Antioxidant and antiglycant properties of different milling fractions of Neltuma ruscifolia, an underutilized species

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Antioxidant and antiglycant properties of different milling fractions of *Neltuma ruscifolia*, an underutilized species

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Abstract

Within the group of neglected and underutilized species (NUS) is Neltuma ruscifolia (formerly *Prosopis*), belonging to the family Fabaceae (or Leguminosae), commonly known as carob trees but unlike other species, it does not have an established or formally defined commercial value. The objective of this study is to evaluate the antioxidant and antiglycant properties, as well as to identify associated bioactive compounds, in extracts derived from different extraction methods (ultrasound and agitation) and grinding fractions of pods of Neltuma ruscifolia, a NUS species. The results showed that the residue fraction extracted by high-intensity ultrasound exhibited the highest bioactivity. Ultrasonic-assisted extraction allowed polyphenolic compounds such as hydroxybenzoic and ellagic acids to be obtained that did not appear with stirring. Other polyphenols (such as chrysin, rutin, kaempferol and cinnamic, coumaric, protocatechuic, ellagic and caffeic acids) were highly related to the bioactivity. This study lays the foundation for the future development of antioxidant/antiglycant additives derived from *Neltuma ruscifolia*, diversifying NUS range

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of natural additives and aligning with the Sustainable Development Goals (SDGs) to safeguard biodiversity, local resources and the planet.

Keywords: NATURAL ADITIVES · BIOACTIVITY · HPLC · NEGLECTED AND UNDERUTILIZED SPECIES · GREEN EXTRACTION METHODS

1. Introduction

Neltuma genus*,* whose species belong in the recent past to *Prosopis* genus [1], is recognized for its ecological resilience and economic potential. Diverse *Neltuma* species all around the world are employed in various cultures as food ingredient for bread, or other meals, for their nutritional value and good sensory acceptability [2-5]. *Neltuma spp.* stands out as a promising contributor to sustainable food solutions, aligning with the global initiatives aimed at addressing food security and promoting biodiversity conservation as established by the Food and Agriculture Organization (FAO) within the Sustainable Development Goals (SDGs).

Neglected and underutilized species (NUS) are wild, cultivated or semi-domesticated, non-commodity, crops non-commercial crops that do not fall under conventional agriculture [6]. They are used for food, medicine, trading, or cultural practices that are significant within their local communities but are not widely commodified or studied as part of the conventional agriculture and survive only in small local or niche markets. However, since they are economically viable, and locally available or adaptable, they may contribute to tackling food and nutrition insecurity and climate change vulnerability [6]. According to FAO experts working for the SDG of zero hunger, NUS have been prioritized as Future Smart Food and have a central role to play in the fight against hunger and malnutrition [7]. The use of wild fruits is part of the application of dynamic local knowledge, sensitive to social and ecological changes [8].

Plants from genus *Neltuma* (ex Prosopis) are considered NUS, since they are wild species, mainly underutilized. *Neltuma* species belong to the economically important family Fabaceae (or Leguminosae). It includes many species native to the Americas, distributed from the southwestern and central United States through Mexico, Central America, the Caribbean, and South America to southern Argentina. When Spanish arrived in America name *Neltuma* plants as carob their similarities with the European carob (*Ceratonia siliqua*), also from the Fabaceae family.

Fabaceae plants are a healthy and affordable source of protein and provide excellent nutritional support to humans and animals. Also, the presence of nodules that contain symbiotic nitrogen fixing Ryzobium bacteria is remarkable characteristic of leguminous plants, and they play a key role in crop rotation [9]. However, unlike other species in this family (beans, soybeans, peas, chickpeas, peanuts, lentils, lupins, mesquite, alfalfa, fenugreek, clove and guar), *Neltuma* species are NUS and do not have an established or formally defined commercial value. Although they are underutilized and barely studied, relegated by research or development efforts, they hold a perceived potential to contribute to nutrition, food security, genetic resistance, or sustainability, since they could have a positive impact on the conservation of native forests and regional economies [8,10], thus preserving biodiversity. According to Hughes et al. (2022), some of the more abundant species of this genus are *Neltuma alba* (Griseb.), *N. nigra* (Griseb.), *N. chilensis* (Molina), *N. flexuosa* (DC.) and *N. affinis* (Spreng.), *Neltuma caldenia* (Burkart) commonly known as white carob, black carob, *tamarugo*, sweet carob or *alpataco*, *ñandubay* and *caldén*, respectively [1].

