Received: 2 September 2023 | Revised: 22 December 2023 | Accepted: 9 January 2024

DOI: 10.1002/aocs.12820

ORIGINAL ARTICLE

AOCS WILEY

Nutrient and bioactive compounds from *Neltuma* spp. seeds

M. Cecilia Cittadini[1] | **Romina Bodoira**[2] | **Damián Barrionuevo**[1] | **Diana Labuckas**[1] | **Marcela Martínez**[1] | **Damián Maestri**[1]

[1]Area Química Agrícola, Instituto Multidisciplinario de Biología Vegetal (IMBIV - Consejo Nacional de Investigaciones Científicas y Técnicas - Universidad Nacional de Córdoba), Córdoba, Argentina

[2]Instituto de Ciencia y Tecnología de los Alimentos Córdoba (ICYTAC – CONICET - UNC), Córdoba, Argentina

Correspondence
Romina Bodoira, Instituto de Ciencia y Tecnología de los Alimentos Córdoba (ICYTAC – CONICET - UNC), Córdoba, Argentina.
Email: romina.bodoira@mi.unc.edu.ar

Abstract

In the search for compounds with nutritional and functional value, seeds from several *Neltuma* species were examined for lipid, protein and phenolic components. Lipid contents ranged between 9.3 and 12.5 g/100 g (seed dry weight), and showed both oleic and linoleic acids predominating largely. Lipids were also a good source of tocopherols (170–603 mg total tocopherols/kg). Proteins accounted for 14.7–35.5 g/100 g seed. Essential amino acids (EAA) comprised 26.1%–40.2% of the total AA content. Based on the EAA score, proteins from all the species analyzed were deficient in leucine, isoleucine and valine, but could meet requirements for histidine, threonine, phenylalanine, tyrosine, and lysine. The qualitative patterns of phenolic compounds were similar each other (predominance of flavonoids, particularly apigenin derivatives). Keeping in mind the examined chemical components, *Neltuma* seeds have potential value as a source of healthy macronutrients and bioactive compounds.

KEYWORDS
amino acids, lipid components, *Neltuma* spp., phenolic compounds, seeds

INTRODUCTION

Cultivated legumes are widely consumed as food worldwide. They provide a good source of protein and many other important nutritional and functional components (Bennetau-Pelissero, 2019; Grela et al., 2017). Several wild legume species have also been investigated as a source of nutrients for humans and livestock (Arinathan et al., 2003; Bhat & Karim, 2009). In many cases, they are present in agroforestry systems where besides providing invaluable benefits such as ecosystem services, can contribute sustainable food resources especially in rural communities.

The species of the genus *Neltuma* (ex *Prosopis*, Fabaceae) are important vegetation elements, particularly in arid and semiarid regions of the world. Many of them produce abundant pods which, since ancient times, have provided basic foodstuffs for man as well as forage for wildlife and domestic herbivores. Most of research on composition and nutritional value of *Neltuma* pods has focused on the mesocarp components (Felker et al., 2013). Studies on seed components are largely unaddressed. This could be due to the belief that the seeds have little nutritional benefit. So, in some regions and countries *Neltuma* foodstuffs are obtained by using only the pods, and seeds are discarded. Currently, there are few reports on seed chemical components of *Neltuma* spp. Fatty acid and sterol compositions have been investigated in *N. alpataco* and *N. denudans* from the Patagonian region in Argentina (Mazzuca & Balzaretti, 2003). Chemical analyses of different parts of *N. alba* fruits indicate that the seeds contain most of the protein; in addition, they provide significant amounts of total polyphenols (Sciammaro et al., 2016). Interestingly, protein concentrates obtained from *N. alba* seeds have been proposed for formulation of functional foods because of their good amino acid profiles and important biological and functional properties (Cattaneo et al., 2014).

In Argentina, several *Neltuma* species have become widespread (Joseau et al., 2013). They can grow and fruit in marginal areas for conventional crops,

Abbreviations: AA, amino acid; DW, dry weight; EAA, essential amino acid; FA, fatty acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TPC, total phenol content; TT, total tocopherol.

under conditions of very low water availability. The present research was primarily aimed to contribute information to nutrient databanks of underexploited plant resources. Thus, four *Neltuma* species were evaluated for unexplored seed components with a focus on nutritional and functional compounds.

MATERIALS AND METHODS

Pods from *Neltuma* species (*N. flexuosa*, *N. nigra*, *N. chilensis*, *N. alba*) were obtained from trees of natural populations in the north-western region of Córdoba province (Argentina). The identity of the named species was previously authenticated by using herbarium specimens from the botanical museum CORD (Argentina). From each species, three pod samples (about 1 kg each)—each obtained from at least five individual trees—were used for analytical determinations. Pods were harvested at full maturity stage and, after drying in darkness, the seeds were separated by hand and stored at −20°C until use. Before proceeding to the different analyses, seeds were ground to a fine powder using a universal cutting mill (Tecno Dalvo, Argentina). Seed dry matter content was determined by oven drying at 80°C (AOCS, 2009).

Lipid analyses

Lipid extraction for total lipid content determination was performed using Soxhlet devices with n-hexane as solvent (AOCS, 2009). Fatty acid (FA) composition was evaluated—as methyl esters (FAME)—by gas chromatography (GC) according to protocols for lipid sample preparation and GC conditions previously reported (Cittadini et al., 2021). Identity of FAME was confirmed by means of GC (Clarus 580, Perkin-Elmer, Shelton, USA)—mass spectrometry (MS, Clarus SQ8S) analysis. Separations were performed on a CP Wax 52 CB (Varian, Walnut Creek, USA) fused-silica capillary column using helium (flow rate 1 mL/min) as carrier gas. The GC oven temperature was initially maintained at 180°C (5 min) and then increased at 2°C/min to 220°C. Both injector and detector temperatures were set at 250°C. The FAME components were identified by mass spectra matching using the Wiley mass spectra search library.

