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CHARACTERIZATION OF THE RAMON TREE SEED (*Brosimum alicastrum* Swartz.) AS A POTENTIAL FOOD SOURCE

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ABSTRACT

The main objective of this work was to study the physical, chemical, and functional properties of the seed of the Ramon tree (*Brosimum alicastrum* Swartz) to evaluate its potential application in the elaboration of food products of high nutritional value. The results were compared with other seeds marketed internationally through an *a posteriori* comparative study of means. Ramon seed yielded 43.99 % of the dietary fiber, with energy value of 183.02 kcal per 100 g seed, cation exchange of 0.0015 meq $[H^+]$ g^{-1} seed, and uptake of organic molecules of 0.91 g oil g^{-1} seed. Trolox equivalent antioxidant capacity had a value of 0.88 mg Trolox g^{-1} of seed. The antioxidant activity of the seed (IC_{50}) was 1.602 mg mL^{-1}, while the tree leaf had an IC_{50} of 0.618 mg mL^{-1}. The content of total phenols and flavonoids was also determined, resulting in 154 mg of gallic acid per 100 g of seed and 72.14 mg of catechin per 100 g of seed, respectively. To evaluate its capacity to produce high-nutritional-value foods, the biscuit test was carried out with Ramon seed whole meal flour, obtaining an expansion factor of 62.8. All the aforementioned tests were performed in triplicate, and the Student's *t*-test was used for the comparison of means between treatments. The hypothesis of the present work was demonstrated since the seed of the Ramon tree represents an alternative as a functional food, since physical, chemical, and functional properties comparable to other seeds of recognized nutritional value were observed.

Keywords: Moraceae, functional foods, endemic seeds of Mesoamerica.

INTRODUCTION

Recent estimates indicate that about 800 million people suffer from hunger in the world, equivalent to 10 % of the global population, while 2370 million face moderate or severe food insecurity, representing 30 % of the world's population. Most of these people are in Asia and Africa (FAO *et al*., 2021). Food security efforts in Mexico are centered on maize (Donovan *et al*., 2022). However, there is a growing need to find alternatives to plant-based crops that demand large water resources and do not favor agrobiodiversity. Recently, chia (*Salvia hispanica* L.), quinoa (*Chenopodium quinoa*

Willd.), and amaranth (*Amaranthus hypochondriacus* L.) seeds have been identified as highly nutritious food sources with great potential for food security (Olmos *et al*., 2022).

Seeds have acquired great relevance in healthy eating as they can serve as an allergen-free substitute and can be used as a condiment or additive in processed foods. Currently, food allergies are the main cause of anaphylactic reactions in Western countries, affecting 1–3 % of the general population and up to 8 % of children. In Europe and the United States, food allergies cause more than 30 000 anaphylactic reactions every year, with 10–18 % of these occurring at school (Cuadrado *et al*., 2021). According to the Food and Agriculture Organization of the United Nations (FAO), the most severe allergenic foods are soy, milk, eggs, peanuts, crustaceans, tree nuts, cereals, and fish, known as the "big eight" (Krisnawati *et al*., 2022).

It has been shown that incorporating seeds provides multiple health benefits in a balanced diet, especially due to their high contents of macronutrients, micronutrients, and polyphenols with antioxidant activity (Munekata *et al*., 2020). The growing international market for seed consumption offers many production opportunities for Mexico, with the potential to have a global impact. In recent years, global food trends indicate a growing consumer interest in food products that offer benefits for human physiological functions, such as overall growth and development, cardiovascular system, antioxidant capacity, and xenobiotic metabolism (Alvídrez *et al*., 2002).

The food industry has an important challenge in developing high nutritional value products for disease prevention. For example, a low intake of dietary fiber has been associated with intestinal diseases, prompting the search for new sources of fiber in foods and justifying the study of seeds to evaluate these qualities. In addition, antioxidants have gained importance in preventive health due to their capability to reduce chronic diseases such as Alzheimer's and Parkinson's (Zeb, 2020). On the other hand, seed protein is of great importance in the food industry, being an important source of essential amino acids and containing functional properties used for obtaining foods with highly valued sensory characteristics (Ananey-Obiria *et al*., 2018). Reserve proteins, which account for half or more of the total protein content in seeds (Fukushima *et al*., 1991; Shewry *et al*., 1995; Kumar *et al*., 2019), are especially relevant in marketing because of their abundance and economic and dietary importance.

The seed of the Ramon tree (*Brosimum alicastrum* Swartz.) of the Moraceae family is an endemic Mesoamerican species known by more than 50 common names (i.e., capomo, apomo, ash, juandiego, nazareno, ojite, ojoche blanco, ojochillo, ox, Ramon blanco, Ramon colorado, ojoche, among others). This tree is considered an emblematic plant of the Maya, as it was used as the main food source along with maize (Puleston, 1982; Peters and Pardo-Tejeda, 2018). Its fruit is a berry with a fleshy, edible pericarp that measures 1.5 to 2.5 cm in diameter, which turns yellowish green to orange in color when ripe, is covered with tiny scales, and contains a single seed.

