Potential of *Leucaena leucocephala* and *Leucaena esculenta* Seeds in Human Nutrition: Composition, Techno-functional Properties, Toxicology and Pretreatment Technologies

Laura Victoria Aquino-González¹, Beatriz Noyola-Altamirano¹, Lilia Leticia Méndez-Lagunas¹, Juan Rodríguez-Ramírez¹ , Sadoth Sandoval-Torres¹ , Luis Gerardo Barriada Bernal²**10.18805/LRF-743**

ABSTRACT

The consumption of legume seeds has benefits for human nutrition and health. Seeds of *Leucaena leucocephala* and *Leucaena esculenta* are fast-growing legumes that are used for a variety of purposes. In general, they are used to preserve/restore the soil; the foliage can be used as fodder for livestock and the seeds for human consumption in rural populations. Leucaena seeds are a source of nutrition, since they contain proteins, carbohydrates and lipids (including omega 3, 6 and 9 unsaturated acids). The protein fraction has shown pharmaceutical effects of interest (analgesic, lipolytic, emollient, anticancer, anti-inflammatory, anti-coagulant and antidiabetic) and some techno-functional properties for the food industry. However, the main limitation to the human consumption of leucaena seeds is the presence of mimosine, an antinutrient that produces side effects such as alopecia, growth retardation, cataracts and infertility in animals. In this sense, some technological processes that could contribute to its decrease. This work was carried out at the Nutraceuticals Department of the Interdisciplinary Research Center for Integral Regional Development, Oaxaca Unit, Mexico. A comprehensive and integrative review of research work carried out in different parts of the world was conducted. The bibliographic search was carried out from January 2020 to January 2022. Several databases such as Scopus, Latindex, Dialnet, Redalyc.org, FAO and Academic Google were explored. A total of 81 documents were used to prepare this review document. This review integrates aspects related to the potential use of leucaena seeds in human nutrition: nutritional composition, pharmacological, techno-functional properties, the processes to improve digestibility and reduce toxicity.

Key words: Anti-nutritive compounds, Leucaena seeds, Macronutrients, Pharmacological activity.

The family *Leguminosa*e include thousands of species distributed in about 750 genera (Hutchinson, 1964). These species are creeping, shrubby or arboreal growth species and their life cycle can be annual or perennial (Sprent and Parsons, 2000; Bianco and Cenzano, 2018). Around 150 legumes have been listed by the *Codex alimentarius* as safe for human consumption. Soybeans (*Glycine max*), common beans (*Phaseolus vulgaris*), lentils (*Lens culinaris*), beans (*Vicia faba*), peas (*Pisum sativum*) and chickpeas (*Cicer arientinum*) are the most important legumes due to their nutritional characteristics and their low ecological impact, except for soybeans (World Health Organization, 2007; Delgado-Andrade *et al*., 2016).

The legumes for human consumption contain a high concentration of carbohydrates and proteins as the main nutritional compounds of interest for the human nutrition (Haytowitz *et al*., 2011). In addition, they contain several compounds of biological activity classified as undesirable or anti-nutritional such as phytates, galactosesoligosaccharides, protease inhibitors, spooning and *etcetera* (Messina, 1999). The biological effect of these anti-nutritional molecules directly affects the digestibility of proteins and carbohydrates.

The leucaenas (also called *guajes* in Spanish) are arboreal legumes distributed throughout many ecosystems, from semi-arid areas, tropical deciduous forest, medium sub-

1 Instituto Politécnico Nacional-Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional; Unidad Oaxaca. Hornos 1003, Col. Noche Buena, C.P. 71230. Santa Cruz Xoxocotlán, Oaxaca, México.

²Consejo Nacional de Humanidades, Ciencia y Tecnología. Hornos 1003, Col. Noche Buena, C.P. 71230. Santa Cruz Xoxocotlán, Oaxaca, México.

Corresponding Author: Luis Gerardo Barriada Bernal, Consejo Nacional de Humanidades, Ciencia y Tecnología. Hornos 1003, Col. Noche Buena, C.P. 71230. Santa Cruz Xoxocotlán, Oaxaca, México. Email: lbarriada@ipn.mx

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evergreen forest and mangroves. They are widely distributed in Mexico, especially in the southeastern center of the country (Zárate, 1994). In Mexico, the seeds of various species are consumed, especially *Leucaena esculenta* and *Leucaena leucocephala*. Protein concentration of these species varies from 26 to 31%, while the carbohydrate concentration range is 57 to 61% (Román-Cortés *et al*., 2014). Additionally, the seeds contain some non-polar vitamins, as well as compounds of phenolic origin that can exert a biological function such as nutraceuticals or probiotics (Román-Cortés *et al*., 2014).