For tocopherol analysis, ground seed samples (20 g each) were extracted with n-hexane (40 mL × three times) at room temperature (22–25°C). The solvent was removed using a rotary vacuum evaporator at 40°C. The lipid samples obtained were analyzed by high performance liquid chromatography (HPLC, Perkin-Elmer, Shelton, USA) following analytical methods used previously (Cittadini et al., 2021). Accurately-weighted lipid samples (1 g each) were diluted with n-hexane to 10 mL. The solutions were filtered through 0.45 μm pore filters. Aliquots of 20 μL of the filtrated solutions were injected into a Supelcosil LC-NH2-NP column (25 cm × 4.6 mm, Supelco, Bellefonte, USA). The mobile phase was n-hexane/ethyl acetate (70/30 vol/vol) with a flow rate of 1 mL/min. UV detection at 295 nm was performed. Individual tocopherols were identified by comparing their retention times with those of authentic standards (α-, γ- and δ-tocopherols, ICN Biomedicals, Costa Mesa, USA), and were quantified by the external standard method. The linearity of the response was verified by fitting to line results of each one tocopherol individuals (eight standard solutions with known concentrations) covering the concentration range from 2 to 800 ppm, with linearity regression coefficients (R^2) varying between 0.98 and 0.99.

Protein analyses

The Kjeldahl method (total nitrogen x 6.25) was used to quantify the seed total protein content (AOAC, 2009). For amino acid (AA) analysis, defatted seed samples (500 mg) were hydrolyzed following the standard AOCS (2009) procedure. Hydrolyzed samples were filtered using 0.45 μm membrane filters and then submitted to pre-column derivatization. For this purpose, accurately-weighted hydrolyzed samples were placed in screw-capped tubes and re-suspended in 500 μL HCl 0.1 M plus 1.8 mL borate buffer, 0.75 mL methanol and 15 μL diethyl ethoxymethylenemalonate (Sigma-Aldrich, St. Louis, USA). The tubes were kept at a constant temperature (25°C) for 24 h. The obtained N-[2,2-bis (ethoxycarbonyl) vinyl] AA derivatives were analyzed by reversed-phase HPLC (Perkin-Elmer, Shelton, USA) using a C18 column (Zorbax Eclipse Plus C18, Agilent Technologies, Santa Clara, USA) thermostated at 40°C. Solvents (A, sodium acetate buffer, 25 mM pH 6; B, methanol/acetonitrile/water, 45/45/10 vol/vol) were used at a flow rate of 0.9 mL/min, according to the following gradient: 100% A for the first 18 min, 57% to 100% B for the next 5 min, 0% B from 23.1 to 30 min. Detection of AA derivatives was performed at 280 nm. Identification was carried out by using authentic AA standards (Sigma-Aldrich, St. Louis, USA), and quantification by means of the external standard method. The linearity of the response was verified by fitting to line results of each one amino acid individuals (five standard solutions with known concentrations). Calibration curves from the tested amino acid standards showed linearity regression coefficients (R^2) ranging between 0.95 and 0.99. The contents of the various identified essential AA (EAA) were compared with the FAO/WHO/UNU (2002) reference pattern. Individual EAA scores were determined according to FAO/WHO/UNU using the formula: AA score = mg of AA in 1 g test protein/mg of AA in requirement pattern. Limiting AA were identified as those having a ratio lesser than 1.

Phenolic compound analyses

Phenolic compounds were obtained from 10 g of ground seed samples previously defatted at room temperature (n-hexane 50 mL × three times). Defatted samples (5 g) were extracted thrice with methanol (80%, 50 mL every time). The extracts were combined and washed twice (50 mL every time) with n-hexane. The hydro-alcoholic phase was recovered, filtrated through 0.45 μm pore filters, concentrated under vacuum below 50°C to a final volume of 1 mL, and further used for both total phenol content (TPC) and HPLC-ESI-MS/MS analyses according to procedures reported elsewhere (Bodoira et al., 2022).

The composition of phenolic extracts was analyzed by means of HPLC-ESI-MS/MS using an Agilent 1200 HPLC Series system (Agilent Technologies, Santa Clara, USA). The chromatographic separations were achieved on a Kromasil (Bohus, Sweden) reversed-phase C18 column (5 μm, 250 mm × 4.60 mm i.d.) under analytical conditions used previously (Bodoira et al., 2022). The MS detector was programmed to perform a MS/MS scan of the most abundant ions, using collision energy of 13.0 eV. The identification of phenolic compounds was based on their retention times (Rt), elution order, and comparison of UV–Vis spectra and mass spectrometry data reported in the literature (Cittadini et al., 2021; Picariello et al., 2017; Singh et al., 2017). The Compass version 3.1 software and DataAnalysis version 4.1 software were used for data acquisition and processing, respectively.

Statistical analysis

Statistical differences between species in the analyzed chemical parameters were estimated from ANOVA test at the 5% level ($p \leq 0.05$) of significance. Whenever ANOVA indicated a significant difference, a pair-wise comparison of means by least significant difference (LSD) was carried out. Statistical analyses were performed using the InfoStat program (InfoStat version 2018, National University of Córdoba, Argentina).

RESULTS AND DISCUSSION

Lipid content and composition

The seed lipid contents of the studied species were in the range 9.33%–12.50% (Table 1). These values result higher than those found in *Prosopis farcta* (3.2%, Lajnef et al., 2015) and *N. alpataco* (7.0%, Mazzuca & Balzaretti, 2003), and similar to those reported by Lamarque et al. (1994) in *N. caldenia* (10.7%) and *N. argentina* (14.7%). By way of comparison with other wild edible legumes, the seed lipid contents from the examined *Neltuma* spp. in many cases far exceed those found in species of *Canavalia* spp. (range 1.4%–12.1%) and *Cassia* spp. (3.8%–7.0%), as well as those of some tree legume species recently investigated which showed seed oil yields ranging from 2.1% to 14.9% (Grygier et al., 2023). On the other hand, seed lipid contents from *Neltuma* were notable

TABLE 1 Lipid content, fatty acid and tocopherol profiles from *Neltuma* spp. seeds.

