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Proceeding Paper

Compositional Changes Associated with Successive Boiling of Wild *Cynophalla retusa* (Indian Bean) Pods Collected from the Paraguayan Chaco †

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Abstract: *Cynophalla retusa*, known as "Indian bean", is an important traditional food for the ethnic groups of the Gran Chaco. However, its contribution of minerals to the diet is unknown and the toxic nature of its raw pods has been reported. The aim of this investigation was to evaluate the composition of minerals, oxalic acid and phytate contents in whole raw and cooked pods, with successive changes of boiling water every 1 h for 4 h in total, as well as the alkaloid content in the cooking water. Bivalent mineral composition determinations (Ca, Fe, Cu and Mg) were made, as well as measurements of the phosphorus and antinutrient contents, such as phytate and oxalic acid, to determine the mineral contribution. The raw pods (*C. retusa*) contained 6.67% ash, with high contents of Ca, Fe, Cu, Mg and P. Loss of minerals occurred with successive boiling and significant decreases in antinutrients, with significant changes after each boiling period (1, 2, 3, 4 h). The boiling improved the bioavailability of Ca by removing oxalic acid from the sample cooked in the fourth boiling period. However, the phytate contents were not reduced to the same extent (only up to 40%). The results show that *C. retusa* pods can be a source of minerals (Ca, Fe, Cu and Mg) under controlled conditions of cooking and decreases in antinutrients like oxalic acid. From this perspective, this food source can be a viable alternative to increase food safety and nutrition, using one of many Paraguayan species that are little-known. Therefore, domestication and conservation studies are necessary.

Keywords: antinutrients; *Cynophalla retusa*; oxalic acid; phytate; neglected and underutilized species; minerals

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1. Introduction

Regional food resources have great value within the framework of food security, and there are many indigenous foods that have been underutilized until now. However, they constitute means of subsistence in indigenous populations of the Central Chaco, as is the case with the "Indian bean", "sacha bean" and "guaicuru bean" (*Cynophalla retusa* (Griseb.) X. Cornejo and H.H. Iltis. This name was recently accepted name for the flora of the Southern Cone, and it is synonymous with *Capparis retusa* Griseb., *Capparis retusa* Griseb. var. velutina, *Capparis cynophallophora* L. var. *cuneata*, *Capparis cynophallophora* L. var. *retusa*). The genus *Cynophalla* (DC.) J. Presl (Capparaceae) comprises 16 woody species, was established to group a series of species previously found in the genus *Capparis* L. [1]. One of the main limitations for its integral use is the lack of knowledge about its real nutritional value and its wild appearance, so studies on its nutritional potential in ancestral consumption conditions and its adaptation to standardized cultivation systems can be a way to improve the nutrition of the population and generate market opportunities in the productive sector of non-traditional crops.

Cynophalla retusa it is a shrub typical of the Gran Chaco ecosystem, 2–7 m high, and with a silicuiform, incurved capsular fruit, with notable strangulations, whose ripening season occurs from December to March. The plant is considered toxic and its fruit, although edible, is cooked in a particular way known by the local communities of the indigenous peoples of the Gran Chaco, such as the Lengua–Maskoy, Tobas, Wichis, Qom, Qomle'ec and Pilagá. Ethnobotanical studies report that, in human food, the boiled fruits of *Cynophalla retusa* are consumed with successive changes of water, in order to extract their "bitter beginning" [2,3]. It has been observed that *C. retusa* raw grains contain antinutrients, such as oxalic acid (87.8–121.0 mg/100 g) and phytate (463.6–535.7 mg/100 g), which can interfere with the bioavailability of minerals [4].

Although plant foods can be important sources of minerals in the diet, they can have the presence of oxalic acid and phytates (*myo*-inositol hexakisphosphate; InsP_6), two important components that can affect the bioavailability of bivalent minerals, because they potentially form complexes with dietary minerals and proteins, such as phytates, or by forming salts with oxalic acid, and decrease their bioavailability. These compounds can be reduced by boiling and solubilization in cooking water, or by treatments such as pre-soaking, germination or fermentation [5]. The choice of method for the reduction in these undesirable compounds depends largely on the type of food and the form of the final product in which that food is consumed. The aim of this investigation was to evaluate the composition of mineral, oxalic acid and phytate contents in whole raw and cooked *Cynophalla retusa* pods, with successive changes to the water in which the *C. retusa* is boiled.

