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Physicochemical analysis, antioxidant activity and research of saponins in fresh and blanched caruru (*Amaranthus deflexus* Linn) and ora-pro-nóbis (*Pereskia aculeata* Miller) leaves

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Abstract
Caruru and ora-pro-nóbis are unconventional food plants rich in proteins, essential amino acids, fibers, vitamins, minerals, and bioactive compounds but contain antinutritional factors. Blanching is one of the recommended methods to eliminate antinutrients, but it can change the chemical composition of foods. Therefore, the study's objective was to evaluate the physicochemical characteristics (pH and acidity), the proximate composition, the bioactive compounds (total phenolic compounds and vitamin C), the antioxidant activity and the presence of saponins in fresh caruru and ora-pro-nóbis leaves and subjected to blanching. The analyzed samples showed significant content of moisture, ash, protein, fiber, total phenolic compounds, vitamin C and antioxidant activity. In caruru, blanching increased pH, total solids, proteins, lipids, and carbohydrates while decreasing moisture, antioxidant activity and saponins. In ora-pro-nóbis, this processing increased pH, fiber, and total phenolic compounds and decreased vitamin C and saponins.

Keywords: PANC, antinutrients, blanching, centesimal composition, bioactive compounds, natural antioxidants.

Graphical Abstract

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ROYALDATASET

1. Introduction

Over the centuries, the number of plants with food potential worldwide has been estimated to reach 75,000 species (Kinupp, 2007; Tangley & Miller, 1991). However, only 4 vegetables - rice, wheat, corn and potato - supply more than 50% of the daily energy needs of the world's population (Bredariol, 2015).

Unconventional Food Plants (UFPs) or "plantas alimentícias não-convencionais" (PANC) in Portuguese are all exotic, native, autochthonous, wild, spontaneous, or cultivated plants that have one or more parts or portions that can be consumed in human food. Also, the acronym PANC includes unconventional parts, portions, or products from conventional plants (Kinupp & Lorenzi, 2014). PANCs were already present in the diet of individuals, but gradually they were forgotten and devalued due to the migration of the rural population to large urban centers and because they do not have expected commercial value (Viana, 2013).

However, interest in PANCs has been growing in the scientific community, given their nutritional richness in terms of proteins, fibers, vitamins, minerals, and bioactive compounds capable of promoting health and preventing and treating pathologies (Kinupp, 2007; Viana, 2013). Furthermore, it is known that the Brazilian population has experienced changes in health and food consumption patterns in recent decades. The reduction in hunger and malnutrition was accompanied by a significant increase in chronic noncommunicable diseases (NCDs) characterized by a chronic inflammatory process (Brazil, 2013).

Despite this scenario, Brazil still faces diseases related to iron, vitamin A and calcium deficiencies, as well as a high prevalence of chronic malnutrition in some population groups (Brazil, 2013, 2011, 2009). Moreover, considering the current context of the COVID-19 pandemic, all these rates of nutritional deficiencies and hidden hunger may have increased as the number of people facing hunger on a daily basis has increased, reaching 33.1 million Brazilians, as indicated by the Brazilian Research Network on Food and Nutrition Sovereignty and Security (PENSSAN Network, 2022).

The *Amaranthus deflexus* Linn is known as caruru, native to South America and occurs in the South and Southeast of Brazil (Kinupp & Lorenzi, 2014). It is a small annual herbaceous plant that belongs to the Amaranthaceae family (Sarker & Oba, 2019; Guimarães, 2017; Kinupp & Lorenzi, 2014). Caruru is considered a pest by farmers, as the maximum vegetative development coincides with conventional crops that are important for the economy (Silva et al., 2016). In addition, the leaves and seeds are edible (Kinupp & Lorenzi, 2014).

Pereskia aculeata Miller, whose popular name is ora-pro-nóbis, is a semi-woody bushy vine that originated in the tropics and spread to other locations worldwide (Trennepohl, 2016; Kinupp & Lorenzi, 2014; Almeida & Corrêa, 2012). In Brazil, it is found from Bahia to Rio Grande do Sul. This plant is resistant to adverse conditions, has vegetative development throughout the year, and adapts to numerous types of soils (Guimarães et al., 2018; Nascimento, 2016; Trennepohl, 2016; Viana, 2013; Almeida & Corrêa, 2012). The edible parts are the leaves, buds, flowers, and fruits (Kinupp & Lorenzi, 2014; Almeida & Corrêa, 2012).

Caruru and ora-pro-nóbis are PANCs whose leaves are sources of proteins, calcium, iron, vitamin C, carotenoids and compounds with antioxidant and anti-inflammatory activity (Moura et al., 2021; Guimarães, 2018; Trennepohl, 2016; Viana, 2013; Almeida, 2012). In this sense, including these vegetables in the diet may be a strategy to reduce the nutritional deficiencies prevalent in Brazil, especially in the early stages of life and more susceptible population groups, as well as to prevent the development of NCDs. However, as PANCs are wild and non-domesticated plants, they may contain antinutritional factors (ANFs), such as saponins, which in excess are harmful to groups vulnerable to nutritional deficiencies (Higashijima, Lucca, Rebizz & Rebizzi, 2020; Nascimento, 2016).