Many of the *Neltuma* species are endemic in Argentina and they are also extremely resistant to adverse environments (heat, drought, alkalinity, and salinity). They contribute

to soil stabilization and improvement, allowing sustainable agriculture [11, 12]. Since their development provides numerous ecologically desirable characteristics, many projects are based on carob trees for the recovery of soils since due to its highly branched root system, it constitutes a protector against erosion [13-15]. The sustainable use of this sub-valuated resource also represents a source of income for the native populations of arid and semiarid areas of South America and other regions of the world and an opportunity for innovation for the food industry [16]. Several works have been published on the collection of pods of different species of the genus and on their use in the manufacture of flour, bread, syrup, lodge, as medicine or as substitute for chocolate or coffee, confirming its importance [17-21]. Due to the limited research efforts devoted to the NUS species and their characterization, it is important to identify the compounds present in selected vegetable extracts and quantify them in order to avoid variations for climate, species, or cultivar.

Neltuma ruscifolia (Griseb.) (commonly called *vinal, visnal, viñal, olkhá, pao de espinho, quilín, tayt* and *yuncumarim*) is comparatively less documented and even more underutilized, in comparison to others species of its genus [1]. Their fruits (pods) are made up of an external part, the exocarp, fibrous in nature, which surrounds the mesocarp or pulp (composed mainly of sugars) and the endocarp (the capsules) also fibrous and much harder than the exocarp. Within the capsules the seeds contain high protein content of good nutritional and good level of fibers [5, 22]. Remarkably, these latter authors proposed alternatives for wheat bread making employing *N. ruscifolia* flours in a batter formulation. Their high protein content makes it suitable for human and livestock consumption as well as to enrich other flours [22]. An interesting feature of leguminous plants is the presence of a polysaccharide in their seeds, many of them with many potential technological applications. The physico-chemical and rheological characterization of the galactomannan

gum extracted from the endosperm of vinal (*Neltuma ruscifolia*) seeds, which is similar to guar gum, have been performed [21, 23-25].

In recent decades, plants phenolic compounds have attracted considerable attention due to their functional and nutritional benefits, including antioxidant and antimicrobial effects. The activitity of polyphenols to counteract lipid oxidation, which is one of the main causes of food deterioration, is well known. Besides, polyphenols may block dicarbonyl compounds generated in the Maillard reaction thus avoiding protein glycation, and those that meet this condition are potential antiglycating agents [26]. Extracts rich in these compounds offer a promising solution to replace artificial additives, meeting the rising consumer demand for natural food products and cleaner labels. The extraction of compounds from natural sources with potential technological application plays a pivotal role in various scientific domains, and extraction should be conducted in optimized “green” or environmentally friendly techniques. These methods not only reduce the environmental impact but also enhance the extraction efficiency, yielding extracts rich in beneficial compounds from many vegetal species, as has been reported for green pepper [27], *Prosopis alba* and *P. nigra* [28], and *N. juliflora* [29].

The primary objective of this study is to assess the antioxidant and antiglycation properties of extracts obtained from various extraction methods and milling fractions of *Neltuma ruscifolia* pods. Additionally, the research aims to identify the associated bioactive compounds and pinpoint the fractions exhibiting the highest concentration of these compounds and utilize these findings in the development of ingredients that enhance the diversification of food sources and contribute to a more sustainable agri-food system.

2. Materials and methods

2.1. Milling of *Neltuma ruscifolia* pods

The vinal fruits were harvested in Santiago del Estero at their optimum ripening stage. They were immediately washed, disinfected in a chlorine solution (5%) and dried in a plate dehydrator (FA 10-MZ, COBOS, Argentina) at 50°C for 3.5 hours. They were then stored at -12°C until use. Grinding was carried out with a grinder (HC-1000 Y, Arcano, China) and sieved through different stainless steel meshes (A.S.T.M N° 5, 7, 10 and 20; Zonytest, Argentina) as reported by Ojeda et al. (2023) [5]. Three fractions (particle size <840 µm) were obtained: Endocarp powder (EP), seed powder (SP) and residue powder (RP-exocarp, mesocarp and residue). All the fractions the powders obtained were stored in polyethylene bags fitted with Ziploc® type fasteners and stored at -12°C until use.

