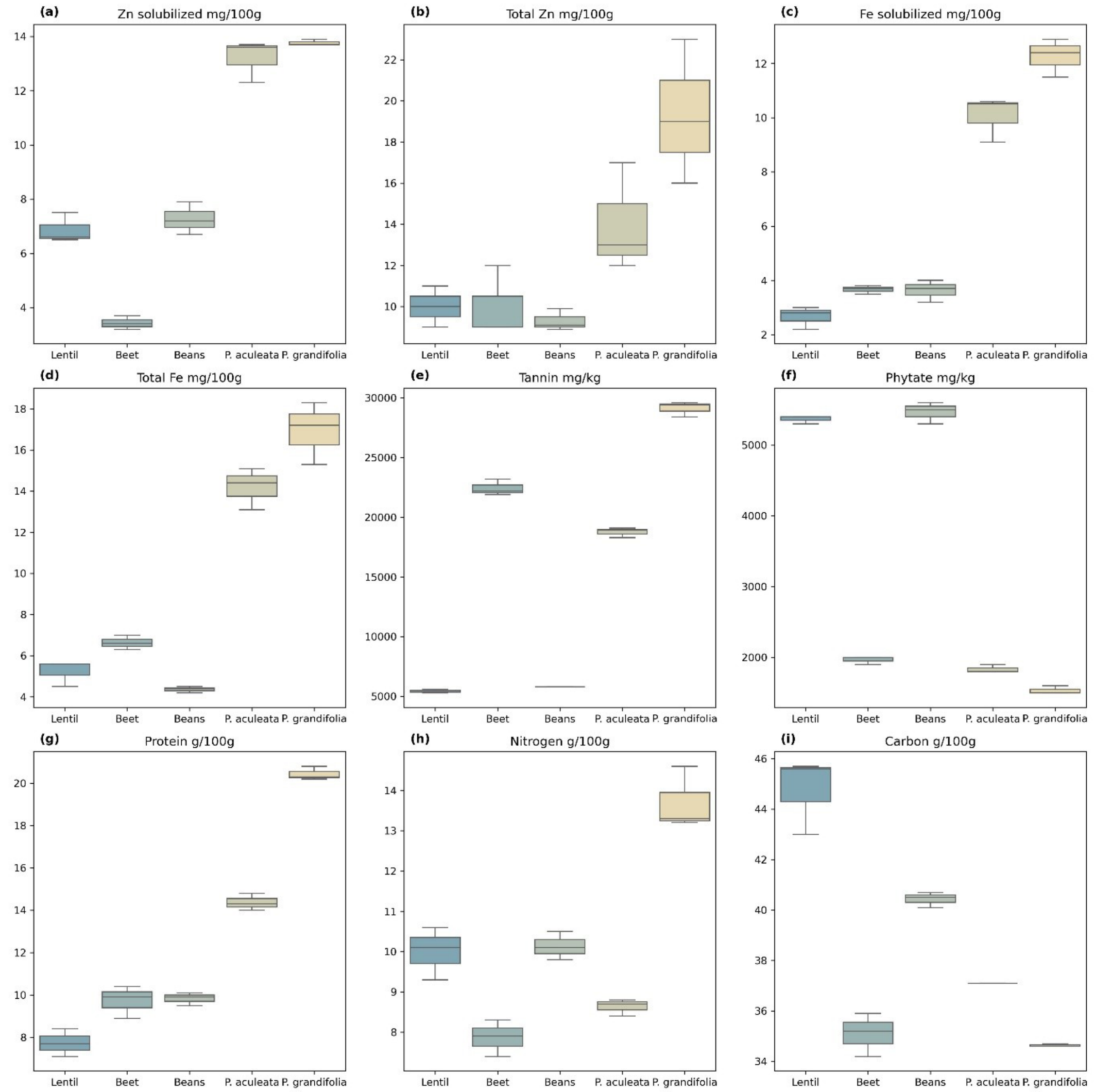

Figure 2. Boxplot analysis illustrating the distribution of various chemical components across different species: Lentil, Beet, Beans, *P. aculeata* and *P. grandifolia.* (a) Zn solubilized (mg/100g); (b) Total Zn (mg/100g); (c) Fe solubilized (mg/100g); (d) Total Fe (mg/100g); (e) Tannins (mg/kg); (f) Phytate (mg/kg); (g) Protein (g/100g): (h) Nitrogen (g/100g); (i) Carbon (g/100g).

high in *P. grandifolia*, may add an additional layer of complexity in interpreting these results. Tannins, known for their ability to chelate and bind to proteins and minerals (Abera et al., 2023), could theoretically compromise the absorption of Fe and Zn, thus limiting their biological availability. Nevertheless, the actual impact of tannins on the bioavailability of these minerals depends on a variety of factors, such as the chemical form of the tannins, the composition of the diet, the food preparation method, and the presence of other dietary components that may affect the solubility and absorption of the minerals (Platel and Srinivasan, 2016; Singh and Prasad, 2023).