Thus, to take advantage of all its health benefits, including PANCs in the daily diet must embrace the removal of ANFs. Blanching is a simple method for enzymatic inactivation, contributing to food preservation and which can also be recommended for removing or reducing ANFs. However, such a process can change the nutritional properties of foods (Higashijima et al., 2020; Nascimento, 2016). To date, there are no studies involving the blanching and leaves of *A. deflexus* and *P. aculeata*.

Therefore, the present study aimed to evaluate the physicochemical characteristics, centesimal composition, bioactive compounds, antioxidant activity and the presence of saponins in fresh and blanched leaves of caruru and ora-pro-nóbis.

2. Material and Methods

This study was carried out during September and October 2021 in Joinville - Santa Catarina, Brazil. Before starting the experiments, plant specimens were certified and deposited at the Herbario Joinvillea.

2.1 Reagents

The following analytical grade reagents were used in the analyzes: sodium hydroxide, sulfuric acid, 4% boric acid, anhydrous copper sulfate, sodium sulfate, methyl red, bromocresol green, ethanol, 70% ethanol, hydrochloric acid, petroleum ether, acetone, mercury iodide, potassium dichromate, sodium thiosulfate, iodine, starch, sodium carbonate, 96% ethanol, methanol (Vetec, São Paulo, Brazil), 2M Folin-Ciocalteu reagent (Êxodo Científica, São Paulo, Brazil), anhydrous gallic acid (Scientific Exodus) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma- Aldrich, São Paulo, Brazil).

2.2 Obtaining samples

The leaves of caruru and ora-pro-nóbis were collected in Joinville, SC, Brazil, at coordinates 26°19’11.O”S and 48°52’43.O”W. The harvest occurred in September and October 2021, corresponding to the spring season, in the early evening to collect fresh leaves.

2.3 Exsiccate

The exsiccate was performed in September 2021 at the Joinvillea Herbarium (JOI), located at the University of the Region of Joinville (Univille), Bom Retiro Campus – Joinville, SC, Brazil. The procedures were performed as described by Fonseca & Vieira (2015) with at least three replications, with a single specimen of each species being prepared at the end. The vouchers were deposited under registration numbers JOI 18875 (*Amaranthus deflexus* Linn) and JOI 18874 (*Pereskia aculeata* Miller).

2.4 Sample preparation

Immediately after harvesting, caruru and ora-pro-nóbis leaves in good condition were washed in running water to remove dirt and separated into two groups: fresh leaves (group 1) and leaves to be blanched (group 2). Then, excess water was removed from the leaves of group 1 with the aid of absorbent paper, which were placed on benches lined with paper towels to dry at room temperature for approximately 6 h. Next, samples from group 2 were subjected to wet cooking in the form of blanching, as described by Gouveia et al. (2015). Thus, the leaves were placed in boiling water, followed by an ice bath for 1 min. Subsequently, the same procedure as in group 1 was performed to remove excess water and dry it at room temperature. Then, the samples from each group were wrapped separately in paper towels, stored in transparent plastic bags, and identified. Group 1 samples were named fresh caruru and fresh ora-pro-nóbis, and the samples from group 2 were called blanched caruru and blanched ora-pro-nóbis (**Fig. 1**).

Fig. 1 Samples from group 1: fresh caruru (a) and fresh ora-pro-nóbis (b). Samples from group 2: blanched caruru (c) and blanched ora-pro-nóbis (d). Source: Authors’ archive (2021).

2.5 Physicochemical characterization

The pH and total titratable acidity (TTA) were analyzed using an aqueous extract of the leaves prepared with 10 g of leaves and 100 mL of distilled water, liquefied (LI-20-N, Skymsen, Brazil) for 15 s. The extract was strained, and the fibrous part was discarded. The pH was measured using the electrometric process described by Instituto Adolfo Lutz - IAL (2008) and Cecchi (2003). TTA was determined by titrimetry for colored foods, according to IAL (2008) and Cecchi (2003), using 0.1 M or 0.01 M NaOH as the titrant, and the results were expressed in percentage.

2.6 Proximate composition

Moisture content was determined from direct drying in an oven at 105 ºC (TE-394/3, Tecnal, Brazil) to constant weight. The total solids (TS) were obtained from the moisture determination, consisting of the remaining dry residue (IAL, 2008). For the other centesimal composition analyses, the samples were dried in an oven at 45 ± 2 °C and ground in an analytical mill (Q298A21, Quimis®, Brazil). The total ash was quantified after the samples' incineration in a muffle furnace (Q318521, Quimis ®, Brazil) at 550 ºC until constant weight. Protein content was determined by quantifying total nitrogen using the Kjeldahl method. Lipids were determined with petroleum ether using a Goldfish fat determination equipment (TE-004, Tecnal, Brazil), in which the extraction is performed by immersion and percolation of the sample with the solvent (Cecchi, 2003; IAL, 2008). The crude fiber content was determined based on the insoluble organic residue of the sample obtained after acidic and alkaline digestion. Finally, carbohydrate content was determined indirectly by subtracting the percentages of moisture, ash, protein, lipids, and crude fiber from 100%. The results were expressed in grams per 100 grams of wet sample (Cecchi, 2003; IAL, 2008).