Parameter	*N. alba*	*N. chilensis*	*N. nigra*	*N. flexuosa*
LC (g/100 g, DW)	9.33 ± 0.37^{a}	10.94 ± 0.16^{ab}	12.50 ± 0.06^{b}	11.99 ± 0.84^{b}
Fatty acids (%)				
16:0 (palmitic)	10.89 ± 0.10^{a}	12.72 ± 0.41^{ab}	15.28 ± 0.71^{b}	11.81 ± 0.48^{ab}
16:1 (palmitoleic)	0.78 ± 0.13^{c}	0.47 ± 0.08^{ab}	0.56 ± 0.01^{bc}	0.28 ± 0.01^{ab}
18:0 (stearic)	4.99 ± 0.10^{a}	5.53 ± 0.24^{a}	4.24 ± 0.26^{a}	5.59 ± 0.86^{a}
18:1 (oleic)	36.25 ± 0.26^{c}	35.56 ± 2.53^{c}	43.69 ± 0.32^{d}	27.21 ± 0.25^{b}
18:2 (linoleic)	41.12 ± 0.19^{b}	39.35 ± 1.60^{b}	32.00 ± 0.35^{a}	51.49 ± 0.04^{c}
18:3 (linolenic)	1.76 ± 0.05^{bc}	1.60 ± 0.07^{b}	2.23 ± 0.11^{d}	1.19 ± 0.01^{a}
20:0 (arachidic)	3.17 ± 0.18^{c}	3.85 ± 0.14^{d}	1.31 ± 0.16^{b}	1.84 ± 0.12^{b}
20:1 (eicosenoic)	1.07 ± 0.10^{d}	0.92 ± 0.10^{d}	0.72 ± 0.03^{c}	0.62 ± 0.04^{b}
ΣSFA	19.05 ± 0.16^{a}	22.10 ± 0.79^{a}	20.82 ± 0.81^{a}	19.23 ± 0.25^{a}
ΣMUFA	38.09 ± 0.40^{c}	36.95 ± 2.45^{c}	44.96 ± 0.35^{d}	28.10 ± 0.28^{b}
ΣPUFA	42.87 ± 0.24^{b}	40.95 ± 1.67^{b}	34.23 ± 0.46^{a}	52.68 ± 0.04^{c}
TT (mg/kg lipid)	252.33 ± 10.0^{b}	170.87 ± 3.16^{a}	435.26 ± 14.14^{c}	603.51 ± 5.41^{d}
α-Tocopherol	247.64 ± 9.89^{b}	167.55 ± 2.98^{a}	418.43 ± 13.32^{c}	544.58 ± 3.10^{d}
γ-Tocopherol	4.69 ± 0.12^{b}	3.32 ± 0.18^{a}	16.83 ± 0.82^{c}	58.93 ± 2.31^{d}

Note: Data are expressed as mean values ± standard deviation. For each chemical parameter, the mean values with different superscript letter are significantly different ($p > 0.05$).

Abbreviations: DW, dry weight; LC, lipid content; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; SFA, saturated fatty acids; TT, Total tocopherols.

higher than those from some cultivated legumes, such as peas (*Pisum sativum*), lentils (*Lens culinaris*) and beans (*Phaseolus vulgaris*) which usually contain about 2 g lipid per 100 g seed (Padhi et al., 2017).

Fatty acid (FA) composition is summarized in Table 1. All *Neltuma* species evaluated showed the same FA components. Palmitic, oleic and linoleic acids were found as major FAs, in general accordance with data from wild and cultivated legumes worldwide (Arinathan et al., 2003; Bhat & Karim, 2009; Grygier et al., 2023; Padhi et al., 2017). *Neltuma nigra* and *N. flexuosa* seeds presented the highest concentrations of oleic and linoleic acids, respectively. On the other hand, *N. alba* and *N. chilensis* had very similar composition; with the exception of palmitoleic and arachidic acids, no significant differences were found between their FA profiles. In general, the concentrations of the main FAs in the examined *Neltuma* species were similar to those reported in other species of the genus. Of note, however, are the lower linoleic acid percentages in *N. alba*, *N. chilensis* and *N. nigra* (about 41%, 39% and 32%, respectively) as compared to those found in *N. argentina* (51.1%) and *N. alpataco* (56.1%) seed oils (Lamarque et al., 1994; Mazzuca & Balzaretti, 2003).

Based on the lipid fraction, a comparison of the FA composition between the *Neltuma* species studied and some cultivated legumes revealed that the former have less PUFA (linoleic and linolenic acids) contents than the later (Padhi et al., 2017). However, when FA concentrations were calculated on the basis of dry seed weight, the PUFA content in *Neltuma* was in the range 4.0–6.3 g/100 g seed, which represent amounts substantially higher than those present in varieties of peas, lentils and beans (1.05, 0.86 and 1.34 g PUFA/100 g seed, respectively), and similar to those found by Padhi et al. (2017) in chickpeas (5.03 g PUFA/100 g seed).

A thorough review of the scientific literature does not show reports on tocopherol content and composition of *Neltuma* seeds. As a striking fact, the seed lipids from the studied species showed meaningful differences in their total tocopherol (TT) contents; the range observed was between 170.87 and 603.51 mg TT/kg lipid (Table 1). In all cases, the tocopherol fraction was composed mostly of α-tocopherol (167.55–544.58 mg/kg lipid) and minor amounts of γ-tocopherol (3.32–58.93 mg/kg lipid). Interestingly, the amount of TT found in *N. flexuosa* (about 600 mg/kg lipid) compares favorably with that found in peanut, a legume intended for oil production, in which the concentration of TT typically amounts to about 500 mg/kg oil (Maestri, 2023). On a DW basis of whole seeds, the range for tocopherol contents from all the species evaluated (from 2.35 mg TT/100 g in *N. chilensis* seeds to 7.21 mg TT/100 g in *N. flexuosa* seeds) is similar to that observed in edible pulses (2.2–5.7 mg TT/100 g) such as beans, lentils and peas (Padhi et al., 2017).

Protein content and composition

Protein content from *Neltuma* spp. accounted for 14.66%–35.46% of the seed dry matter content (Table 2); the highest level was observed in *N. flexuosa*. The values recorded are in line with those reported in other species of the genus such as *P. argentina* (26.9%) and *P. caldenia* (32.2%), (Lamarque et al., 1994). Moreover, they resemble the values found in some tribal pulses (12.9%–24.7%, Arinathan et al., 2003), and in cultivated peas (*Lathyrus* spp., 17.7%–25.6%) and lentils (*Vicia* spp., 20.1%–32.0%), (Pastor-Cavada et al., 2011a,b).