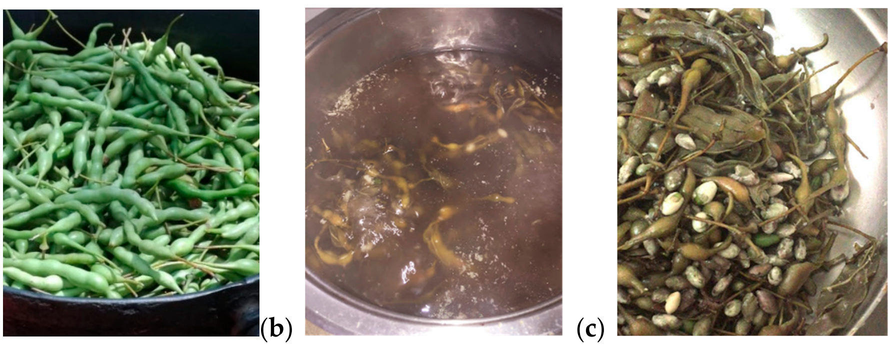
2. Materials and Methods

2.1. Raw Material

The samples used for the study were the closed mature capsules of *Cynophalla retusa* from Philadelphia, Chaco, Paraguay, collected in 2021. Once in the Food Biochemistry department laboratory (FCQ-UNA), they were preserved at −20 °C until the time of analysis.

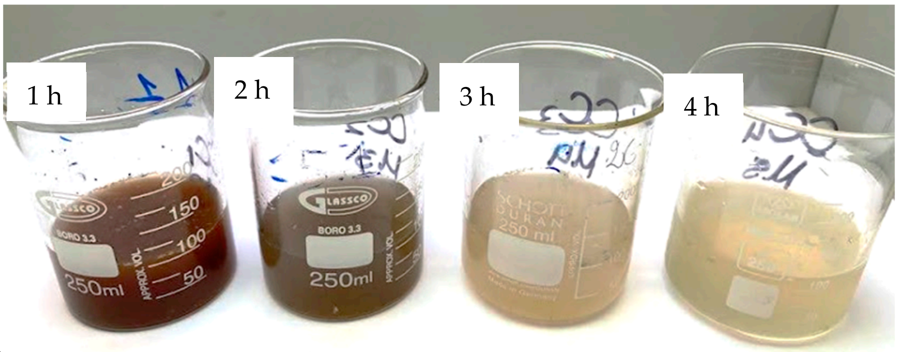
2.2. Experimental Design

A triplicate experiment was performed at a laboratory scale, according to the cooking process reported in the literature [3] for the raw mature whole capsules of *Cynophalla retusa*, with successive changes of water every 1 h; here, the capsules were boiled for 4 h in total (Figure 1). Bivalent mineral composition determinations (Ca, Fe, Cu and Mg) were made, as well as measurements of phosphorus and antinutrient contents, such as phytate and oxalic acid, to determine the mineral contribution in the cooking samples. The presence of alkaloids was also determined in the cooking water. The mineral, oxalic acid and phytate contents in whole raw and cooked *C. retusa* pods were evaluated.



(a) **(b)** **(c)**

Figure 1. *Cont*.



(**d**)

Figure 1. Process of sample cooking: (**a**) *Cynophalla retusa* fresh whole pods. (**b**) Pods boiled with water. (**c**) Samples drained after the first boiling period (1 h). (**d**) Appearance of the cooking broth after water changes at 1 h, 2 h, 3 h and 4 h.

2.3. Analysis

Minerals (Ca, Fe, Mg, Cu) were determined by official AOAC 968.08 method [6] by atomic absorption spectrophotometry (AAS) in a SHIMADZU model AA 6300 equipment (Kyoto, Japan). Results were expressed in mg/100 g. The phosphorus content was analyzed by the AOAC 970.39 method, measuring the percentage of transmittance, at a wavelength of 400 nm UV, on a SHIMADZU device, model UV-1800 (Kyoto, Japan). Results were expressed in mg/100 g. The phytate content was determined by the method previously described by García et al. (1982), by complexometric titration of the excess Fe (III) with EDTA as the titrating chelating agent and 5-sulfosalicylic acid as an indicator. Results were expressed in phytic acid equivalents (PAE/100 g). The determination of oxalic acid was performed according to the method described by Dona and Vercheret [7], by spectrophotometry at 305 nm, due to dissociation of the zirconia (IV)–chloranilate complex.