2.7 Total Phenolic Compounds (TPC)

The TPC was determined by the spectrophotometric method using the Folin-Ciocalteu reagent (Singleton & Rossi, 1965). First, an aliquot of 1 g of dried and ground sample was extracted using 35 mL of 50% (v/v) ethanol in an ultrasonic bath (Q3350, Quimis ®, Brazil) for 60 min at 32 ± 2 °C. Then, the ethanolic extract was filtered using qualitative filter paper (nº 3). Next, 150 μL of ethanolic extract, 7500 μL of distilled water and 750 μL of Folin-Ciocalteu reagent were mixed in a test tube. After 3 min, 2250 μL of 15% sodium carbonate (w/v) and 4350 μL of distilled water were added, and the absorbance was measured in a spectrophotometer (SP-1105, Tecnal, Brazil) at 765 nm after 2 h of incubation in the absence of light. The results were calculated using a standard curve of gallic acid at concentrations of 0.10, 0.30, 0.50, 0.70, 0.90, and 1.10 mg mL^{-1} and were expressed in mg of gallic acid equivalent (GAE) per g of dry sample.

2.8. Vitamin C

Vitamin C content was determined using the method described by Zambiazi (2010). First, in an Erlenmeyer flask, 20 mL of aqueous extract of the samples (as described in item 2.5) and 3 mL of 12 N sulfuric acid were added to decrease the pH, and 3 mL of 0.5% starch solution was used as an indicator. Next, the mixture was titrated with 0.01 N iodine solution until the dark color appeared; then, it was titrated again, using 0.01 N sodium thiosulfate, until the dark color disappeared. Afterward, a new titration with 0.01 N iodine solution was performed until the black color appeared. Finally, the results were expressed in mg of ascorbic acid in 100 g of wet sample, according to **Eqs. 1** and **2**:

$$Y = (Vi \times F_1) - (Vt \times F_2) \quad \textbf{Eq. 1}$$

where : Vi = total spent volume of iodine solution; F_1 = iodine solution correction factor; Vt = volume of thiosulphate used in the titration; F_2 = thiosulfate solution correction factor.

Considering that each mL of 0.01 N iodine solution corresponds to 0.88 mg of ascorbic acid:

$$\text{mg of ascorbic acid 100 mL}^{-1}\text{ of extract} = \frac{(Y \times 0.88)}{Ve} \quad \textbf{Eq. 2}$$

where: Y = result of **Eq. 1**; Ve = volume of extract used.

2.9 Antioxidant activity (AA)

The ability of the compounds in the samples to inhibit the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was measured according to the method described by Brand-Williams et al. (1995). First, 100 μL of each ethanol extract (as described in item 2.7) was added to 3.9 mL of DPPH (0.1 mmol L^{-1}) and analyzed before and after 60 min protected from light in a spectrophotometer (SP-1105, Tecnal, Brazil) at 517 nm. Then, the results were expressed as an inhibition percentage (IP) of the DPPH radical per mL of extract, according to **Eq. 3**:

$$IP = \frac{(Abs_{initial} - Abs_{final})}{Abs_{initial}} \times 100 \qquad \textbf{Eq. 3}$$

2.10 Presence of saponins

The research of saponins in the samples was carried out through the qualitative foam test, according to the methodology described by the Brazilian Society of Pharmacognosy (2009). In a test tube, an aliquot of 1 g of dried and ground sample was boiled with 10 mL of distilled water for 3 min. Then, the solution was vigorously shaken vertically for 15 s and left to rest for 15 min. After this time, the height of the foam was marked and measured using a ruler. The persistence of foam (positive reaction) after 15 min indicates the presence of saponins in the sample, while its disappearance (negative reaction) indicates the absence of saponins. Foam height was compared between fresh and blanched samples to assess the effect of blanching on saponins. The results were expressed in millimeters.

2.11 Data analysis and processing

The results were expressed as mean ± standard deviation and submitted to ANOVA analysis of variance and Tukey mean comparison tests with 95% confidence and $p < 0.05$, using the Statistica ® 7.0 software (StatSoft Inc., Tulsa, OK, USA).

3 Results and Discussion

3.1 Exsiccate

Fig. 2 shows the specimens prepared for caruru and ora-pro-nóbis, whose species were confirmed and deposited at the Herbario Joinvillea.

3.2 Physicochemical characteristics

The main physicochemical properties of *A. deflexus* and *P. aculeata* can be seen in **Table 1**. The measurement of pH is important to verify the deterioration of food due to the growth of microorganisms, the activity of enzymes and the state of maturation of vegetables (IAL, 2008). In this sense, foods with low acidic pH need greater control during storage, as they are more susceptible to the growth of bacteria harmful to health (Guimarães, 2018).

Fig. 2 Exsiccate of *Amaranthus deflexus* Linn. (a) and *Pereskia aculeata* Miller (b). Source: Authors' archive (2021).

Mean pH values were statistically different between samples, with lower values in fresh samples as compared to blanched leaves. This indicates that the heat treatment makes caruru and ora-pro-nóbis more vulnerable to microbiological contamination. The values found for fresh and blanched caruru were close to neutrality, while ora-pro-nóbis samples were classified as acidic (**Table 1**). The mean pH found by Moura et al. (2021) in fresh caruru leaves (6.8) was similar to that observed for blanched caruru (6.8). Comparing the average pH value found for fresh ora-pro-nóbis (5.1) with studies by Silveira (2015) (pH 5.4), who used the same methodology as this research, Guimarães (2018) (pH 5.3), Trennepohl (2016) (pH 4.9) and Viana (2013) (pH 4.4), who evaluated the pH of ora-pro-nóbis by potentiometry, through direct immersion of the electrode of the digital pHmeter in the crushed and homogenized fresh leaves, it was verified that despite the methodological differences of the last three studies, the results found were similar.