Amino acid (AA) composition observed in the present study is shown in Table 2. Except for isoleucine—which was below the detection limit we used—and leucine—not quantified in *N. nigra* seeds—, the species analyzed showed the protein AA profiles usually reported in cultivated legume species (Bennetau-Pelissero, 2019; Grela et al., 2017; Pastor-Cavada et al., 2011a,b). The percentage of essential AA (EAA), which is considered an indicator of protein nutritional quality, ranged between 26% and 40% of the total AA concentration. The values found in *N. alba* and *N. chilensis* are similar to those present in whole-wheat flour (about 27%), which is a widespread and valuable source of proteins (Shewry & Hey, 2015). Meanwhile, the values from *N. nigra* and *N. flexuosa* compared well with those of cultivated beans such as *Lathyrus* and *Vicia* species (39%–42%), (Pastor-Cavada et al., 2011a,b).

An exhaustive bibliographic survey shows little information about AA composition of *Neltuma* seeds. Seed protein isolates obtained from *N. alba* showed all EAA (Cattaneo et al., 2014), in quantities above the established requirements for human adults (FAO/UNU/WHO, 2002). Besides the absence of isoleucine, proteins from all the species we studied were deficient in leucine and valine, but could meet requirements for histidine, threonine, phenylalanine + tyrosine, and lysine. Seed proteins from *N. alba* and *N. flexuosa* could also satisfy methionine requirements (Table 2). Worthy of mention are the relatively high amounts of histidine, threonine and lysine, particularly in *N. nigra* and *N. flexuosa* (12.75–13.22, 6.84–9.15, and 10.52–11.17 g/100 g protein, respectively). These contents are substantially higher than those present in several *Lathyrus* and *Vicia* species which showed histidine, threonine and lysine contents ranging between 2.2–3.3, 4.0–6.2 and 6.4–8.0 g/100 g protein, respectively (Pastor-Cavada et al., 2011a,b). While histidine has been associated with factors that improve the so-called metabolic syndrome in humans (Moro et al., 2020), threonine plays a vital role in the modulation of nutritional metabolism, macromolecular biosynthesis, and gut homeostasis (Tang et al., 2021). The requirement for lysine is also a matter of interest as it is limiting in cereals, especially wheat. So, the high contents of these AA in *N. nigra* and *N. flexuosa* highlight the

TABLE 2 Protein content and amino acid characterization from *Neltuma* seeds.

Parameter	*N. alba*	*N. chilensis*	*N. nigra*	*N. flexuosa*
PC[A]	24.90 ± 0.85[b]	23.20 ± 1.27[b]	14.66 ± 0.41[a]	35.46 ± 2.60[c]
Asp + Asn[B]	2.04 ± 0.32[a]	2.32 ± 0.28[a]	1.56 ± 0.49[a]	3.05 ± 0.53[a]
Glu + Gln	3.99 ± 0.56[b]	4.39 ± 0.51[b]	1.20 ± 0.35[a]	4.59 ± 0.20[b]
Ser	0.46 ± 0.11[a]	0.86 ± 0.08[b]	0.43 ± 0.01[a]	0.67 ± 0.01[ab]
His	0.66 ± 0.14[a]	0.60 ± 0.07[a]	1.50 ± 0.13[a]	3.35 ± 0.64[b]
Gly	1.34 ± 0.17[a]	1.27 ± 0.18[a]	0.99 ± 0.11[a]	1.76 ± 0.62[a]
Thr	1.02 ± 0.17[a]	1.35 ± 0.01[a]	0.77 ± 0.23[a]	1.72 ± 0.52[a]
Ala	2.59 ± 0.21[b]	1.20 ± 0.29[a]	0.97 ± 0.26[a]	2.54 ± 0.10[b]
Arg	1.06 ± 0.42[a,b]	2.54 ± 0.16[c]	0.84 ± 0.05[a]	0.91 ± 0.10[a]
Tyr	0.37 ± 0.08[a]	0.38 ± 0.01[ab]	0.39 ± 0.07[ab]	0.79 ± 0.20[b]
Cys	0.48 ± 0.08[a]	0.59 ± 0.13[a]	0.28 ± 0.10[a]	0.47 ± 0.33[a]
Val	0.17 ± 0.03[b]	0.51 ± 0.13[c]	0.09 ± 0.01[a]	0.13 ± 0.01[a]
Met	0.40 ± 0.13[a]	0.32 ± 0.45[a]	0.14 ± 0.10[a]	0.41 ± 0.10[a]
Pro	1.72 ± 0.43[b]	1.64 ± 0.01[b]	0.51 ± 0.05[a]	1.04 ± 0.07[ab]
Phe	0.79 ± 0.11[c]	0.47 ± 0.07[ab]	0.36 ± 0.01[a]	0.72 ± 0.04[bc]
Leu	0.66 ± 0.18[ab]	1.12 ± 0.06[b]	ND	0.71 ± 0.33[b]
Lys	1.10 ± 0.01[a]	1.45 ± 0.15[a]	1.49 ± 0.52[a]	2.75 ± 0.32[b]
% EAA[C]	26.2 ± 1.32[a]	27.5 ± 0.71[ab]	40.2 ± 6.09[c]	38.7 ± 1.64[bc]

EAA	EAACD	EAASE	EAAC	EAAS	EAAC	EAAS	EAAC	EAAS	MPR[F]
His	3.71 ± 0.44	2.47	2.93 ± 0.23	1.95	12.75 ± 0.71	8.50	13.22 ± 3.71	8.81	1.5
Thr	5.24 ± 1.10	2.28	6.60 ± 0.18	2.87	9.15 ± 1.86	3.98	6.84 ± 1.08	2.97	2.3
Val	0.86 ± 0.03	0.22	2.50 ± 0.72	0.64	0.40 ± 0.06	0.10	0.49 ± 0.07	0.12	3.9
Met	2.08 ± 0.75	1.30	1.52 ± 0.15	0.95	1.18 ± 0.82	0.73	1.62 ± 0.34	1.01	1.6
Phe	4.04 ± 0.33		2.29 ± 0.25		3.04 ± 0.01		2.51 ± 0.26		3.8 (Phe + Tyr)
Leu	4.02 ± 1.97	0.68	5.45 ± 0.12	0.92	NQ		2.58 ± 1.21	0.44	5.9
Lys	5.65 ± 0.33	1.25	7.07 ± 0.49	1.57	11.17 ± 2.12	2.48	10.52 ± 2.86	2.33	4.5