Conversely, the antioxidant properties of tannins suggest a protective role against lipid peroxidation and cellular damage caused by free radicals (Rudrapal et al., 2022), potentially offering health benefits that may counterbalance their antinutritional effects. Various studies have investigated the antioxidant capacity of *Pereskia* species, confirming their significant potential (Pinto et al., 2012; Maciel et al., 2021). Indeed, the role of tannins as antioxidants is especially critical in conditions of increased oxidative stress, contributing to the prevention of chronic diseases (Ramos-Lopez et al., 2022; Marcucci et al., 2023).

In addition to tannins, phytates, found in lower quantities in *Pereskia* species as opposed to higher concentrations in beans and lentils, also exhibit both antinutritional and antioxidant characteristics (Zhang et al., 2022). While they can form complexes

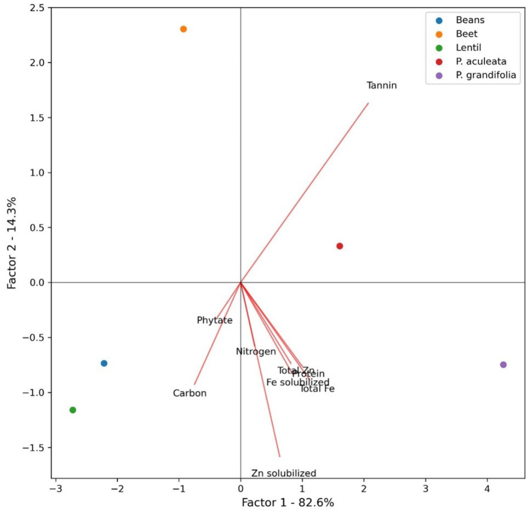

Figure 3. Biplot representation from Principal Component Analysis (PCA) showcasing the assessed species (bean, lentil, beet, *P. aculeata*, and *P. grandifolia*) and the evaluated nutrients (phytate, carbon, iron, solubilized iron, total zinc, solubilized zinc, protein, nitrogen, and tannin).

with essential minerals, reducing their bioavailability, their antioxidant properties may offer protective benefits against oxidative stress, similar to tannins (Pires et al., 2023). Understanding the dual nature of both tannins and phytates is crucial; while they may impede the bioavailability of certain minerals, they also play a protective role in cellular health (Cianciosi et al., 2022).

Additionally, the bioavailability of iron and zinc can be modulated through various strategies aimed at reducing the impact of antinutritional compounds such as phytates and tannins. Among these strategies, food processing methods like fermentation, germination, and cooking have demonstrated efficacy in degrading phytates, thereby promoting greater mineral release for absorption (Feizollahi et al., 2021). The addition of ascorbic acid (vitamin C) to meals can also significantly enhance the absorption of non-heme iron, partially neutralizing the inhibitory effects of phytates (Bester, 2023).

In the specific case of tannins, techniques such as water soaking and hydrothermal processing (boiling, steaming, and pressure cooking) significantly reduce tannin content in legume seeds, thereby improving the availability of proteins and minerals. Studies have shown