Table 1 Physicochemical characteristics and centesimal composition of fresh (*in natura*) and blanched caruru (*Amaranthus deflexus* Linn) and ora-pro-nóbis (*Pereskia aculeata* Miller) leaves.

Analyzes	Sample			
	Fresh caruru	Blanched caruru	Fresh ora-pro-nóbis	Blanched ora-pro-nóbis
pH	6.6±0.0 [a]	6.8±0.1 [b]	5.1±0.0 [c]	5.6±0.2 [d]
Total titratable acidity (%)	1.5±0.4 [a]	1.9±0.4 [a]	1.7±0.0 [a]	1.4±1.0 [a]
Moisture (g 100 g^{-1})	84.4±0.3 [a]	80.9±0.5 [b]	91.0±0.3 [c]	90.6±1.2 [c]
Total solids (g 100 g^{-1})	15.6±0.3 [a]	19.1±0.5 [b]	9.0±0.3 [c]	9.4±1.2 [c]
Ash (g 100 g^{-1})	2.2±0.6 [a,b]	2.5±0.1 [a]	1.4±0.2 [b]	1.6±0.5 [a,b]
Proteins (g 100 g^{-1})	5.0±0.0 [a]	6.2±0.5 [b]	0.9±0.2 [c]	1.3±0.1 [c]
Lipids (g 100 g^{-1})	0.3±0.0 [a]	0.7±0.3 [b]	0.1±0.0 [a]	0.2±0.1 [a]
Carbohydrates (g 100 g^{-1})	7.0±0.4 [a]	8.4±0.6 [b]	5.9±0.3 [b,c]	5.6±0.3 [c]
Crude fiber (g 100 g^{-1})	1.2±0.0 [b]	1.3±0.2 [b]	0.7±0.1 [a]	1.2± 0.1 [b]
Energy value (kcal 100 g^{-1})	50.5±1.5 [a]	64.9±2.8 [b]	28.5±1.1 [c]	29.8±1.3 [c]

*Results expressed on a wet basis. Different letters on the same row indicate a significant difference between the samples at the 95% confidence level and $p < 0.05$.

The acidity content results from the organic acids present in the vegetable matrix; however, some vegetables may have low acidity and, therefore, are more susceptible to bacterial deterioration (Pereira et al., 2015).

The values found for total titratable acidity (TTA) were not statistically different, suggesting that caruru and ora-pro-nóbis have similar content of organic acids and that blanching could not interfere with this parameter. Silveira (2015) evaluated the TTA in fresh ora-pro-nóbis leaves and found a similar mean value (1.3%) to the present study (1.7%). The research by Pereira et al. (2015) carried out the chemical characterization of conventional leafy vegetables grown organically, such as lettuce, spinach and arugula. In fresh samples of lettuce, the pH was around 5.8, while in spinach and kale, a pH of 5.6 was obtained. As for TTA, the percentages were 0.1% for lettuce and 0.2% for kale and spinach (Pereira et al., 2015). Compared with the present study's findings, it was observed that the pH values of these vegetables were close to those of fresh and blanched ora-pro-nóbis, and the acidity was lower than the studied samples.

3.3 Centesimal composition

The results of the centesimal composition are shown in **Table 1**. For the moisture content, there was a statistical difference between the samples of caruru and ora-pro-nóbis, and blanching did not change the moisture content significantly in the ora-pro-nóbis. All samples showed high moisture contents, which were higher in ora-pro-nóbis. Fresh caruru samples had higher moisture (84.4 g 100 g^{-1}), corroborating the findings of Moura et al. (2021), Nepa & Unicamp (2011) and Jiménez-Aguilar & Grusak (2017), which found 88.5, 87.6 and 82.0 g 100 g^{-1} of moisture, respectively, in fresh leaves. Also, Oyedeji et al. (2014) studied caruru in Nigeria, obtaining 63.9 g 100 g^{-1} of moisture.

The results for fresh ora-pro-nóbis (91.0 g 100 g^{-1}) were higher than that found by Guimarães (2018) (87.5 g 100 g^{-1}), Trennepohl (2016) (87.3 g 100 g^{-1}), Viana (2013) (83.3 g 100 g^{-1}) and Oliveira et al., (2013) (87.0 g 100 g^{-1}). Ora-pro-nóbis belongs to the Cactaceae family, and its leaves are succulent, resulting in greater water retention in plant cells (Guimarães, 2018). Therefore, the high moisture content in fresh (91.0 g 100 g^{-1}) and blanched (90.6 g 100 g^{-1}) samples of ora-pro-nóbis matches this characteristic. A reduced TS content was observed in all samples, with higher values in caruru than in ora-pro-nóbis. Blanching increased this parameter in caruru, but it was not significant in ora-pro-nóbis.

Viana (2013) found 16.7 g 100 g^{-1} TS in fresh ora-pro-nóbis, close to the results obtained for blanched caruru (19.1 g 100 g^{-1}) in this study. According to Botrel et al. (2020), moisture and TS values are important indices, as they can interfere with stability (chemical, biochemical and microbiological reactions) and texture and, consequently, with the shelf life of the food.