Currently, the seed of the Ramon tree is still consumed in Mexico and parts of Mesoamerica. However, its consumption is scarce due to the lack of knowledge of

its nutritional value (CONABIO, 2020). Therefore, it is important to diversify the commercialization of seeds with high nutritional value in order to depend less on imports and even to open up export opportunities. To this end, it is important to recognize the seeds produced in rural areas of Mexico and support the economic growth of the communities.

The hypothesis of the present work was that Ramon tree seeds represent a functional food alternative to other seeds currently commercialized in international markets, such as amaranth, chia, and quinoa. The main objective of the present research was to characterize the main physical, chemical and functional properties of the Ramon tree seed as a potential source of nutritional food, including its proximal chemical analysis, water retention capacity, cation exchange, absorption of organic molecules, antioxidant capacity in the seed, phenol, and reserve protein content from the flour of this seed. Finally, to evaluate the food production capacity, a biscuit test was carried out with Ramon seed whole meal flour.

MATERIALS AND METHODS

Ramon tree seed, flour, and leaf were used, acquired in Yucatán, and supplied by Agroalef S.A. de C.V. Chia (*Salvia hispanica* L.), quinoa (*Chenopodium quinoa* Willd.), and amaranth (*Amaranthus hypochondriacus* L.) seeds, vegetable shortening, sugar, salt, and olive oil were obtained from supermarkets in Mexico City.

Proximal chemical analysis (PCA)

Moisture

It was determined by the AOAC Official Method 925.10 Solids (Total) and moisture in flour (AOAC International, 2005). The sample was dried in an oven at a temperature of 130 °C for approximately 2 h. The moisture content was calculated as the difference between the initial and final weights.

Ash

It was determined by the AOAC Official Method 923.03 Ash of flour (AOAC International, 2005). The sample was placed in a muffle at 600 °C for 2 h. Subsequently, it was incinerated with a burner until it stopped giving off smoke and was again introduced into the muffle at 500 °C for 2 h until white or grayish ashes were obtained, which were allowed to cool and finally weighed.

Fat

It was determined by the AOAC Official Method 963.15 Fat in cocoa products. Soxhlet extraction Method (AOAC International, 2005). Anhydrous petroleum ether was used as solvent (Sigma Aldrich®), which was heated, volatilized, and condensed, dripping on the sample. It was then siphoned into the heating flask to start the process again. The fat content was quantified by weight difference.

Protein

It was determined by AOAC Official Method 920.152 Protein in fruit products (Kjeldahl Method) NX6.25 = % protein (AOAC International, 2005). For digestion, the sample was placed in concentrated sulfuric acid (JT Baker®). The mixture was placed in a boiling chamber and concentrated sodium hydroxide was added to release the ammonia. The distillate was titrated with hydrochloric acid (JT Baker®).

Dietary fiber

It was determined by AOAC Official Method 992.16 Total dietary fiber. Enzymatic-gravimetric Method (AOAC International, 2005). Sulfuric acid (Sigma Aldrich®) was added to the defatted sample and allowed to boil for approximately 30 min. Subsequently, sodium hydroxide (Sigma Aldrich®) was added and again boiled for another 30 min. It was filtered and washed with distilled water. The residue was transferred to a porcelain capsule, dried, and calcined in a muffle at 550 °C, allowed to cool and then weighed.

Digestible carbohydrates

Carbohydrates were quantified by percentage difference of moisture, protein, lipids, ash, and fiber, according to the following equation:

$$100 = (\%\ moisture + \%\ ash + \%\ lipids + \%\ protein + \%\ dietary\ fiber)$$

Water-holding capacity (WHC)

It was determined using the method by McConell *et al*. (1974). Two grams of dried and defatted Ramon, chia, quinoa, and amaranth seeds were weighed. Each sample was placed in 50 mL Falcon-type tubes. Subsequently, excess water was added, and they remained at rest for 24 h. Then the samples were centrifuged (PrO-Research Centrifuge by Centurion Scientific Lid, Chichester, UK) at 3000 rpm for 15 min, excess water was separated and weighed. WHC was determined with the following equation:

$$WHC = \frac{Wet\ sample\ weight - Dry\ sample\ weight}{Dry\ sample\ weight}$$

Swelling capacity (CH)

Two grams of dried Ramon, chia, quinoa, and amaranth seeds were weighed and placed in 50 mL test tubes. The occupied volume was measured and distilled water was added in excess, manually shaken, and left to stand for 24 h. Subsequently, the final volume of the samples was measured (Robertson, 2000). The CH content was determined using the following equation:

$$SC = \frac{V_1 - V_0}{Sample\ weight}$$

where V_0 is the initial volume and V_1 the final volume.