The tannins, trypsin inhibitors, phytic acid, tannins, oligosaccharides and mimosine have been reported as unwanted/anti-nutritive compounds in leucaena seeds. The biological effect of those anti-nutritive molecules directly affects the digestibility of protein and carbohydrates (Román-Cortés *et al*., 2014; Tran *et al*., 2016). The non-protein amino acid called mimosine, acid (S)-α-Amino-β-[1-(3-hydroxy-4 oxopyridin), inhibits some intracellular processes (Tran *et al*., 2016; Chaichun *et al*., 2019) and can be used as an immunosuppressive, antiarrhythmic and hypocholesterolemic agent (Syamsudin *et al*., 2010). The use of leucaena seeds as an ingredient in livestock feed has been widely evaluated (Barác *et al*., 2015), but their use in human food has not been formally studied. However, they have been consumed ancestrally in the south of Mexico (Almeida-Costa *et al*., 2006).

This review evaluates, from de human nutrition point of view, the published data on the nutritional composition, pharmacological and techno-functional properties and toxicity of *Leucaena leucocephala* and *Leucaena esculenta* seeds and compares them with other important edible legumes.

Nutritional composition

Protein content

Legume flours, concentrates and protein isolates are used in several food matrices (Barác *et al*., 2015). The average crude protein content in leucaena seeds ranges from 31-33% (Table 1) and these values are higher than those reported for legumes of widespread consumption such as *Glycine max* (soy) or *Phaseolus vulgaris* (bean), where the protein content ranges between 18-25% (Almeida-Costa *et al*., 2006). On the other hand, protein values of Leucaena seeds are lower than that reported for other legumes of lower commercial use (*e.g.* several species of *Lupinus* spp and *Inga paterno*) where the protein content ranges from 32-56% (Carvajal-Larenas *et al*., 2016; Sánchez-Mendoza *et al*., 2017).

In legumes, the protein fraction is constituted by different sub fractions: globulins, the highest concentration protein sub fraction and albumins, glutelins and prolamins, the lowest concentration protein sub fractions (Desphande,

1990). Within these sub fractions, the biologically active polypeptides are the lectins and the enzyme inhibitors (Hall *et al*., 2017). In cereals, the glutelins and prolamins are the main protein sub fractions. In leucaena seeds, the globulins constitute the main protein sub fraction, \approx 43.5% of proteins (Chandrasekhara-Rao *et al*., 1984; Sethi and Kulkarni, 1995). The concentration of albumins is \approx 28.4%, glutelins \approx 25% of the protein fraction; and the prolamins <4% (Sethi and Kulkarni, 1995). Similar distributions have been reported for other legumes of widespread consumption such as *Glycine max* and/or *Phaseolus vulgaris*, where the fraction of the globulins represent the 54-79% and 85-95% of the protein fraction, while in *Lupinus album* the concentrations range was reported of 80-90% of the protein fraction (Almeida-Costa *et al*., 2006; Carvajal-Larenas *et al*., 2016).

In the legume seeds, their nutrition/biological potential must be evaluated beyond the quantification of the protein concentration and the protein amino acid residues constitution. Although the correlation between a higher concentration of protein and a better attributes from the nutritional point of view is not correct, these polypeptides may be enzymes with unwanted biological functions, such as trypsin inhibitors and lectins (Sánchez-Mendoza *et al*., 2017).

Amino acids

Deficiency of methionine and cysteine residues is *characteristic* in all legume protein fractions; therefore, when legumes are used in human nutrition it must be enriched with protein fractions from cereals (Desphande, 1990). Leucaena seeds are particularly rich in glutamic acid, arginine and leucine (Table 2).

In *Glycine max* seeds, the amino acids with the highest concentrations are glutamic acid, aspartic acid and proline (Colina *et al*., 2017; Duke, 2022) while in the seeds of *Phaseolus vulgaris*, the amino acids of higher concentration correspond to glutamic acid, leucine and serine (Menchú and Méndez, 2012). In other legumes such as lupine (*Lupinus* spp); glutamic acid, arginine and aspartic acid are the amino acids with the highest concentration (Carvajal-Larenas *et al*., 2016).