The ash content obtained in the analyzed samples was similar to the trend observed for fresh vegetables, in which the total ash ranges from 0.4 to 2.1 g 100 g^{-1} (Cecchi, 2003). However, no statistical difference was found between the studied samples, even after blanching, suggesting that this thermal treatment does not promote a significant loss of minerals in these samples. Thus, consumption of fresh or blanched caruru and ora-pro-nóbis leaves, as a food additive, may potentially prevent the deficiencies of micronutrients evidenced in the Brazilian population, mainly iron and calcium (Brazil, 2013, 2011, 2009). However, further studies are needed to evaluate the antinutritional factors in these samples to indicate the best way to consume these foods and obtain their health benefits.

Moura et al. (2021), Oyedeji et al. (2014), and Nepa & Unicamp (2011) reported higher values in fresh caruru (3.17, 6.97 and 2.6 g 100 g^{-1}, respectively). Trennepohl (2016) obtained 2.1 g 100 g^{-1} of ash in fresh ora-pro-nóbis, a result superior to the present study.

For protein content, higher values were observed in caruru samples after blanching, which did not occur in ora-pro-nóbis. In this sense, caruru proved to be a better source of protein when compared to ora-pro-nóbis and may be included in the diet as a complement, aiming to reach daily protein goals or to complete another vegetable protein due to the presence of limiting amino acids in these food matrices (Cozzolino, 2016). Furthermore, the results found in fresh caruru were greater than those reported by Moura et al. (2021) and Nepa & Unicamp (2011), which found 3.1 and 3.2 g 100 g^{-1}, respectively. Oyedeji et al. (2014), in turn, verified 8.4 g 100 g^{-1} of proteins in fresh caruru.

Although presenting a low protein value compared to caruru, ora-pro-nóbis can offer more protein in the form of flour. The study by Guimarães (2018), for example, found an average of 20.4 g 100 g^{-1} of protein in ora-pro-nóbis dry leaves, of which about 92.5% are digestible and used by the human body, which is an important fact, as plant-based proteins tend to be poorly bioavailable (Guimarães, 2018; Cozzolino, 2016; Gomes, 2010). In addition, it should be noted that ora-pro-nóbis leaves are excellent sources of essential amino acids, such as tryptophan, phenylalanine, tyrosine, isoleucine, leucine, threonine, and lysine, except methionine, which is a limiting amino acid in this species (Guimarães, 2018).

Also, Trennepohl (2016) found 3.4 g 100 g^{-1} of proteins in fresh ora-pro-nóbis leaves. In this sense, from a nutritional point of view, caruru and ora-pro-nóbis leaves may contribute to reducing rates of protein-based malnutrition in Brazilian society (Brazil, 2013).

Low lipid content was observed in all samples, corresponding to the trend observed in vegetables, whose values fall within the range from 0.1 to 1.2 g 100 g^{-1} (Cecchi, 2003). The content of this nutrient was similar in fresh and blanched samples of caruru and ora-pro-nóbis, with higher values in the blanched caruru. Thus, ingestion of caruru and ora-pro-nóbis may be beneficial in lipid-restricted diets, for example. The findings for fresh caruru corroborate with Moura et al. (2021) and Nepa & Unicamp (2011), which showed 0.3 and 0.6 g 100 g^{-1}, respectively. However, Oyedeji et al. (2014) found high values, around 4.8 g 100 g^{-1}, in fresh caruru. The study by Trennepohl (2016) found 3.4 g 100 g^{-1} of lipids in fresh ora-pro-nóbis leaves.

The carbohydrate content was low in the analyzed samples. However, an increase in this nutrient was observed after caruru blanching, while ora-pro-nóbis leaves did not show any difference with this thermal process. The results for fresh (7.0 g 100 g^{-1}) and blanched caruru (8.4 g 100 g^{-1}) were higher than those found in studies with fresh caruru leaves, as that of Moura et al. (2021) (5.0 g 100 g^{-1}) which analyzed the total carbohydrates including the fiber content, and Nepa & Unicamp (2011) (6.0 g 100 g^{-1}). Oyedeji et al. (2014) obtained high values (10.5 g 100 g^{-1}) in fresh caruru compared to the present study. Trennepohl (2016) found low carbohydrate content (1.4 g 100 g^{-1}) in fresh ora-pro-nóbis when compared to that observed in fresh (5.9 g 100 g^{-1}) and blanched (5.6 g 100 g^{-1}) ora-pro-nóbis. These variations can be explained by the differences between the cultivation soils and the season of the year in which they were harvested (Almeida et al., 2014). Also, it is worth remembering that, due to the methodology used in the present work to calculate the carbohydrate content, the difference in the values of the other components results in the interference of this parameter.

Among the analyzed samples, fresh ora-pro-nóbis had the lowest fiber content (0.7 g 100 g^{-1}). The content of this nutrient was similar in samples of fresh caruru, blanched caruru and blanched ora-pro-nóbis. There was also an increase in fibers after blanching ora-pro-nóbis. No studies were found analyzing the crude fiber content in caruru; however, Oyedeji et al. (2014) and Nepa & Unicamp (2011) determined the dietary fiber in fresh samples of caruru, finding higher values (5.5 and 4.5 g 100 g^{-1}, respectively) when compared to the present study. However, these findings corroborate the employed methodologies. According to IAL (2008), the fibers correspond to the organic residue obtained after specific extraction methods. Hipsley proposed the term dietary fiber, which Trowell defined as the components of plant cell walls included in the human diet that resist the action of gastrointestinal tract secretions. In the analysis of foods intended for human consumption, it is recommended to determine the dietary fiber content instead of crude fiber, as the food is treated with several physiological enzymes to simulate the conditions of the human intestine, unlike crude fiber, in which the use of strong acids and bases can lead to the loss of part of the fiber content (IAL, 2008).