Note: Abbreviations and units: [A]PC, protein content (g/100 g seed, DW); [B]amino acid concentrations (g/100 g seed); [C]EAA, essential amino acids; [D]EAAC, essential amino acid concentrations (g/100 g protein), [E]EAAS, essential amino acid scores, [F]MPR, minimum physiological requirements for human adults (g/100 g protein) according to FAO/WHO/UNU (2002). Data are expressed as mean values ± standard deviation. Amino acid concentrations with different superscript letters are significantly different ($p > 0.05$) among *Neltuma* species.
Abbreviations: ND, not detected; NQ, not quantified.

potential of their seed meals to improve the AA profile of cereal flour-based foods.

In line with findings reported from several seed proteins—including those of legumes and cereals—the most abundant non-EAA were those from the two pairs namely aspartic acid + asparagine and glutamic acid + glutamine. Interestingly, the aminograms also revealed higher amounts of arginine than those usually present in edible pulses (Margier et al., 2018). Arginine is considered a semi-essential AA involved in multiple metabolic processes and biological functions (Morris, 2006).

Phenolic compounds

The *Neltuma* species studied showed meaningful differences in the content of total phenolic compounds (Table 3); the highest one was found in *N. nigra* (about 96 mg/100 g seed). Compared with values reported for cultivated legumes, such content is similar to those found in cowpeas (about 100 mg/100 g) and pigeon pea seeds (78–120 mg/100 g), but lower to those from chickpea and lentil varieties (Singh et al., 2017).

A total of 39 phenolic compounds were detected in the examined *Neltuma* seeds (Table 3). With the exception of compound 14, which was assigned tentatively as ferulic acid derivative, the remaining components were identified as flavonoids and structurally related substances. Apigenin derivatives were found to show the highest contents in all *Neltuma* species studied. Apigenin 8-C and 6-C glucosides—commonly named vitexin and isovitexin—were by far the most abundant components reaching concentrations near to 40 mg/100 g (DW) in *N. nigra* and *N. flexuosa* seeds. Such concentrations are within the ranges found in some cultivated legumes such as the mung bean

TABLE 3 Mass spectral data, identification and concentration of phenolic compounds from *Neltuma* spp. seeds.

CN (RT)	[M-H]⁻(m/z) theoretical	[M-H]⁻(m/z) experimental	MS^2 (m/z)	Identification	Chemical group	*Prosopis* spp.			
						N. Alba	*N. Chilensis*	*N. Nigra*	*N. Flexuosa*
1 (11.1)	283.0679	283.0671	151	Apigenin-7-methyl ether (Acacetin)	Flavone	0.04 ± 4.05E−03	0.09 ± 9.36E−03	0.11 ± 3.99E−02	0.21 ± 5.57E−02
2 (11.6)	305.0656	305.0675	219–261-179	Gallocatechin	Flavanol	0.19 ± 1.75E−02	0.53 ± 5.32E−02	0.63 ± 1.86E−01	4.92 ± 7.24E−01
3 (12.5)	725.1923	725.1983	563–545-353	Apigenin 6-C-(6''-O-glc) arab-8-C-glc (Isoschaftoside hexoside)	Flavone	0.57 ± 1.36E−02	0.52 ± 4.12E−02	0.24 ± 3.55E−02	0.29 ± 9.16E−01
4 (12.8)	725.1923	725.2006	563–545-353	Apigenin 6-C-(6''-O-glc) arab-8-C-glc (Schaftoside hexoside)	Flavone	1.18 ± 6.08E−02	0.87 ± 1.21E−01	0.52 ± 6.35E−03	4.60 ± 1.68E−01
5 (13.0)	289.0706	289.0732	245	Catechin	Flavanol	ND	ND	ND	0.11 ± 5.04E−03
6 (13.5)	593.1501	593.1558	473–431	Apigenin 6,8-di-C-glucoside (Vicenin I)	Flavone	0.70 ± 2.29E−02	0.65 ± 5.93E−02	1.20 ± 1.34E−01	5.69 ± 8.85E−01
7 (13.9)	593.1501	593.1552	473–431	Apigenin 6,8-di-C-glucoside (Vicenin II)	Flavone	1.25 ± 1.72E−01	0.44 ± 5.54E−02	1.94 ± 4.89E−02	6.11 ± 1.56
8 (14.0)	579.1344	579.1400	459	Luteolin 6-C-arab-8-C-glc	Flavone	1.38 ± 3.39E−02	1.41 ± 5.16E−03	1.03 ± 2.83E−02	ND
9 (15.1)	563.1395	563.1396	503–473	Apigenin 6-C-arab-8-C-glc (Isoschaftoside)	Flavone	4.67 ± 1.0E−04	14.41 ± 1.0E−04	39.49 ± 2.42	3.26 ± 1.0E−04
10 (15.5)	563.1395	563.1345	503–473	Apigenin 6-C-glc-8-C-arab (Schaftoside)	Flavone	6.07 ± 1.0E−04	16.52 ± 1.0E−04	43.75 ± 4.21	1.78 ± 1.0E−04
11 (15.8)	625.1399	625.1453	300	Quercetin dihexoside	Flavonol	0.26 ± 1.46E−02	ND	ND	ND
12 (16.0)	447.0922	447.0949	357–327-285	Luteolin hexoside	Flavone	ND	40.34 ± 4.52	ND	ND
13 (16.1)	577.1552	577.1586	527–457-337	Chrysin 6-C-glu-8-C-glc	Flavone	ND	ND	0.17 ± 1.52E−02	ND
14 (16.2)	273.000	273.0089	193	Ferulic acid derivative	HCA	0.45 ± 1.0E−04	1.78 ± 2.20E−01	1.28 ± 1.45E−01	ND
15 (17.1)	769.1974	769.2033	545–425	Apigenin 6-C-feruloyl glc-8-C-glc (Isomer I)	Flavone	0.11 ± 1.30E−02	0.09 ± 1.74E−02	0.07 ± 9.30E−03	ND
16 (17.2)	595.1294	595.1323	300	Quercetin hexoside pentoside	Flavonol	0.04 ± 3.08E−03	ND	ND	ND
17 (17.4)	431.0972	431.0997	311	Apigenin 8-C-glucoside (Vitexin)	Flavone	45.2 ± 4.38E−01	151.9 ± 2.89	386.7 ± 1.99	419.37 ± 8.46
18 (17.5)	739.1869	739.1923	545–425	Apigenin 6-C-feruloyl arab-8-C-glc	Flavone	0.01 ± 5.02E−03	0.15 ± 2.45E−02	0.24 ± 4.17E−03	1.59 ± 8.31E−02
19 (17.6)	547.1446	547.1475	503–427-337	Chrysin 6-C-arab-8-C-glc (Isomer I)	Flavone	ND	ND	0.38 ± 4.05E−02	ND
20 (17.6)	609.145	609.1504	609–301	Quercetin rutinoside (Isomer I)	Flavonol	0.07 ± 1.0E−04	ND	ND	ND
21 (17.7)	533.1289	533.1320	443	Apigenin 6-C-arab-8-C-pentoside (Isomer I)	Flavone	0.18 ± 9.88E−04	0.44 ± 3.73E−02	0.38 ± 1.31E−02	ND