The quality evaluation of leucaena protein by the DIASS criterion, referred to the quality of the protein of the whole milk powder (UN-FAO, 2013) is 8.73% for infants, 17.45% for child and 22.48% for adolescents and adults. Based in

Table 1: Comparative nutrient composition of some legume seeds.

Fatty acids (%) Ash $(%)$	Lipid $(%)$	Fiber $(%)$	Carbohydrate (%)	Protein content (%)				
2.98 8.05	5.36	15.26	61.31	31.70	Leucaena leucocephala			
8.44 5.34	3.65	10.55	57.89	33.12	Leucaena esculenta			
7.44 5.44	20.00	6.69	18.00	36.82	Glycine max			
1.98 4.69	2.56	12.08	57.10	6.25	Phaseolus vulgaris			
1.10 3.40	10.3	11.6	33.85	45.70	Lupinus spp ^a			

^aLupinus spp refers to the average value reported for the species of *Lupinus mutabilis*, *Lupinus albus*, *Lupinus angustifolia*, *Lupinus termis* and *Lupinus mutabilis*.

the DIAAS quantity criteria, the protein fraction must be catalogued as a high protein source, but based in the DIAAS quantity and quality criteria, the protein faction must be catalogued as a low-quality protein source for the human nutrition; then, its use must be part of a food matrix constituted by a mix with a cereal/animal proteins (UN-FAO, 2013).

Carbohydrates

In legumes, the carbohydrates are considered the molecules of highest concentration. They make up 50% of the dry weight of the seed (Ter Meulen *et al*., 1979; Middelbos and Fahey, 2008; Ahmed and Abdelati, 2009). In leucaena seeds, the carbohydrate concentration ranges from 35-60%, (Table 1) (Sethi and Kulkarni, 1995; Román-Cortés *et al*., 2014) and the metabolic conversion rate in animal models range from 10.7 to 12.4 kilojoule per gram on a dry basis, kj $g_{\scriptscriptstyle{\text{db}}}^{}$. These values are lower than those reported by *Glycine max,* 20.95 kj g_{db}1 (Middelbos and Fahey, 2008; Hall *et al.*, 2017) and *Lupinus* spp. 20 kj g_{db}1 (Carvajal-Larenas *et al*., 2016).

There is no information regarding the complete profile and concentration of oligosaccharides present in leucaena seeds. Sethi and Kulkarni (1995) and Román-Cortes *et al.* (2014) suggest that the carbohydrate fraction is composed mainly of non-structural polysaccharides.

Plouvier (1962) and Beveridge *et al.* (1977) report the presence of oligosaccharides such as raffinose, glucose, sucrose, stachyose, D-pinal and Myo-inositol.

Fatty acids

Within the fatty acid profile of leucaena seeds, as shown in Table 3, the fraction with the highest concentration corresponds to polyunsaturated fatty acids, 50-86% (Ter Meulen *et al*., 1979; Babar *et al*., 1989; Sethi and Kulkarni, 1995; Ahmed and Abdelati, 2009; Imededdine *et al*., 2014; Lafont *et al*., 2014; Carvajal-Larenas *et al*., 2016; Badal, 2017; Alam *et al*., 2017). The saturated acids represent between 20 -25% of the fraction of total fatty acids, with palmitic acid as the predominating fatty acid (Sathe *et al*., 1984).

According to Sethi and Kulkarni (1995), other components of the un-saponifiable fraction of *Leucaena leucocephala* seed are phytosterols (35% of the un-saponifiable fraction), tocopherols (17% of the un-saponifiable fraction) and carotenoids (20% of the un-saponifiable fraction). Within the no saponifiable seed fraction of *Leucaena leucocephala*, Chen and Wang (2010) reported several steroidal molecules: 5α, 8α-epidioxy-(24ξ)-ergosta-6, 2, 2-dien-3β-ol; as well as several phytosterols β-sitosterol, β-sitostenon and stigmasterol.

^a*Lupinus* spp refers to the average value reported for the species of *Lupinus mutabilis*, *Lupinus albus*, *Lupinus angustifolia, Lupinus termis* and *Lupinus mutabilis*.

b Recommended intake for older child (>6 years), adolescents and adults.

ND No reported values.

NC Non considered values for the recommended intake by UN-FAO.