3. Results and Discussion

Mineral Composition

The raw pods (*C. retusa*) contained 6.67% ash, with high contents of Ca, Fe, Cu, Mg and P. According to the contents of the bivalent minerals (Ca, Fe, Cu and Mg), a significant decrease could be observed after 1 h of cooking (Table 1). Variable values were observed in each cooking hour in water. However, at the end of the experiment, all the mineral concentrations were significantly lower in the cooked samples. Data on the mineral composition of this food are limited in the literature; to our knowledge, this is the first work on the effect of cooking on the mineral content of *Cynophalla retusa* capsules. Taking into account that the species *Cynophalla retusa* is considered synonymous with *Capparis retusa*, comparisons were made with other species of the *Capparis* genus. Thus, in the raw samples of *C. retusa*, the Ca content was comparable to that of *Capparis spinosa* “capers” (21.8–154 mg/100 g), and much higher compared to what has been reported in fruits of *Capparis decidua* (14.1–35.1 mg/100 g). The Fe contents reported for other Capparis fruits, such as *C. ovata* (5.2–43.9 mg/100 g), *C. spinosa* (6.9–25.4 mg/100 g) and *C. decidua* (12.3–81.8 mg/100 g) are higher than those observed in this work (1.57 $\pm$ 0.25 mg/100 g) [8].

Table 1. Contents of minerals, phytic acid and oxalic acid and presence of alkaloids in the raw and cooked samples.

Compound	Cooking Time in Water at 100 °C (h)				
	0 (raw)	1	2	3	4
Ca (mg/100 g)	87.1 ± 6.8 ^{a}	33.7 ± 5.3 ^{b}	38.1 ± 6.1 ^{b}	50.8 ± 2.3 ^{c}	39.1 ± 6.3 ^{bc}
Fe (mg/100 g)	1.57 ± 0.25 ^{a}	0.55 ± 0.19 ^{b}	0.370 ± 0.050 ^{bc}	0.34 ± 0.09 ^{bc}	1.06 ± 0.64 ^{bc}
Cu (mg/100 g)	0.73 ± 0.06 ^{a}	0.600 ± 0.028 ^{b}	0.51 ± 0.04 ^{c}	0.64 ± 0.01 ^{bd}	0.71 ± 0.03 ^{a}
Mg (mg/100 g)	48.3 ± 4.9 ^{a}	5.90 ± 0.78 ^{b}	8.78 ± 1.01 ^{bc}	11.9 ± 0.7 ^{c}	7.26 ± 1.13 ^{bc}
P (mg/100 g)	98.1 ± 4.2 ^{a}	24.3 ± 0.94 ^{b}	19.4 ± 0.7 ^{bc}	17.1 ± 0.7 ^{c}	14.4 ± 0.9 ^{c}
PA (PAE/100 g).	1950 ± 316 ^{ab}	1183 ± 365 ^{b}	989 ± 126 ^{b}	908 ± 25 ^{b}	790 ± 180 ^{b}
OA (mg/100 g).	191.1 ± 1.4 ^{a}	34.16 ± 6.83 ^{b}	25.6 ± 1.5 ^{bc}	21.1 ± 5.1	Not detectable
Alkaloids	+++	-	-	-	-

Note: The results are expressed as average ± SD. Different letters in the same row demonstrate statistically significant differences between means (single-factor ANOVA, post-Tukey test; $p < 0.05$). PAE: phytic acid (PA) equivalent, OA: oxalic acid. (+++) abundant presence, (-) absence/not detected.