TABLE 3 (Continued)

CN (RT)	[M-H]⁻(m/z) theoretical	[M-H]⁻(m/z) experimental	MS^2 (m/z)	Identification	Chemical group	*Prosopis* spp.			
						N. Alba	*N. Chilensis*	*N. Nigra*	*N. Flexuosa*
22 (17.9)	577.1552	577.1581	457–353	Apigenin rutinoside (Isomer I)	Flavone	ND	0.09 ± 1.87E−02	ND	ND
23 (18.7)	593.1501	593.1517	285	Kaempferol rutinoside	Flavonol	ND	ND	1.37 ± 9.47E−02	0.94 ± 2.41E−01
24 (19.0)	431.0972	431.0991	311–341	Apigenin 6-C-glucoside (Isovitexin)	Flavone	34.2 ± 1.92E−01	168.1 ± 1.71	441.2 ± 1.61	345.73 ± 9.18
25 (19.6)	547.1446	547.1463	503–427-337	Chrysin 6-C-arab-8-C-glc (Isomer II)	Flavone	ND	0.05 ± 5.67E−03	0.29 ± 4.08E−02	ND
26 (19.7)	533.1289	533.1324	443	Apigenin 6-C-pent-8-C-arab (Isomer II)	Flavone	ND	0.52 ± 1.01E−02	0.08 ± 2.76E−03	ND
27 (19.8)	609.145	609.1473	609–301	Quercetin rutinoside (Isomer II)	Flavonol	0.08 ± 0.001	0.11 ± 1.71E−02	0.11 ± 2.53E−03	0.94 ± 1.81E−01
28 (20.2)	577.1552	577.1570	457–353	Apigenin rutinoside (Isomer II)	Flavone	ND	0.08 ± 1.38E−02	0.13 ± 5.34E−03	0.10 ± 6.50E−02
29 (20.6)	769.1974	769.2012	545–425	Apigenin 8-C-feruloyl glc-6-C-glc (Isomer II)	Flavone	0.06 ± 2.64E−03	0.17 ± 4.11E−02	0.06 ± 4.15E−03	ND
30 (20.7)	729.2389	729.2400	545–425	Apigenin derivative (Isomer I)	Flavone	0.06 ± 1.0E−04	0.04 ± 1.47E−02	ND	ND
31 (21.1)	607.1657	607.1675	299	Kaempferol methyl ether hexoside rhamnoside	Flavonol	ND	ND	ND	0.14 ± 8.11E−02
32 (21.2)	739.1869	739.1919	545–425	Apigenin 6-C-feruloyl glc-8-C-arab	Flavone	0.05 ± 6.93E−03	0.16 ± 2.94E−02	0.16 ± 3.07E−03	0.82 ± 1.54E−01
33 (22.3)	623.1606	623.1615	315	Isorhamnetin 3-O-rutinoside	Flavonol	ND	ND	0.40 ± 1.45E−01	0.11 ± 6.91E−02
34 (22.9)	299.0596	299.0549	284	Chrysoeriol	Flavone	0.01 ± 1.0E−04	0.02 ± 2.34E−03	0.06 ± 4.34E−03	0.03 ± 9.66E−03
35 (23.4)	447.0922	447.0948	285	Kaempferol hexoside	Flavonol	ND	6.25 ± 8.03E−01	40.75 ± 1.15	ND
36 (23.8)	729.2389	729.2397	545	Apigenin derivative (Isomer II)	Flavone	0.03 ± 1.0E−04	ND	0.02 ± 2.33E−03	ND
37 (25.5)	271.0601	271.0610	227	Naringenin chalcone	Other	ND	ND	0.17 ± 8.97E−04	0.01 ± 3.42E−03
38 (26.1)	285.0393	285.0407	247	Kaempferol	Flavonol	0.03 ± 1.0E−04	ND	ND	ND
39 (26.5)	315.0499	315.0500	300	Isorhamnetin	Flavonol	0.06 ± 1.0E−04	0.02 ± 8.42E−03	ND	0.29 ± 1.0E−04
Total phenolic compounds						96.98[d]	405.75[c]	962.93[b]	797.04[a]

Note: Concentrations are expressed as mg/kg seed (DW); mean values ± standard deviation. Mean values of total phenolic compounds with different superscript letter are significantly different ($p > 0.05$).
Abbreviations: CN, compound number; HCA, hydroxycinnamic acid; ND, not detected; RT, retention time (min).

(17.0–62.4 and 22.6–73.6 mg/100 g, for vitexin and isovitexin, respectively), which is known as a major source of polyphenols (Hou et al., 2019). As a matter of interest, the protective effects of these compounds in several oxidative stress-related diseases are widely recognized, and have been reviewed in a recent published review (Babaei et al., 2020).