The energy value (EV) represents the energy provided by proteins, carbohydrates, and lipids. All samples showed low EV, higher in blanched caruru due to the increase in the macronutrients verified after blanching this vegetable, and lower in both samples of ora-pro-nóbis. The reduced EV in the samples could be due to the reduced content of carbohydrates and lipids.

The values found in fresh caruru (50.5 kcal 100 g^{-1}) were higher than Nepa & Unicamp (2011) (34 kcal 100 g^{-1}). On the other hand, fresh ora-pro-nóbis showed higher EV (28.5 kcal 100 g^{-1}) than that reported by Trennepohl (2016) (17.8 kcal 100 g^{-1}).

Nepa & Unicamp (2011) determined the centesimal composition of conventional leafy vegetables, such as chard, watercress, iceberg lettuce, kale, spinach, and arugula, with the results expressed on a wet basis. Compared to fresh and blanched caruru, it was verified that these vegetable matrices have a higher content of TS, ashes, proteins, lipids and carbohydrates and lower moisture than all leafy vegetables mentioned. In addition, the fiber content was higher than chard and iceberg lettuce but lower than other vegetables.

The fresh and blanched ora-pro-nóbis samples presented moisture and TS similar to kale and higher ash and carbohydrates than all vegetables. The protein content was higher than iceberg lettuce, while lipids were similar for all vegetables except kale. The fiber content of fresh ora-pro-nóbis was inferior to all vegetables. In blanched ora-pro-nóbis, this nutrient was higher only in chard and iceberg lettuce (Nepa & Unicamp, 2011).

The EV of the samples of caruru and ora-pro-nóbis were higher than all conventional leafy vegetables mentioned, and in ora-pro-nóbis, the values were close to watercress and spinach (Nepa & Unicamp, 2011).

3.4 Bioactive compounds and antioxidant activity

The results on the antioxidant potential of caruru and ora-pro-nóbis can be seen in **Table 2**. A high concentration of TPC was observed in all analyzed samples, with higher values verified in blanched ora-pro-nóbis (36.2 mg GAE g^{-1}) compared to fresh ora-pro-nóbis (16.3 mg GAE g^{-1}). Thus, blanching positively affected the TPC for ora-pro-nóbis, increasing 122.1% of these compounds compared to the fresh sample. However, blanching was not significant regarding TPC in caruru. Concerning vitamin C, there was a higher concentration in fresh ora-pro-nóbis (339.6 mg 100 g^{-1}) than in fresh caruru (131.9 mg 100 g^{-1}). As seen in the TPC, thermal processing only interfered with the vitamin C values of ora-pro-nóbis, reducing 75.2% of these compounds.

Table 2 Bioactive compounds and antioxidant activity (AA) of fresh and blanched caruru (*Amaranthus deflexus* Linn) and ora-pro-nóbis (*Pereskia aculeata* Miller)

Analyzes	Sample			
	Fresh caruru	Blanched caruru	Fresh ora-pro-nóbis	Blanched ora-pro-nóbis
TPC (mg GAE g^{-1})**	9.9±0.5^{a}	6.9±0.4^{a}	16.3±0.2^{b}	36.2±3.0^{c}
Vitamin C (mg 100 g^{-1})*	131.9±82.5^{a}	22.1±3.5^{a}	339.6±101.4^{b}	84.1±39.8^{a}
AA (%)**	59.7±2.7^{a}	42.6±1.3^{b}	76.6±3.5^{c}	84.8±0.8^{c}

*Results expressed on a wet basis. **Results expressed on a dry basis. Different letters on the same row indicate a significant difference between the samples at the 95% confidence level. TPC, total phenolic compounds.

The daily recommendation for vitamin C in males varies from 45 to 90 mg/day, while in females, it varies from 45 to 120 mg/day, being higher for infants (Padovani et al., 2006). Considering the concentrations obtained for vitamin C in caruru and ora-pro-nóbis, it appears that the consumption of 100g of caruru and blanched ora-pro-nóbis leaves contributes to achieving daily needs at all stages of life.

Furthermore, vitamin C favors the absorption of non-heme iron found in these vegetables and promotes the other functions of this nutrient in the human body (Padovani et al., 2006). However, depending on the stage of life, it is necessary to consume other food sources of vitamin C to meet the daily needs of vitamin C since this nutrient was not high in blanched leaves compared to the fresh samples.

The AA was higher in fresh ora-pro-nóbis samples (76.6%) and blanched ora-pro-nóbis (84.8%), followed by fresh caruru (59.7%) and blanched caruru (42.6%). Despite the increase in TPC after blanching, there was no statistical difference for the AA in ora-pro-nóbis. Considering that several phenolic compounds are polymers, the heat treatment may have caused the hydrolysis of these compounds, resulting in greater content in the blanched ora-pro-nóbis samples (Silveira, 2015). In addition, ora-pro-nóbis leaves are rich in soluble fibers of the mucilage type (Sharif et al., 2013; Alves et al., 2011) and are visually thicker than caruru. Therefore, such characteristics may have contributed to the retention of TPC in the plant matrix and increased contact with heat, leading to TPC hydrolysis. In this sense, further studies on the TPC in these plant matrices are needed, such as chromatographic analyses that verify the size of the molecules in the sample extracts (Cecchi, 2003).