However, previous data reported [4] indicate that in general, the grains of *C. retusa* have a potential contribution of magnesium (160–165 mg/100 g), iron (3.70–4.40 mg/100 g) and phosphorus (294–305 mg/100 g), with concentrations higher than those observed in the whole pods in this work. The P content in the raw samples (98.1 ± 4.2 mg/100 g) (Table 1) also decreased significantly in the first 1 h cooking period, with values much lower than those of the fruits of species such as *C. ovata* and *C. decidua* (290–3073 and 701–808 mg/100 g, respectively) [8]. The concentration of phytate decreased after 1 h of cooking, but then remained stable (Table 1). All the phytate values were higher than those reported in the grains of *C. retusa* 463.6–535.7 mg/100 g [4], which could indicate that the phytate content is higher in the pod that surrounds the grains in the fruit capsule. The oxalic acid content in the crude samples (191.1 ± 1.4 mg/100 g) was also higher than that reported for grains of *C. retusa* (87.8–121.0 mg/100 g) [4], and decreased from its initial value in the raw samples to levels not detectable after 4 h of cooking, with successive water changes. These results show that oxalic acid is lost in the cooking broth. However, phytates are more stable in the matrix and remain even with a boiling treatment of 4 h. The content of P found in the form of phytate was calculated to determine the amount of bioavailable free phosphorus in the samples, and the percentage of phosphorus as phytic acid is shown in Table 2. It is known that the limit value for the molar ratio of AF/Ca = 0.2, and that values above this would mean that PA compromises the good absorption of Ca [9]. On the other hand, a PA/Fe molar ratio > 0.4 also compromises the good absorption of Fe [10]. The results obtained allow us to understand that the bioavailability of Ca and Fe in both raw and cooked samples can be affected by phytate content (Table 2).

Table 2. Phytic acid and molar ratio between the antinutrients and minerals Ca and Fe.

Relation	Boiling Time (h)				
	0 (raw)	1	2	3	4
% P as PA	5.61 ± 0.94 ^{a}	13.7 ± 4.4 ^{b}	14.4 ± 2.2 ^{b}	14.9 ± 4.0 ^{bc}	15.6 ±4.5 ^{c}
PA/Ca	1.30 ± 0.23 ^{a}	1.20 ± 0.35 ^{a}	1.23 ± 0.15 ^{a}	1.00 ± 0.29 ^{ab}	0.75 ± 0.09 ^{b}
PA/Fe	116 ± 15 ^{ab}	206 ± 28 ^{c}	257 ± 38 ^{cd}	396 ± 298 ^{ad}	79 ± 26 ^{a}
OA/Ca	0.84 ± 0.04 ^{c}	0.23 ± 0.05 ^{b}	0.21 ± 0.01 ^{b}	0.15 ± 0.02 ^{a}	-

Note: The results are expressed as average ± standard deviation. Different letters in the same row demonstrate statistically significant differences between means (single-factor ANOVA, post-Tukey test; $p < 0.05$). % P as PA indicates the percentage of phosphorus as phytic acid. PA/Ca: phytic acid/Ca molar ratio. PA/Fe: phytic acid/Fe molar ratio. OA/Ca: oxalic acid/Ca molar ratio. (-) absence/not detected.

This can be explained in part by the fact that phytates are thermostable, and therefore a significant reduction in their content cannot be expected during the cooking process. A Phytate has traditionally been considered an antinutrient in animal feed and human diets, due to its potent chelating capacity. However, more recently, phytate has been recognized as a natural antioxidant and as a nutraceutical, and classified by the Food and Drug Administration (FDA) as Generally Recognized As Safe (GRAS), it has potent antioxidation and anti-inflammatory actions and it has been shown to be effective in treating or preventing certain diseases. Recommending a diet high in phytate can exert multiple health benefits, with no harm [9].

On the other hand, the ability of oxalic acid to interact with Ca, being a dicarboxylic acid that forms insoluble salts with calcium, produces a decrease in the bioavailability of this metal if the AO/Ca ratio > 1. It is considered that 2.5 g of oxalic acid precipitates with 1 g of calcium. Therefore, the bioavailability of calcium is determined by the oxalic acid/calcium molar ratio, and when this ratio is greater than 2.25, the consumption of the food can be decalcifying, since the calcium of the observed food not only complexes but, concomitantly, binds to calcium from other foods in the digestive tract, as well as precipitating Ca ions present in the intestinal lumen [9]. The calculation of the AO/Ca ratio made it possible to demonstrate that the water cooking treatment and the successive water changes were efficient for the removal of oxalic acid from the crude sample and improved the bioavailability of the calcium (Table 2). From this perspective, more studies on the optimization of cooking treatments are necessary to achieve the nutritionally efficient use of the minerals present in this native food resource, such as with the previous use of phytase enzymes, without ruling out that this food source can be a viable alternative to increase food security and nutrition, in local communities in situations of scarcity, following the traditional procedures validated here. It is necessary to design conservation programs and use plans to improve the sustainability of food systems in which cooking methods are used in an ancestral way.