Another relatively abundant polyphenolic compounds in *Neltuma* seeds were the apigenin 6-C-glc-8-C-arab and 6-C-arab-8-C-glc isomers, which are usually known as schaftoside and isoschaftoside, respectively. They reached concentrations close to 4 mg/100 g in *P. nigra* seeds. All *Neltuma* spp. examined also contained minor amounts of further glycosylated derivatives (hexosides) of both compounds (peaks number 3 and 4). Schaftoside and isoschaftoside have been found in high amounts (41–61 and 337–518 mg/100 g, respectively) in the seed germ flour from some *Neltuma* species and in *Ceratonia siliqua* seeds (Picariello et al., 2017). Interestingly, all these compounds have been described as potent α-glucosidase inhibitors (Zhao et al., 2019) thus suggesting that seeds from all the named plant materials could be used as a source of bio-actives contributing to modulate carbohydrate digestion in humans.

Compounds number 6 and 7 were identified as apigenin 6,8-C-di-glucosides. The occurrence of two compounds with identical $[M-H]^-$ signal and major fragment ions suggests the possibility of stereoisomers already described and sometime named as vicenin (Picariello et al., 2017). Both compounds were relatively abundant in *N. flexuosa* but scarce in the other species. Nevertheless, the amounts found in *N. flexuosa* seeds (about 1.2 mg/100 g of both substances) were lesser to those reported in the seed germ flour (18–51 mg/100 g) from other *Neltuma* species (Picariello et al., 2017).

Kaempferol aglycone was almost absent in the materials studied but some derivatives—particularly kaempferol hexoside—were abundant in *N. nigra* seeds. The presence of these compounds is worthy of mention due to their wide range of bioactive and pharmacological properties including antioxidant, anti-cancer, anti-inflammatory, and cardioprotective effects (Imran et al., 2019). Other common flavonoids, such as luteolin, quercetin, chrysin, chrysoeriol and isorhamnetin, were generally present—mostly as glycosylated forms—in the examined *Neltuma* seeds, but always in small quantities.

CONCLUSIONS

Seeds from the studied *Neltuma* spp. presented valuable lipid contents showing a well-balanced fatty acid composition (low percentage of saturated fatty acids, moderate or high MUFA and PUFA concentrations) and good amount of tocopherols. Proteins accounted for about 15%–35% of dry seeds, and included 26%–40% of essential amino acids. Based on their AA scores, they were deficient in leucine, isoleucine and valine. Proteins from *N. nigra* and *N. flexuosa* presented meaningful quantities of the EAA histidine, threonine and lysine. In general, the qualitative patterns of phenolic compounds were similar each other (predominance of apigenin derivatives), although they differed markedly in the amounts of the individual components. Overall, seeds from the examined *Neltuma* species represent an undervalued source of healthy macronutrients and functional components. Moreover, as a matter of interest, these species can produce fertile hybrids (Joseau et al., 2013), so they may be a source of great genetic diversity. This in turn opens up the possibility of searching for interspecific hybrids with improved composition of target seed metabolites.

AUTHOR CONTRIBUTIONS

M.C.C. conceived and designed the study. R.B., M.C.C., D.B. and D.L. carried out the experiments and analytical methods. M.M. worked out almost all of the technical details and performed the numerical calculations. D.M. participated in the planning, supervision and financing of the study. D.M. and R.B. wrote the first draft of the manuscript. All authors discussed the results and contributed to the final manuscript.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Romina Di Paola Naranjo (ICYTAC-CONICET) for her assistance with the HPLC-ESI-MS/MS analysis.

FUNDING INFORMATION

This research was financed with funds from SECyT (Secretaría de Ciencia y Tecnología de la Universidad Nacional de Córdoba—Argentina).

CONFLICT OF INTEREST STATEMENT

The authors have declared no conflict of interests.

ETHICS STATEMENT

This article does not contain any studies with human and animal subjects.

ORCID

Romina Bodoira https://orcid.org/0000-0002-3808-7899

Damián Maestri https://orcid.org/0000-0002-2102-308X

REFERENCES

AOCS. Official methods and recommended practices of the American Oil Chemists' Society. 5th ed. Champaign, USA: AOCS Press; 2009.

Arinathan V, Mohan VR, John De Britto A. Chemical composition of certain tribal pulses in South India. Int J Food Sci Nutr. 2003;54:209–17. https://doi.org/10.1080/09637480120092026

Babaei F, Moafizad A, Darvishvand Z, Mirzababaei M, Hosseinzadeh H, Nassiri-Asl M. Review of the effects of vitexin in oxidative stress-related diseases. Food Sci Nutr. 2020;8: 2569–80. https://doi.org/10.1002/fsn3.1567

Bennetau-Pelissero C. Bioactive molecules in food. In: Mérillon JM, Ramawat K, editors. Reference series in phytochemistry. Switzerland: Springer; 2019. p. 223–65.

Bhat R, Karim A. Exploring the nutritional potential of wild and underutilized legumes. Compr Rev Food Sci Food Saf. 2009;8: 305–31. https://doi.org/10.1111/j.1541-4337.2009.00084.x

Bodoira R, Rossi Y, Velez A, Montenegro M, Martínez M, Ribotta P, et al. Impact of storage conditions on the composition and antioxidant activity of peanut skin phenolic-based extract. Int J Food Sci Technol. 2022;57:6471–9. https://doi.org/10.1111/ijfs.15964

Cattaneo F, Sayago JE, Alberto MR, Zampini IC, Ordoñez RM, Chamorro V, et al. Anti-inflammatory and antioxidant activities, functional properties and mutagenicity studies of protein and protein hydrolysate obtained from *Prosopis alba* seed flour. Food Chem. 2014;161:391–9. https://doi.org/10.1016/j.foodchem.2014.04.003

Cittadini MC, García-Estévez I, Escribano-Bailón MT, Bodoira R, Barrionuevo D, Maestri D. Nutritional and nutraceutical compounds of fruits from native trees (*Ziziphus mistol* and *Geoffroea decorticans*) of the dry Chaco forest. J Food Compos Anal. 2021;97:103775. https://doi.org/10.1016/j.jfca.2020.103775

FAO/UNU/WHO. Technical report series 935 protein and amino acid requirements in human nutrition. Report of a joint WHO/FAO/UNU expert consultation. Geneva, Switzerland: WHO; 2002.