2.2. Color of the flours from *Neltuma ruscifolia* pods

The color was measured directly in the milled pods with a Hunter Lab MiniScan EZ hand-held colorimeter (Leicestershire, United Kingdom) and using an illuminant D65 and observation angle of 2°. The parameters L, a^*, b^* were obtained in the CIELAB homogeneous color space [30]. The color parameters chroma (C^*) and hue (h) were calculated according to Eq. (1) and (2) according to García (202) [31].

$$\text{Eq.(1)} \qquad C = (a^*)^2 + (b^*)^2$$

$$\text{Eq.(2)} \qquad h = \tan^{-1}(b^*/a^*)$$

2.3. Preparation of ethanolic extracts

The plant extracts were obtained following the methodology proposed by Favre et al. (2020) from the different fractions using 1:1 ethanol-water solutions [27]. For ultrasound-assisted extraction, an ultrasonic bath Julabo (D-7633, Germany), 35 KHz/maximum intensity was used at 40°C for 40 minutes (LIU). The second treatment (high intensitiy ultrasound–HIU) was performed using an ultrasonic UP100H (Hielscher Ultrasonics GmbH, Germany) equipped with an MS2 sonotrode and a Cheung et al. (2012) modified method.

An acoustic power density of 600 W/cm^2 (0.5 cycles), amplitude of 220 µm (100%), for 5 minutes and 40°C. The third extraction was done by agitation (TA) in a water bath at 40°C for 24 hours. All the extracts obtained were centrifuged (30 min, 6372 rcf, 4°C) and the supernatant was collected for analysis. Figure 1 summarizes the milling and extraction process for *Neltuma ruscifolia* pods.

(Fig. 1)

Fig. 1 *Neltuma ruscifolia* pods milling and extraction process

2.4. Total polyphenolic content (TPC)

The Folin Ciocalteu method was done using a standard curve of gallic acid (Merck, Darmstadt, Germany) with 6 points between 0 and 0.5 mg/mL (R^2 = 0.9907) [32]. A spectrophotometer Jenway 6505 ultraviolet–visible (Burlington, New Jersey, USA) was used to measure absorbance at 765 nm. The results were expressed as mg equivalent of gallic acid (GAE)/g of dry basis (d.b.).

2.5. Radical scavenging capacity (TEAC)

TEAC was determined using 7 mM of $ABTS^{\cdot+}$ solution prepared by dissolving 0.0194 g of 2,2 azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid), (ABTS·+) in 0,0033 g potassium persulfate 2.5 mM and kept overnight in the darkness at room temperature [33]. Absorbance was measured at 734 nm. Solutions of Trolox standard (was obtained with 6 points between 0.02–0.12 mg/mL) were used to construct the calibration curve (R^2 = 0.9980). The results were expressed as mg of Trolox per g of dry matter (mg Trolox/g powder d.b.).

2.6. Identification and quantification of polyphenols by High-pressure liquid chromatography (HPLC)

For the identification of polyphenols, the technique reported by Schenk et al. (2021) with slight modifications was used [34]. The extracts of *Neltuma ruscifolia* pods were filtered using a 0.45µm nylon membrane filter (NY 14831, Corning®, NY, EE. UU) and injected (20µL) into an HPLC system (Waters 1525, USA), equipped with a matrix detector photodiode. (Waters 2996, USA). A Lichrospher® 100 column, RP-18e, (250 mm×4mm, 5µm) an automatic injector (Waters 2707, USA), and a 200-450 nm detection range were used. Mobile phase A was composed of methanol - acetonitrile (1:1) while mobile phase B consisted of water - phosphoric acid (99:1). The staggered gradient program was optimized by the percentage change of mobile phases and set as follows: Time(min)/mobile phase-A:B (%): T0/5:95, T3/5:95, T8/20:80, T15/20:80, T18/30:70, T33/30:100, and T37/5:95 at a flow rate of 1 ml/min.