4. Conclusions

In the sample of the raw whole fruit, a high content of Ca, Fe, Cu, Mg and P was observed. During the cooking process for 4 h with successive water changes, it was observed that all these minerals presented significant differences in their concentrations throughout the experiment in the cooked sample. However, although this process decreases the mineral content in the feed, it improves the bioavailability of Ca due to the reduction in antinutrients such as oxalic acid. The oxalic acid content was removed up to undetectable values after the 4 h cooking treatment. However, the phytate was only 40% removed. The results show that *C. retusa* pods can be a source of minerals (Ca, Fe, Cu and Mg) under controlled cooking conditions and decreases in antinutrients such as oxalic acid.

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References

1. Abraham de Noir, F.; Bravo, S. *Frutos de leñosas nativas de Argentina*; 1a; Universidad Nacional de Santiago del Estero—UNSE, Facultad de Ciencias Forestales: Santiago del Estero, Argentina, 2014; ISBN 978-987-1676-19-4.

2. Schmeda-Hirschmann, G.; Theoduloz, C.; Jiménez-Aspee, F.; Echeverría, J. Bioactive constituents from south american prosopis and their use and toxicity. *Curr. Pharm. Des.* **2020**, *26*, 542–555. [CrossRef] [PubMed]
3. Arenas, P.; Scarpa, G.F. Edible Wild Plants of the Chorote Indians, Gran Chaco, Argentina. *Bot. J. Linn. Soc.* **2007**, *153*, 73–85. [CrossRef]
4. Mereles, L.; Caballero, S.; Coronel, E.; Villalba, R.; López, J.; Piris, P.; Wiszovaty, L.; Delmás, G.; Friesen, A. Recursos alimentarios autóctonos del Chaco. Una mirada a su potencial nutritivo. *Rojasiana*; Special issue No. 6. 2022, pp. 1–65. San Lorenzo, Paraguay. Available online: https://qui.una.py.vxsct57016.avnam.net/wp-content/uploads/2024/07/1.-ROJASIANA-Serie-especial-6-DIGITAL-FINAL-.pdf (accessed on 12 November 2024).
5. Sathe, S.; Venkatachalam, M. Influence of Processing Technologies on Phytate and Its Removal. In *Food Phytates*; CRC Press: Boca Raton, FL, USA, 2001; p. 32. ISBN 978-0-429-13488-3.
6. Horwitz, W.; Chichilo, P.; Reynols, H. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 17th ed.; AOAC: Gaithersburg, MA, USA, 2000.
7. Dona, A.-M.; Verchère, J.-F. Analytical Applications of Oxocarbons. Part 3. Specific Spectrophotometric Determination of Oxalic Acid by Dissociation of the Zirconium(IV)–Chloranilate Complex. *Analyst* **1991**, *116*, 533–536. [CrossRef]
8. Iqbal, A.; Anwar, F.; Nadeem, R.; Sultana, B.; Mushtaq, M. Proximate Composition and Minerals Profile of Fruit and Flower of Karir (Capparis Decidua) from Different Regions of Punjab (Pakistan). *Asian J. Chem.* **2014**, *26*, 360–364. [CrossRef]
9. Pujol, A.; Sanchis, P.; Grases, F.; Masmiquel, L. Phytate Intake, Health and Disease: "Let Thy Food Be Thy Medicine and Medicine Be Thy Food". *Antioxidants* **2023**, *12*, 146. [CrossRef]
10. Abdulwaliyu, I.; Arekemase, S.O.; Adudu, J.A.; Batari, M.L.; Egbule, M.N.; Okoduwa, S.I.R. Investigation of the Medicinal Significance of Phytic Acid as an Indispensable Anti-Nutrient in Diseases. *Clin. Nutr. Exp.* **2019**, *28*, 42–61. [CrossRef]