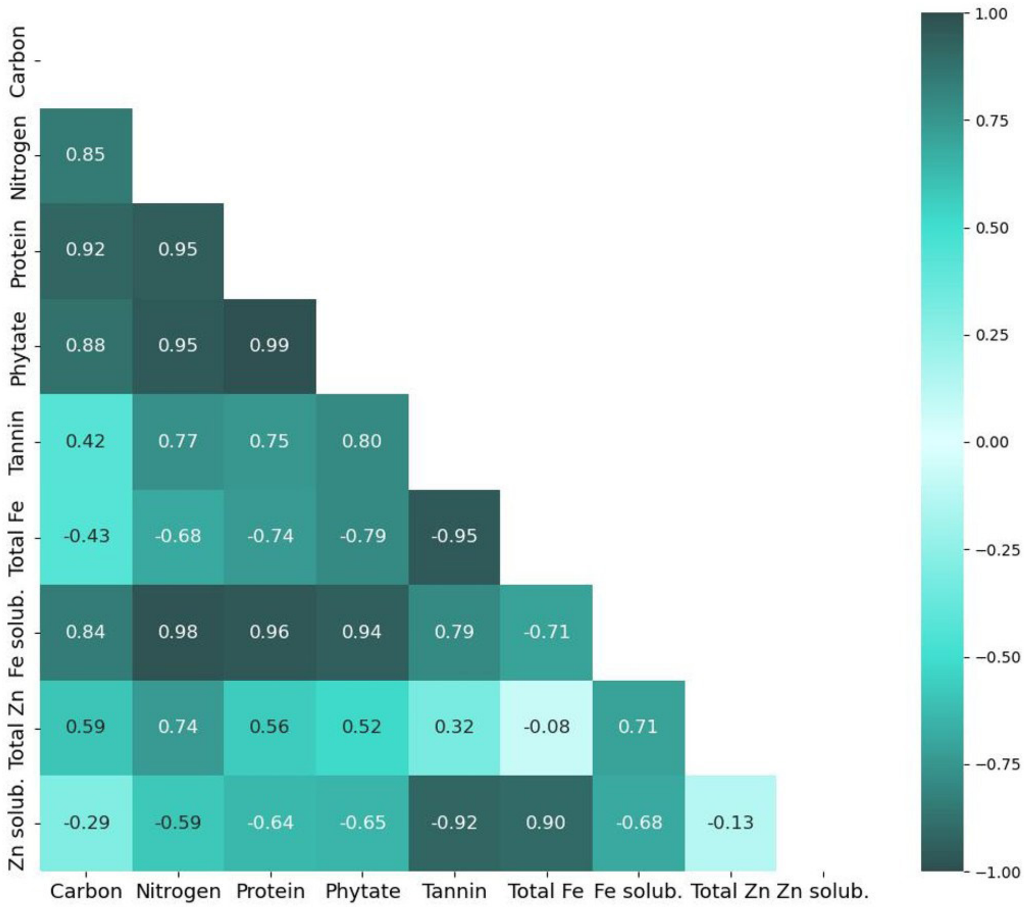

Figure 4. Pearson correlation matrix illustrating the interrelationships among the evaluated variables: iron, solubilized Fe, total Zn, protein, tannin, phytate, carbon, and nitrogen. Correlations are categorized into high (values ranging from 1 to 0.7 or -1 to -0.7), medium (values from 0.69 to 0.4 or -0.69 to -0.4), and low (values closer to 0), both in positive and negative directions.

that soaking at varying hydration levels and employing hydrothermal techniques can result in reductions of up to 78% in tannin content, facilitating the absorption of essential nutrients (Ojo, 2022).

Therefore, the implementation of these culinary and dietary practices can mitigate the antinutritional effects of phytates and tannins present in *P. grandifolia* and *P. aculeata*, optimizing mineral utilization and reinforcing the potential of these species as important nutritional resources.

Therefore, it is essential to consider the interaction between tannins, phytates and micronutrients from a comprehensive perspective, considering not only the antinutritional potential but also their antioxidant benefits and the influence of other dietary factors on the bioavailability of Fe and Zn. A careful assessment of these parameters can elucidate the true impact of tannins and phytates on mineral nutrition and assist in the development of strategies that optimize the utilization of nutrients contained in *P. grandifolia* and *P. aculeata*.

Assessing the data from a more global perspective, utilizing more robust statistical analyses such as Principal Component Analysis (PCA) and the Pearson correlation matrix, we were once again able to confirm the nutritional significance of the *Pereskia* species. In addition to a positive correlation with the levels of iron and zinc (both total and solubilized), a positive association was also observed with protein content in both *P. grandifolia* and *P. aculeata*, endorsing the hypothesis that these species can be classified as superfoods (Fernandes et al., 2022). Furthermore, multivariate analysis reveals a notable phenomenon: an inverse correlation between phytates and iron absorption. This suggests that the presence of phytates in these *Pereskia* species may exert a less pronounced antinutritional effect on the bioavailability of iron compared to conventional sources rich in phytate, such as beans and lentils. This distinction implies that *Pereskia* species may offer an expanded nutritional profile, potentially enhancing the bioavailability of critical minerals and protein, which are vital to human health.

The findings underscore the importance of these species for their traditional uses and remarkable nutritional properties as rich sources of iron and zinc. This work also emphasized the need for future research on nutrient bioavailability and the impact of compounds like tannins and phytates on mineral absorption. Additionally, the genetic study pointed out intraspecific complexity, suggesting variations that could directly influence nutritional characteristics. Thus, the results reaffirm the value of Pereskia as NCFPs and pave the way for future research that could expand the use of these species in dietary and therapeutic contexts.

Future research should address the limitations of this study, particularly the collection of *Pereskia* samples from a single location (UNIPAR), which may not account for environmental influences such as soil type, climate, and seasonal variations on the plant's nutritional composition. Expanding the sampling to different regions and including edaphic analyses will be essential to correlate soil characteristics with iron and zinc content. Additionally, it is fundamental to conduct *in vivo* studies, such as those using anemic rat models, to accurately assess the absorption and bioavailability of nutrients, especially iron and zinc. These investigations will provide a deeper understanding of the nutritional potential of *P. grandifolia* and *P. aculeata*, reinforcing their role as valuable non-conventional food plants (NCFPs) and supporting their broader use in dietary and therapeutic applications.

5. Conclusion

The conclusion of the study emphasizes the significance of *P. grandifolia* and *P. aculeata* species as Non-Conventional Food Plants with substantial nutritional potential, which stand out for their high concentrations of iron and zinc, essential micronutrients for human health. Although the presence of compounds such as tannins and phytates in their composition could potentially reduce the bioavailability of these minerals, they also offer antioxidant benefits that may protect against chronic diseases and oxidative stress. Therefore, a deeper understanding of the interactions between these compounds and nutrients is crucial for optimizing their use in food and the formulation of new bioproducts.

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