It is known that blanching promotes the disruption of the cell walls of plant cells, favoring the extraction of nutrients and bioactive compounds inside the cells (Palermo et al., 2014). Thus, the less thick leaves of caruru may have favored the loss of TPC and vitamin C by oxidation and/or leaching due to the exposure of these substances to the high temperature during boiling and contact with boiling water and the water bath ice, respectively (Silveira, 2015; Palermo et al., 2014; Pigoli, 2012; Campos et al., 2009). These results corroborate the lower AA in blanched caruru since TPC and vitamin C have antioxidant properties (Viana, 2013). Furthermore, the increase or decrease in TPC, vitamin C and AA after heat treatment can be explained by morphological, genetic, and chemical differences between the leaves studied, the processing used, the sample preparation, the way of extraction and the chemical nature of the compounds present in plant matrices (Nayak et al., 2015; Silveira, 2015; Oliveira et al., 2013).

The study by Moura et al. (2021) analyzed TPC and AA in the aqueous extract of dried caruru leaves and vitamin C in fresh caruru leaves, obtaining 0.05 mg GAE g^{-1} of extract, EC_{50} of 139.62 ± 1.88 μg mL^{-1} and 28.5 mg 100 g^{-1}, respectively. The TPC and vitamin C results were lower than those observed in the present study (9.9 mg GAE g^{-1} and 131.9 mg 100g^{-1}, respectively, for fresh caruru).

Nepa & Unicamp (2011) also found low levels of vitamin C in fresh caruru leaves (5.4 mg 100 g^{-1}). However, these differences may be due to the method of extraction and methodology used, in addition to factors related to cultivation, such as soil and climate of the place of cultivation (Trennepohl, 2016; Silveira, 2015). Jiménez-Aguilar & Grusak (2017) analyzed TPC, vitamin C and AA in fresh caruru, finding similar values, such as 4.4 mg GAE g^{-1}, 100 mg 100 g^{-1} of vitamin C and 54 μmol Trolox equivalent per g in the fresh sample, respectively. Finally, Guimarães (2018) evaluated the TPC in the acetone extract of dried leaves of ora-pro-nóbis, finding 6.4 AGE g^{-1}. The same author obtained 142.5 mg 100 g^{-1} of vitamin C in fresh ora-pro-nóbis leaves.

Viana (2013) evaluated the AA in the methanolic extract of dried ora-pro-nóbis samples, verifying 92.0, 75.5 and 19.6% of the inhibition percentage (IP) at 1, 0.1 and 0.01 mg mL^{-1}, respectively. Trennepohl (2016) analyzed the crude ethanolic extract of raw leaves of ora-pro-nóbis, finding a TPC concentration equal to 136.5 GAE g^{-1} of the crude ethanolic extract and AA of 87.05% in relation to the rutin standard. On the other hand, Oliveira et al. (2013) found 192.2 mg 100 g^{-1} of vitamin C in fresh ora-pro-nóbis leaves.

Silveira (2015) studied the effect of wet cooking (addition of fresh leaves in boiling distilled water (in a 1:4 ratio)) and mixed cooking (30 mL of soybean oil and 50 g of sample) at different cooking times (1, 2.5, 5 and 10 min for wet cooking and 1, and 2.5 min for mixed cooking) on fresh ora-pro-nóbis leaves, followed by an ice bath to cool the vegetable. On fresh ora-pro-nóbis leaves, this author obtained 475.2 mg 100 g^{-1} of vitamin C on a wet basis and 34.9 mg GAE g^{-1} on a dry basis, results close to those verified in the present study. Furthermore, there was an increase in TPC and a reduction in vitamin C after wet cooking at all times, finding a higher TPC content (53.7 mg GAE g^{-1}) and a lower vitamin C content (179.2 mg 100 g^{-1}) in 10 min of wet cooking. Mixed cooking also promoted the same effect. In this sense, it is clear that the results by Silveira (2015) are consistent with the trend observed in ora-pro-nóbis for TPC and vitamin C.

3.5 Presence of saponins

The results related to the presence of saponins in caruru and ora-pro-nóbis leaves can be seen in **Table 3**. Antinutritional factors (ANFs) are compounds so named because they interfere with the digestibility, absorption, or use of nutrients, cause adverse reactions in the gastrointestinal tract, are toxic and can cause damage to health when ingested in large quantities, and even be lethal. Besides saponins, there are other ANFs, such as oxalic, phytic and hydrocyanic acids, protease and amylase inhibitor proteins, tannins, nitrites, and nitrates (Higashijima et al., 2020; Nascimento, 2016).

Analyzing the data in **Table 3**, caruru has more saponins than ora-pro-nóbis, with the highest amount found in fresh caruru due to the greater foam formation in this vegetable matrix. However, it is important to point out that several compounds can contribute to foam formation, but since saponins are one of them, the reduction of foam after blanching may indirectly indicate a reduction in the saponin content.