2.7. Oxidation onset temperature by DSC - Antioxidant capacity (AC)

The oxidation onset temperature (OOT) was determined by differential scanning calorimetry (DSC) using a Mettler Toledo 822 equipment (Mettler Toledo AG, Switzerland) and STARe Thermal Analysis System version 3.1 software (Mettler Toledo AG). Temperature and melting enthalpy calibrations were performed using standard compounds of defined melting point (156.6 and 419.7 °C for indium and zinc, respectively) and heat of melting (28.45 J g−1 for indium). All measurements were taken at least in duplicate with 4 µL extract and 10 uL of calendula oil, using perforated aluminium pans of 40 µL inner volume (Mettler Toledo AG, Zurich, Switzerland). An empty pan covered with a holed lid was used as a reference. The OOT was determined by heating the samples in oxidative conditions (with air flux) from 20°C to 150°C at 5°C/min (standard method ASTM E2009) (ASTM, 2012). The onset was taken as the intersection of the baseline and the tangent to the oxidation peak [35].

2.8. Antiglycant capacity (AG)

The antiglycant capacity was determined by analyzing the inhibition of Maillard intermediates and browning development in a bovine serum albumin (BSA) + glucose (GLU) exposed to high and moderate temperatures [27]. The samples were placed in glass vials, sealed and heated in an oven at different temperatures (100ºC for 6 h; and 55ºC for 7 days). The absorbance at 420 and 290 nm was measured in a Jasco V-630 UV-Vis spectrophotometer (JASCO Inc., Easton, MD, USA), and fluoresce was measured in a (Denovix DS-1 Fluorometer, New Zealand; Excitation: 375 nm-Emission: 435-485 nm). The rates of browning were determined using equation (3). Aminoguanidine (1mg/mL) was used as a standard with comparison purpose.

$$F = a + k\,t \quad \text{Eq. (3)}$$

Where: a is a constant value, k is the first order kinetic constant, and t is the storage time**.** The correlation coefficients obtained when describing experimental data through equation (3) were $R^2 > 0.80$.

2.9. Statistical analysis

All determinations were performed in triplicate and mean values and standard deviations were reported. Statistical analysis of results was performed through ANOVA for a level of signifcance (α) of 0.05 followed by LSD Fisher post hoc test to identify signifcant differences among systems. All statistical analysis and regressions were performed using the Statgraphics Centurion XV software (V 2.15.06, 2007, Statpoint Technologies, Inc., USA). The correlation analysis of the bioactive properties was conducted using a multivariate principal component analysis with InfoStat 4 software.

3. Results and discussion

3.1. Color analysis of the milled pod fractions

Table 1 shows the color parameters for the pod powders. The SP was lighter than the rest of the fractions (higher L^*) and it was characterized by yellow tones (higher b^* values) possibly due to the presence of the germ (in agreement with [5]. Both EP and SP exhibit more reddish - brown tones (higher a^* values) than the RP, and similarly to the results reported by [28] for *Prosopis nigra* and *P. alba*. RP was the darkest fraction (lowest L^*) and it also presented the lowest reflectance values for a^* and b^*. This fraction contains the epicarp, which is the outermost barrier, rich in polyphenolic compounds, which can partly explain its darker color [36]. The obtained values for the seed are consistent with those published by [37]. for *Prosopis leavigata*. The (C_{ab}) obtained values indicate that the EP and RP exhibited the most saturated color. Meanwhile the dominant wavelengths were similar between all fractions (Table 1). The hue angle increased from the samples corresponding to the outer towards the inner part of the pods, with the hue angle being higher for HS. The hue angle increased in the order of RP < EP < SP, progressing from the outer to the inner part of the pod.

Table 1 Determination of color in powder and extracts of different fractions of *Neltuma ruscifolia*. RP (residue powder); EP (endocarp powder); SP (seed powder); RE (residue extract); EE (endocarp extract) and SE (seed extract). Different letters indicate statistical difference (p<0,05) between columns for the same raw

(Table 1)

3.2. Polyphenolic content of the extracts

Figure 2 shows the antioxidant activity of *Neltuma ruscifolia* powder extracts. The total polyphenol contents (TPC) were similar for the EE-HIU and RE-HIU (Figure 2A). These values did not show significant differences with those obtained for the EE-TA. However,

the RE-TA presented the highest TPC value. It is worth noting that the lowest TPC were detected in the SE in all treatments.