Vitamins

Little information is available about the vitamin content in leucaena seeds, where the tocopherols (especially the α tocopherol) and carotenoids are the vitamin family of the higher concentration (Table 4). The lipophilic molecules from grains and legumes, such as carotenoids and tocopherols, have been cited as anti-cardiovascular and eye pathologies molecules (Boschin and Arnoldi, 2011; Monge-Rojas, 2013). The recommended intake of tocopherol (vitamin E as αtocopherol) is of 5-6 mg $d⁻¹$ for kids and 9-12 mg $d⁻¹$ for adult females/males (Institute of Medicine, 2007). In humans, the tocopherols (especially the α -tocopherol, the most abundant and the γ -tocopherol, with the highest antioxidant activity) and their physiological role depends on their ability to quench free radicals in cell membranes and other lipid environment, preventing the autoxidation of polyunsaturated fatty acids (Bramley *et al*., 2000). The recommended intake of vitamin A (as retinol) is 0.76-0.87 mg d⁻¹ for kids and 0.66-0.76 mg d⁻¹ for adult females/males (Institute of Medicine, 2007). They

Table 3: Comparison of fatty acid profile from legume seeds.

are considered as an antioxidant molecules with therapeutic value in photosensitivity disorders and leukoplakia pathologies (Olson, 1999). The recommended intake of vitamin K is 6-7 mg d^{-1} for kids and 11-15 mg d^{-1} for adult females/males (Institute of Medicine, 2007). In humans, the vitamin K is a cofactor for the function of the enzyme γ -glutamyl carboxylase, necessary for the activation of multiple vitamin K dependent-proteins (VKDPs). The VKDPs proteins have been correlated with bone, vascular and reproduction health as well as in cancer progression (Fusaro *et al*., 2019).

Minerals

Regarding minerals, phosphorus and potassium have the highest concentration (Table 5). Information about the mineral profile and concentration of leucaena seeds is limited. As other legumes, an intra/inter specific variations have been reported due to agronomic aspects (sowing/ harvest variables), the extraction/pre-treatments and genetic flow, among other reasons.

^a*Lupinus* spp refers to the average value reported for the species of *Lupinus mutabilis*, *Lupinus albus*, *Lupinus angustifolia, Lupinus termis* and *Lupinus mutabilis*.

ND No reported values.

Table 4: Comparison of vitamins from legume seeds lipid fraction.

Vitamins (mg/100 g of lipid fraction)	Leucaena leucocephala	Glycine max	Phaseolus vulgaris	Lupinus spp ^a
α - tocopherol	175.47	16.70	ND	1.35
β - tocopherol	12.71	1.60	ND	1.4
γ - tocopherol	14.22	44.80	3.79	8.45
δ - tocopherol	2.23	18.25	0.11	1.84
K	PR	0.04	0.005	ND

^a*Lupinus* spp refers to the average value reported for the species of *Lupinus mutabilis*, *Lupinus albus*, *Lupinus angustifolia, Lupinus termis* and *Lupinus mutabilis*.

ND No reported values.

PR Qualitative presence.

Anti-nutritive compounds

*Leucaena leucocephala s*eeds contain mimosine as its main ant nutrient (Table 6). Mimosine [3-N-(3-hydroxy-4-pyridone) ao-aminopropionic acid], present in the Leucaena family, is considered one of the most important anti-nutritional factors, given side effects (alopecia, growth retardation, cataracts and infertility in animals) that occur when the permitted intake dose is exceeded (Sethi and Kulkarni, 1995).

In human diet, the intake of unprocessed legumes has been related to human pathologies and nutritional disorders. These pathologies are caused by molecules called antinutrients (De Wreede and Wayman, 1970; Sethi and Kulkarni, 1995). For this reason, it is essential to perform a thermal, chemical or physical pretreatment, in order to ensure that the side effects are not harmful to human health. In processed legumes, the remaining anti nutrients concentration after the pre-treatments (*e.g*. germination, cooking or soaking) is lower than in unprocessed legumes (Elizalde *et al*., 2009).

Pharmacological uses

Tran *et al.* (2016) reported the absence of significant adverse effects in the consumption of tea made from dried leaves of *Leucaena leucocephala*. Lim (2012) indicated that the ethno pharmacological use of roasted seeds of *Leucaena leucocephala* as an emollient in humans has no side effects.

Villaseñor *et al.* (1997) evaluated the effects of the compound of the non-polar extract *of Leucaena leucocephala* seeds, alkaloids, observing analgesic effects comparable to those of mefenamic acid - depressive effects on the central nervous system- and anti-mutagenic effects in murine models. The same extracts showed vermifuge effects against *Ascaris suum*.