Felker P, Takeoka G, Dao L. Pod mesocarp flour of north and south American species of leguminous tree *Prosopis* (mesquite): composition and food applications. Food Rev Int. 2013;29: 49–66. https://doi.org/10.1080/87559129.2012.692139

Grela E, Kiczorowska B, Samolińska W, Matras J, Kiczorowski P, Rybiński W, et al. Chemical composition of leguminous seeds: part I- content of basic nutrients, amino acids, phytochemical compounds, and antioxidant activity. Eur Food Res Technol. 2017;243:1385–95. https://doi.org/10.1007/s00217-017-2849-7

Grygier A, Chakradhari S, Ratusz K, Rudzińska M, Patel SK, Lazdiņa D, et al. Lipophilic profile of mature seeds of unconventional edible tree legumes. Eur Food Res Technol. 2023;249: 1543–50. https://doi.org/10.1007/s00217-023-04234-9

Hou D, Yousaf L, Xue Y, Hu J, Wu J, Hu X, et al. Mung bean (*Vigna radiata* L.): bioactive polyphenols, polysaccharides, peptides, and health benefits. Nutrients. 2019;11(6):1238. https://doi.org/10.3390/nu11061238

Imran M, Rauf A, Shah ZA, Saeed F, Imran A, Arshad MU, et al. Chemo-preventive and therapeutic effect of the dietary flavonoid kaempferol: a comprehensive review. Phytother Res. 2019;33: 263–75. https://doi.org/10.1002/ptr.6227

Joseau M, Verga A, Díaz M, Julio N. Morphological diversity of populations of the genus *Prosopis* in the semiarid Chaco of northern Cordoba and southern Santiago del Estero. Am J Plant Sci. 2013;4:2092–111. https://doi.org/10.4236/ajps.2013.411261

Lajnef HB, Mejri H, Feriani A, Khemiri S, Saadaoui E, Nasri N, et al. *Prosopis farcta* seeds: potential source of protein and unsaturated fatty acids? J Am Oil Chem Soc. 2015;92:1–8. https://doi.org/10.1007/s11746-015-2660-1

Lamarque AL, Maestri DM, Grosso NR, Zygadlo JA, Guzmán CA. Proximate composition and seed lipid components of some *Prosopis* (Leguminosae) from Argentina. J Sci Food Agric. 1994;66:323–6. https://doi.org/10.1002/jsfa.2740660309

Maestri D. Groundnut and tree nuts: a comprehensive review on their lipid components, phytochemicals, and nutraceutical properties. Crit Rev Food Sci Nutr. 2023;1:1–25. https://doi.org/10.1080/10408398.2023.2185202

Margier M, Georgé S, Hafnaoui N, Remond D, Nowicki M, Du Chaffaut L, et al. Nutritional composition and bioactive content of legumes: characterization of pulses frequently consumed in France and effect of the cooking method. Nutrients. 2018; 10(11):1668. https://doi.org/10.3390/nu10111668

Mazzuca M, Balzaretti VT. Fatty acids, sterols and other steroids from seeds of Patagonian *Prosopis* species. J Sci Food Agric. 2003; 83:1072–5. https://doi.org/10.1002/jsfa.1510

Moro J, Tomé D, Schmidely P, Chalvon-Demersay T, Azzout-Marniche D. Histidine: a systematic review on metabolism and physiological effects in human and different animal species. Nutrients. 2020;12(5):1414. https://doi.org/10.3390/nu12051414

Morris SM. Arginine: beyond protein. Am J Clin Nutr. 2006;83(2): 508S–512S.

Padhi EM, Liu R, Hernández M, Tsao R, Dan RD. Total polyphenol content, carotenoid, tocopherol and fatty acid composition of commonly consumed Canadian pulses and their contribution to antioxidant activity. J Funct Foods. 2017;38:602–11. https://doi.org/10.1016/j.jff.2016.11.006

Pastor-Cavada E, Juan R, Díaz J, Alaiz M, Vioque J. Nutritional characteristics of seed proteins in 15 *Lathyrus* species (Fabaceae) from southern Spain. Food Sci Techno. 2011a;44:1059–64. https://doi.org/10.1016/j.lwt.2010.09.021

Pastor-Cavada E, Juan R, Díaz J, Alaiz M, Vioque J. Nutritional characteristics of seed proteins in 28 *Vicia* species (Fabaceae) from southern Spain. J Food Sci. 2011b;76:1118–24. https://doi.org/10.1111/j.1750-3841.2011.02336.x

Picariello G, Sciammaro L, Siano F, Volpe MG, Puppo M, Mamone G. Comparative analysis of C-glycosidic flavonoids from *Prosopis* spp. and *Ceratonia siliqua* seed germ flour. Food Res Int. 2017;99:730–8. https://doi.org/10.1016/j.foodres.2017.06.058

Sciammaro L, Ferrero C, Puppo MC. Chemical and nutritional properties of different fractions of *Prosopis alba* pods and seeds. J Food Meas Charact. 2016;10:103–12. https://doi.org/10.1007/s11694-015-9282-z

Shewry PR, Hey SJ. The contribution of wheat to human diet and health. Food Energy Secur. 2015;4:178–202. https://doi.org/10.1002/fes3.64

Singh B, Singh JP, Kaur A, Singh N. Phenolic composition and antioxidant potential of grain legume seeds: a review. Food Res Int. 2017;101:1–16. https://doi.org/10.1016/j.foodres.2017.09.026

Tang Q, Tan P, Ma N, Ma X. Physiological functions of threonine in animals: beyond nutrition metabolism. Nutrients. 2021;13(8): 2592. https://doi.org/10.3390/nu13082592

Zhao H, Liu Y, Chen J. Screening of α-glucosidase inhibitors from natural flavonoids by an in-capillary assay combining PMMA and EMMA. Anal Methods. 2019;11:1371–8. https://doi.org/10.1016/j.canlet.2021.12.018

How to cite this article: Cittadini MC, Bodoira R, Barrionuevo D, Labuckas D, Martínez M, Maestri D. Nutrient and bioactive compounds from *Neltuma* spp. seeds. J Am Oil Chem Soc. 2024. https://doi.org/10.1002/aocs.12820