(Fig. 2)

Fig. 2 Antioxidant activity of *Neltuma rucifolia* extracts determined by Total Polyphenol content (A) and Trolox equivalents radical scavenging Capacity (B). RE (residue extract, black); EE (endocarp extract, gray) and SE (seed extract, light gray). Agitation (TA); Light Intensitiy Ultrasound (LIU) High Intensitiy Ultrasound (HIU). Different letters indicate statistical difference between extracts ($p<0,05$). [38] Data extracted from Suárez-Rebaza et al. (2023) for *P. pallida and from* [36] Villalba et al. (2022) for *P. alba*

Figure 2B presents the radical scavenging capacities (by TEAC) obtained for the different extracts for fractions of *Neltuma ruscifolia*. Treatments with ultrasound (high and low intensity) are capable of extracting the highest TEAC values from the residual fractions. Additionally, the EP and RP subjected to agitation, as well as the EP-HIU exhibited similar values. After all extraction treatments, and similarly to TPC results, the SP of *Neltuma ruscifolia* showed the lower TEAC values.

Table 2 Comparison between Total Polyphenol Content (CTP) and Trolox equivalent Radical scavenging (TEAC) among *Neltuma ruscifolia* extracts (this study) and other *Neltuma* (ex-*Prosopis)* species. Results are expressed on dry basis (d.b.)

(Table 2)

As shown in Table 2, lower TPC and TEAC values were found for pods from *Prosopis alba* and *Prosopis pallida,* using 45% ethanol, than for the RE or EE of *Neltuma ruscifolia*. Also, TPC values for *Prosopis laevigata* seed flour [39] were lower than those found in this study for *Neltuma ruscifolia* powders, using extractions with lower ethanol concentration (40% ethanol). In addition, lower values of TPC for *P. alba* and *P. chilensis (*brown and yellow) were reported than those determined in methanolic extracts [40]. These differences not only depend on the botanical source analyzed but also origin variations, the extraction process, the method used to determine the bioactive compounds as well as the solvent

used also influences the results. According to the classification proposed by Vasco et al. (2008) [41], based on TPC, *Neltuma ruscifolia* is among the species with high content of soluble polyphenols (72 ± 3 mg GAE/g d.s.).

There is consensus that antioxidant capacity may be directly correlated with the quantity of present phenolic compounds [42-44]. In the extracts obtained from *Neltuma ruscifolia*, this direct relationship between these two characteristics was observed, although the correlations obtained were directly influenced by the extraction process. For the agitation treatment, the correlation was 0.77; for high-intensity ultrasound, this figure was 0.90; while for low-intensity ultrasound, it was 0.14. Based on this, we can appreciate that the agitation method and high-intensity ultrasound allow for obtaining extracts whose antioxidant capacity is primarily related to the presence of polyphenols. Conversely, the low-intensity ultrasound method produces extracts whose antioxidant capacity cannot be related to the low content of polyphenols extracted.

3.3. Quantification of polyphenols by HPLC

Table 3 shows the polyphenol profile found in the extracts of *Neltuma ruscifolia* after applying different extraction treatments. It can be seen that caffeine (15,3 ± 0,69 ppm) was detected in the EE-TA. vanillic acid was detected in all fractions and all treatments, with the highest concentration for SE-TA and EE-TA, and in very low concentration for the RE. Vanillic acid has been found not only in green tea infusions but also in other infusions such as Hibiscus, grapefruit, and basil [45]. On the other TA hand, Protocatechuic acid was identified in RE for the three treatments and in the EE through ultrasound technologies, indicating it is easily extractable in the RP than in the EP. Hydroxybenzoic acid was detectable by using ultrasound, and the concentrations of this compound were higher with the higher intensity treatment. It is noteworthy that Theobromine was found in all seed

fractions and also appeared in the residue fraction only when applying LIU. Only by using HIU, ellagic acid was extracted and detected in all three fractions, with its highest content in the RE. Quercetin was also present in all HIU extracts and additionally in the EE-TA. It can be observed that some compounds like kaempferol, hydroxybenzoic acid, ellagic acid, apigenin, sinapic acid, resveratrol, and galangin cannot be extracted by TA, but by ultrasound. Undoubtedly, TA does not exhibit sufficient efficiency to allow cells to release compounds as in the case of low and high-intensity ultrasound. It can be interpreted that the frequency and concentration at which these compounds appear depend on the extraction treatment used, and that each fraction presents a different polyphenolic profile.

Table 3 Quantification of polyphenols by High-pressure liquid chromatography (HPLC) - RE (residue extract); EE (endocarp extract) and SE (seed extract). Agitation (TA); Light Intensitiy Ultrasound (LIU) High Intensitiy Ultrasound (HIU). Different letters indicate statistical difference. ($p<0,05$).