Gamal-Eldeen *et al*. (2007) reported chemo-preventive and/or anticancer effect of some polysaccharide-protein complexes isolated from *Leucaena leucocephala* seeds that inhibited/modulated the action of cytochrome P450 1A (CYP1A) and the transduction of glutathione-S-transferases (GST). Souza-Pinto *et al.* (1995), reported that trypsin 1 (LLTI-1) and trypsin 2 (LLTI-2), inhibitors isolated from the seed protein fraction of *Leucaena leucocephala*, showed an anti-inflammatory effect in murine models. Oliva *et al.* (2000) found that other serine proteinase inhibitor isolated from *Leucaena leucocephala* seeds, modified the activity of plasmin, plasma trypsin and human chymotrypsin. Syamasudin *et al.* (2010) reported that galactose as well as other several glycose compounds extracted from *Leucaena leucocephala* seeds, showed a hypoglycemic effect in mouse models where a diabetic pathology was previously induced. *In vitro* studies showed some effects on mitotic frequency and incorporation of thiamine in healthy human cells, as well as in cell division and proliferation in human carcinoma without significant effects on the mechanisms of protein synthesis and DNA synthesis (Tsai and Ling, 1971; Krude, 1999; Kubota *et al*., 2014).

Kuppusamy *et al.* (2014) reported that aqueous extracts of leucaena seeds could be used as complementary therapy in the treatment of type 2 diabetes due to its hypoglycemic

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ND No reported values.

Table 6: Anti-nutrient molecules from leucaena seeds.

ND No reported values.

effect as well as in weight control treatments due to its adipogenesis and lipolytic properties. Pendyala *et al.* (2010) reported that *Leucaena leucocephala* gum (galactomanase gums) can serve as a drug-carrying suspensions and emulsions. In addition, these gums can act as antitumor, hemo binder and anticoagulant with similar actions to those reported from soybeans (Deodhar *et al*., 1998).

Techno-functional properties

The water holding capacity (WHC) and oil holding capacity (OHC) of the protein fraction in commercial legumes are in the range from 1.8-6.8 and 3.5-6.8 grams per gram of protein (g g-1), respectively. The legume protein fractions exhibit good foaming formation, emulsion capacity and solubility (Hassan *et al*., 2009; Pendyala *et al*., 2010). These properties were preserved in a wide range of process/variable values (*e.g.* extreme pH, 2.0 and 10.0) (Pendyala *et al*., 2010) .

Leucaena seed flour has a good oil absorption capacity, 2.2±0.2 g of oil/g of flour, an important quality in the formulation of fried foods, bakery and confectionery products. This attribute also helps to reduce the development of oxidative rancidity and consequently increases the shelf life of the products (Bravo-Delgado *et al*., 2019).

Sethi and Kulkarni (1994) evaluated the functional and organoleptic properties of *Leucaena leucocephala* seed protein isolate. They found an oil and water absorption capacity very similar to soybean protein isolate and that these values are higher than those presented by other edible legumes such as bean, pea, chickpea and fava bean. They also carried out successful organoleptic tests of cake and mayonnaise supplemented with protein isolate as a replacement for egg protein. Deodhar *et al.* (1998) and Pendyala *et al.* (2010) analyzed the techno-functional properties of gums from leucaena seeds and proposed other possible industrial applications (*e.g.* as pharmaceutical tablet formulation, as a carrier in modified release dosage forms and as a suspending agent and emulsifying agent owing to its pseudo plastic and thixotropic properties).

Processes used to reduce the toxicity of leucaena seed

Various methodologies are used to reduce the antinutritional molecules concentration in leucaena seeds, most of them being widely used in other legumes, *e.g. Phaseolus vulgaris*. These are based on two fundamental principles, the high solubility in polar solutions (water) and the thermolabile behavior of those molecules.

The human protease inhibitors from legume seeds exhibit thermo-sensitive properties (Hegarty *et al*., 1964), while other compounds such as those of phenolic origin (phenols, flavonoids, tannins and anthocyanins) and alkaloids exhibit good solubility in polar solvents.

Regarding mimosine, several authors have evaluated the use of extraction processes (by aqueous extractions, methane sulfonate and ethanol) and biological processes (germination) as a practical and effective process to reduce its concentration (Tangendjaja *et al*., 1984; Sethi and Kulkarni, 1995; Alabi and Alausa, 2006; Mohamed *et al*., 2014).