Table 3 Qualitative test of saponins in fresh and blanched caruru (*Amaranthus deflexus* Linn) and ora-pro-nóbis (*Pereskia aculeata* Miller) leaves

Analyzes	Sample			
	Fresh caruru	Blanched caruru	Fresh ora-pro-nóbis	Blanched ora-pro-nóbis
Foam height after stirring (mm)*	28.0±2.8 [b]	3.5±0.7 [a]	3.5±0.7 [a]	1.3±0.6 [a]
Foam height after rest (mm)**	21.0±5.7 [b]	3.5±0.7 [a]	2.5±2.1 [a]	0.5±0.0 [a]

*Shaking period: 15 s. **Rest period: 15 min. Different letters on the same row indicate a significant difference between the samples at the 95% confidence level.

In this sense, it was found that blanching potentially reduced this antinutritional factor only in caruru samples. Although this effect did not occur in ora-pro-nóbis samples, the study by Silveira (2015) showed that boiling ora-pro-nóbis leaves for 1 min promoted a reduction of other antinutritional factors, such as protease inhibitors up to 6 times.

Saponins are glycosides of polycyclic steroids or terpenes. The chemical structure presents an amphiphilic property, part of the molecule is lipophilic (triterpene or steroid), and the other is hydrophilic (sugars). This characteristic determines the property of reducing the surface tension of water, as well as detergent and emulsifying actions (Schenkel et al., 2007).

Due to their amphiphilic behavior, saponins can form complexes with steroids, proteins, and membrane phospholipids, enabling numerous biological actions. In cell membranes, they can change permeability but also lead to destruction, determining hemolytic, ichthyotoxic, molluscicidal and antiparasitic activities (Schenkel et al., 2007).

Furthermore, they irritate the body's mucous membranes and have a hypocholesterolemic effect. This mechanism is explained by the formation of complexes with sterols, such as cholesterol, resulting in increased excretion of cholesterol or bile acids through feces and increased use of cholesterol for bile synthesis. Another assumption for this effect considers the irritating properties of saponins, which form complexes with cholesterol in the membranes of the intestinal mucosa cells, resulting in the exfoliation of this layer of the gastrointestinal tract with loss of function and reduction of the absorption area (Podolak et al., 2010; Schenkel et al., 2007; Sparg et al., 2004).

The adverse effects of saponins are related to the increased permeability of intestinal mucosal cells, which can increase the absorption of foreign substances that would usually not be absorbed, in addition to predisposing individuals to an increased risk of sensitization to dietary antigens. Also, the tendency to form complexes with steroids and proteins can decrease the digestibility of foods (Podolak et al., 2010; Sparg et al., 2004).

Thus, the use of this processing technique in the analyzed plants can increase safety during consumption, considering the adverse effects of saponins and other antinutritional factors in the body, as well as encouraging the inclusion of blanched caruru and ora-pro-nóbis leaves. as an additive in culinary preparations in order to obtain its health benefits. However, the results found are qualitative. Therefore, quantitative studies on the content of saponins in the studied samples are necessary to formulate safe consumption recommendations.

4 Conclusion

The caruru and ora-pro-nóbis leaves showed significant moisture content, ash, protein, fiber, TPC, vitamin C and AA, with higher levels of vitamin C in fresh leaves. In addition, the high foam height in fresh leaves suggests higher saponin content in fresh caruru and ora-pro-nóbis. In the context of mineral deficiencies that affect Brazilians, such as iron and calcium, the consumption of blanched caruru and ora-pro-nóbis leaves may contribute to reducing levels in vulnerable groups, such as children, adolescents, women of childbearing age, the elderly, individuals with dietary restrictions, among others, as these samples had a high ash content and fewer saponins. Also, blanching did not interfere with the ash content. In order to increase protein intake, bleached caruru leaves could be a good option since they have a higher concentration of this nutrient. Although vegetable proteins are not complete, it is possible to complement them with other vegetables in the same meal to reach the daily recommendations. Considering the concentrations obtained for vitamin C in caruru and ora-pro-nóbis and the possible lower content of saponins in blanched leaves, it appears that the consumption of

100g of caruru and blanched ora-pro-nóbis leaves contributes to achieving daily needs at all stages of life. For a higher intake of TPC and higher antioxidant activity in the body, blanched ora-pro-nóbis may be a good choice. Furthermore, considering the reduced EV of caruru and ora-pro-nóbis, it is possible to include these blanched plants in energy-restricted diets. Therefore, considering that most physicochemical parameters evaluated increased or did not change after blanching, that the presence of saponins was lower in blanched caruru leaves and that other studies show the reduction of antinutritional factors in ora-pro-nóbis leaves after wet cooking, it was found that blanching makes caruru and ora-pro-nóbis leaves safer for consumption and, at the same time, makes it possible to obtain their health benefits.

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Authors' Contributions

G. Reinert: Conceptualization, investigation, data analysis, methodology, resources, writing – original draft, revision and editing. AR Almeida: Research, data analysis, methodology, writing – proofreading and editing. RD Armas: Acquisition of financial support, resources, writing – proofreading and editing. MVOB Maciel: Conceptualization, investigation, methodology, data analysis, writing – revision and editing, project administration. All authors read and approved the final manuscript.

Availability of data and materials

Data are available under request from the corresponding author.

Ethics approval and consent to participate.

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Informed Consent Statement

Not applicable.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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