(Table 3)

3.4. Antioxidant capacity (AC)

Figure 3 shows the antioxidant capacity of *Neltuma ruscifolia* extracts by analyzing the oxidation pattern of in marigold oil by differential scanning calorimetry. It was observed that the onset temperature of oxidation (OOT) for marigold oil was 198.1ºC. Upon adding the SE the OOT slightly increased to 198.9ºC. In the case of other fractions, it was noted that the OOT significantly differed from that of marigold oil (control), being 206.5ºC and 207.5ºC, for RE and EE, respectively. Therefore, the presence of natural antioxidants in *Neltuma ruscifolia* extracts enhanced the oxidative stability of marigold oil.

Some authors also found that *P. Involucratum* extracts prevented sheep sebum oxidation [46]. Additionally, it has been reported that natural antioxidants from *Inca mint* leaves had

a better antioxidant effect than TBHQ in soybean oil during frying, significantly improving the oxidative stability of soybean oil during frying [47].

(Fig. 3)

Fig. 3 Determination of the oxidation onset temperature (OOT) by differential scanning calorimetry (DSC) in marigold oil (control dotted line) with the addition of extracts from different fractions of *Neltuma ruscifolia.* RE (residue extract, black); EE (endocarp extract, gray) and SE (seed extract, light gray)

3.5. Antiglycant capacity (AG)

Figure 4 (A-B and C) shows the ability of *Neltuma ruscifolia* extracts to inhibit the formation of Maillard intermediate and products. In Figure A (290 nm), the data followed a trend until day 3, after which a change in the antiglycation capacity was detected. The control exhibited the highest color formation, followed by RE, EE, STD, and SE. Table 4 shows the first order kinetic rate constants (K). As observed in Figure 4B (420 nm, brown pigments, visible region), the higher color formation rate was observed for the control sample consistently with the higher rate constant (K=0.37). The EE fraction showed greater color formation than the RE fraction, followed by SE and finally aminoguanidine. This indicated a good antiglycation capacity of SE fraction, but the aminoguanidine showed the greatest antiglycation power.

The intermediate stages of the Maillard reaction measured by fluorescence development, are represented in Figure 4C. The trend followed by the fractions until day 3 was as follows: RE, EE, SE, STD, and control. This trend reverses after day 3, with the EE fraction exhibiting the highest antiglycation capacity, followed by RE, SE, and lastly, STD, in the fluorescent range.

The capacity to inhibit the formation of brown compounds (intensely reddish-brown pigments that do not decolorize) generated in the final stage of the Maillard reaction, was

calculated as a percentage and measured in the visible spectrum at 420 nm between 4 and 6 hours of high-temperature thermal treatment (100ºC). In Figure 4D it can be seen that *Neltuma ruscifolia* HIU extracts are capable of inhibiting the formation of brown compounds in accelerated processes, with the RE fraction being the one that presents the greatest inhibition up to 4 hours, followed by EE. It is necessary to clarify that the control inhibits at 0%, and the extracts reach more than 60%. But, if the reaction is observed up to 6 hours, the seed fraction (SE) exceeds the residue fraction, which would mean that the antiglycant capacity presented by this fraction (SE) is slower but is more stable over time.(Fig. 4)**Fig. 4** Antiglycant capacity (AG) of *Neltuma ruscifolia* extracts: RE (residue extract, black); EE (endocarp extract, gray) and SE (seed extract, light gray). Abs 290 nm (A), Abs 420 nm (B), Fluorescence emission (435-485) and % inhibición Abs 420 nm

Table 4 First order rate constants (K) associated with the Maillard reaction, showcasing measurements of absorbance at 290 nm (indicative of intermediates), fluorescence (excitation at 370 nm/emission at 410 nm), and absorbance at 420 nm (colored compounds) over a three-day period in a model system (glucose + BSA)

(Table 4)

3.6. Relationship of the bioactive features through multivariate analysis

A multivariate principal component analysis (Figure 5) was conducted to examine the relationships between the concentrations of polyphenolic profiles obtained by HPLC and the values of bioactive properties (TEAC and TPC) of the samples. It was determined that the four principal components (PC) explain 81% of the total variation among the samples. Specifically, in the first two components, PC1 and PC2 (Figure 5 A), 54% of the variation is explained. It will be observed that the quantified polyphenols, such as Chrysin, Rutin, Kaempferol and Cinnamic, Coumaric, Protocatechuic, Ellagic and Caffeic acids, are mainly related to bioactivity, since they are located at the top of the graph, while the rest show a less association as they are located at the bottom.