A complete degradation of mimosine was reported by Tangeudjaja *et al.* (1984) in an aqueous slurry at pH 8.0 and 45° C in 10 minutes. Heating the intact leaf at 70° C resulted in 90% reduction of mimosine in 15 minutes (Labadan, 1969; Ter Meulen *et al*., 1979; Padmavathy and Shobha 1987). Washing with water and soaking, the leaves and seeds had a significant effect in lowering their mimosine contents. Prolonged soaking (48 hours) in 30°C water was most effective in reducing virtually all the mimosine in the leaves (Wee and Wang, 1987). One of the most effective reagents for extracting 95% mimosine is 0.05 N sodium acetate (Tawada, 1988; Tawata, 1990). Studies were carried out by treating *L. leucocephala* seeds with several reagents. Urea and sodium hydrogen carbonate completely removed mimosine. The protein content of the mimosine-free seed mass was reduced to 80% of the original after treatment with urea and 88% after treatment with sodium hydrogen carbonate solution (Hossain *et al*., 1991). In the livestock industry, the reduction of mimosine toxicity of *Leucaena leucocephala* has been reached in leaf and seed feed by adding iron and zinc salts (Chang, 1987). The use of bacteria that produce lactic acid (Inafuku and Pongo, 1992), bacteria of the species *Rhizobium* sp., Soedarjo *et al.* (1994) as well as native microorganisms in the rumen of animals. Allison *et al.* (1992) and Domínguez-Bello and Stewart (2020) have been proven alternatives that can significantly decrease the effect of mimosine in ruminant animal feed. Its mechanism of action consists of the degradation of mimosine to 3-hydroxy-4(4H)-pyridone (Arjona-Alcocer *et al*., 2012; Contreras-Hernández *et al*., 2013). It has been determined that 3-hydroxy-4(4H)-pyridone is also toxic in ruminant animals, but with biological effects of lesser magnitude than those caused by mimosine (Ruiz-Ruiz, 2013).

Sethi and Kulkarni (1994) found that derived from the preparation process of leucaena protein isolates, the mimosine content decreased by 93%. The resulting isolates were used as egg replacers in the preparation of cake and mayonnaise.

Future trends

The UN-FAO estimates that for 2050 will be necessary to increase food production from animal and vegetable sources by nearly 26% from 2014 levels (Mulhollen, 2017). In the same way, the agency estimates a rapid increase in the demand of protein for human food of animal origin, mainly, associated with the expected increase in the human population. This means an increase in the consumption of cereals and legumes for livestock production, altering the balance between the consumption of legumes and cereals by humans and agricultural production systems.

Some economic analyses indicate a consistent increase in the demand for plant-based protein, estimating a global market of USD 75.8 billion in 2025 (Hexa Research, 2019). The industry is then concerned about how introduce a large variety of legume-based foods. Marr (2022) suggests that plant-based meat alternatives could represent 10 per cent of the global meat industry by 2029.

Nowadays, the market is dominated by soy protein derived food, but multiples factors limit their production (mainly the environmental impact of the production) and acceptance (associated health risks in human population exposed to the glyphosate and chemical additives of the acrylamide type). Other vegetable proteins such as *Pisum sativum*, *Vicia faba* or *Phaseolus vulgaris* exhibit a minor market penetration, although the presence of anti-nutrient compounds and their effect on the human nutrition/health does not differ substantially from those found in soybeans. Probably, the less penetration of these legumes is due to various socio-cultural factors (*e.g.* the decrease in its consumption, cultivated area and a stagnation in production of *P. vulgaris*).

CONCLUSION

The uses of non-traditional legumes seeds, as leucaena or prosopis, in human nutrition are limited, mainly due to their potential being unknown. Therefore, research and development should focus on the following aspects:

- Genetic/agronomic selection of varieties with desirable nutritional potential.
- Genetic improvement: breeding of wild spices/varieties with desirable nutritional potential.
- Establishment of the maximum daily intake of mimosine referenced to specific nutritional requirements associated with sex and age.
- The biological effects of prolonged mimosine intake below the determined maximum level.
- Research and development of industrial anti nutrient extraction process and compounds with no desirable biological activity.
- Techno-functional properties of proteins and carbohydrate fractions (starches) present in seeds to improve food matrices.
- Evaluation of polypeptides with desirable biological activity.

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Ethics and conflict of interest

The authors report no ethics conflicts, due no biological (human or animal) was employed.

The authors report that there are no academic, labor, or intellectual conflicts of interest with any person, private industry or goverment.

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