Focusing on PC2, a slight relationship between the origin of the extract and bioactivity is noticed, with an ordering of SE < EE < RE. However, extracts obtained from seeds are positioned in a sector opposite to the increase of antioxidant characteristics, despite containing compounds such as Rutin and Caffeic acid that were directly related to this characteristic. This may be due to the higher content of polyphenols such as Sinapic, Galangin, Apigenin, Hydroxybenzoic Acid, and Theobromine, which inversely correlated with the values obtained for TPC and TEAC. In this same sector, representing the lowest values of bioactive properties, extracts obtained by LIU are found, suggesting that this treatment does not favor the extraction of compounds that maximize functional characteristics.

Regarding the last two components, PC3 and PC4 (Figure 5 B), which explain 27% of the total variation, a differentiation between the samples according to the extraction method is evident. Samples obtained through the HIU process are located in the same zone as the bioactive properties (right side), while those obtained by other methods are mostly located on the opposite side (left side). This suggests that PC3 explains the variations related to the type of process applied, highlighting that HIU proved to be the most effective, followed by TA, while LIU was the least efficient. Furthermore, samples obtained by LIU are quite separated from each other (located in three different quadrants), indicating that for this process, the origin of the sample is even more relevant and that the treatment was very discreet.

The results indicate that the different fractions of *Neltuma Ruscifolia* pods are primarily responsible for the variations in the antioxidant properties of the extracts, while the extraction method has a secondary but significant impact.

(Fig.5)

Fig. 5 Differentiation of the samples through multivariate principal component analysis, based on the quantification of the polyphenol profile and its relationship with antioxidant properties (TEAC and TPC). Gal.a (Gallic acid), Vai.a (Vanillic acid), Chl.a (Chlorogenic acid), Caf.a (Caffeic acid), Caff (Caffeine), Fer.a (Ferulic acid), Que (Quercetin), Pro.a (Protocatechuic acid), Cou.a (Coumaric acid), Rut (Rutine), Cin.a (Cinnamic acid), Chr (Chrysin), The (Theobromine), Kae (Kaempferol), Hyd.a (Hydroxybenzoic acid), Ella.a (Ellagic acid), Api (Apigenin), Sin.a (Sinapic acid), Res (Resveratrol), Gal (Galangin). The circles represent agitation (TA), the diamonds represent high-intensity ultrasound (HIU) and the triangles represent low-intensity ultrasound treatment. The black colors represent the residue extracts (RE), the gray colors the endocarp extracts (EE) and white seed extracts (SE)

4. Conclusions

It is feasible to obtain extracts with antioxidant and antiglycation capabilities from the milling fractions of *Neltuma ruscifolia*. Variations were observed among different fractions and extraction methods, with the residue fraction and high-intensity ultrasound extraction exhibiting the highest bioactivity in both antioxidant and antiglycation properties. The findings highlight that the diverse fractions of *Neltuma Ruscifolia* pods play a pivotal role in influencing the bioactive properties of the extracts, while the extraction method secondarily significantly impacts the outcomes. The use of technologies such as low and high intensity ultrasound allows obtaining polyphenolic compounds (for example hydroxybenzoic acid) that cannot be released through agitation extraction. The obtained seeds extracts contained compounds such as rutin and caffeic acid that are among those highly related to bioactivity properties.This study establishes the basis for future development of antioxidant or antiglycation additives derived from *Neltuma ruscifolia*, diversifying the array of natural additives from NUS and aligning with the Sustainable Development Goals (SDGs) to safeguard biodiversity, local resources, and the planet.

Supplementary Information

Giuliana Seling: Formal analysis, Investigation, Data curation, Roles/Writing - original draft.
Roy Rivero: Data curation, Formal Analysis, Roles expert in multivariate analysis -
Verónica Busch: Funding acquisition, Project administration, Resources, Visualization, Roles/Writing - original draft.
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Data Availability

https://drive.google.com/drive/folders/1nDc29nBRHXhc6gFfV0urmGe3XKqxL1ri?usp=drive_link

Declarations

Competing Interests.

All authors declare that there is no conflict of interest or competing interests.

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Figures

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

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