Cation exchange capacity (CEC)

It was determined according to the method proposed by McConell *et al*. (1974). One gram of Ramon, chia, quinoa, and amaranth seeds were immersed in excess 2N HCl (Sigma Aldrich®) for 24 h. The excess HCl was washed away with saturated NaCl (Sigma Aldrich®). Finally, the samples were suspended in 50 mL of distilled water and titrated with 0.47 N NaOH (Sigma Aldrich®). The CEC was determined as follows:

$$NaOH\left(\frac{0.47\ mol}{1L}\right)\left(\frac{1\ mol\ HCl}{1\ mol\ NaOH}\right)\left(\frac{1}{g\ sample}\right) = H^{+}\ g^{-}1$$

Adsorption Capacity of Organic Compounds (ACOC)

It was determined according to the method proposed by McConell *et al*. (1974). Two grams of Ramon, chia, quinoa, and amaranth seeds were weighed in Falcon tubes. Olive oil was added in excess and left to stand for 24 h. Then they were centrifuged (PrO-Research Centrifuge by Centurion Scientific Lid, Chichester, UK) at 3000 rpm for 15 min. Excess oil was separated and weighed. The ACOC was determined according to the following equation:

$$ACOC = \frac{Wet\ sample\ weight - Dry\ sample\ weight}{Sample\ weight}$$

Antioxidant capacity by inhibition of free radicals

For colorimetric stability of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), the extraction of 1 g of Ramon seed meal was carried out with 10 mL of methanol (JT Baker®) for 24 h (stock solution 100 g L^{-1}). For the determination of leaf antioxidant capacity, 1 g of leaf was weighed and extracted with 10 mL of methanol (JT Baker®) for 24 h (stock solution 100 g L^{-1}).

Subsequently, 2.9 mL of DPPH and 0.1 mL of the stock solution (DPPH 0.005 g L^{-1} in methanol/water (1:1)) was added to seed and leaf samples, which were placed in different concentrations in test tubes, shaken and left to stand for 30 min; this procedure was carried out in triplicate. Then absorbance was measured at 517 nm in the spectrophotometer (Thermo Scientific Vis/UV-Vis GENESYS™ 40, Waltham, USA). To obtain the inhibition percentage, the following equation was used:

$$inhibition\ \% = \frac{h_0 - h_c}{h_0}\ 100$$

where h_o is the absorbance of the highest peak of the blank and h_c the absorbance of the highest peak of the sample. To compare the data in relation to the inhibitory capacity of Trolox, a standard curve was prepared at different concentrations (20, 40, 60, 80, and 100 ppm).

Total phenols content

It was determined by using the Folin Ciocalteu method with a calibration curve of gallic acid as a reference standard (Sigma Aldrich®) at concentrations of 0, 1, 2, 2, 3, 4, and 5 mg L^{-1}. Ramon seed meal extraction was performed in methanol (JT Baker®) (0.93 mg mL^{-1}) for 48 h. From that solution 0.5 mL were taken and mixed with 0.75 mL of Folin Ciocalteu 1 N reagent (Sigma Aldrich®) and allowed to stand for 5 min. Subsequently, 0.75 mL of 20 % sodium carbonate (Sigma Aldrich®) were added, shaken, and allowed to stand for 90 min; this procedure was performed in triplicate. The absorbance was measured at 760 nm in spectrophotometer (Thermo Scientific Vis/UV-Vis GENESYS™ 40, Waltham, USA).

Flavonoid content

The concentration of total flavonoids was determined using a catechin calibration curve (Sigma Aldrich®) with concentrations of 50, 100, 300, 700, and 1000 ppm, by adding 600 µL of a Ramon seed solution (6.5 mg mL^{-1}) to 2.6 mL of solution A ($NaNO_2$ 0.06 M, Sigma Aldrich®). The mixture was left to react for 5 min. Then, 180 µL of $AlCl_3$ (Sigma-Aldrich®) were added and allowed to stand for 1 min. Finally, 2.5 mL of solution B (NaOH 1.7 M (Sigma Aldrich®)) was added. The assay was performed in triplicate and the absorbance was read at 415 nm in spectrophotometer (Thermo Scientific Vis/UV-Vis GENESYS™ 40, Waltham, USA).

Determination of protein by the Bradford method

A standard curve was prepared from the BSA solution 0.1 mg mL^{-1} (Sigma Aldrich®). Different concentrations were made and allowed to stand for 5 min. The absorbance was read at 595 nm. In order to perform the experiment, 200 µL of Bradford reagent (Sigma Aldrich®) and 100 µL of the extracted protein fractions were taken, brought to a final volume of 1 mL with distilled water, and performed in triplicate. They were allowed to stand for 5 min and after this time the absorbance was read at 595 nm in the spectrophotometer (Thermo Scietific Vis/UV-Vis GENESYS™ 40, Waltham, USA).

Storage proteins

For albumin determination, 10 g of defatted flour were weighed and shaken with 100 mL of deionized water for 1 h under refrigeration. It was centrifuged (PrO-Research Centrifuge by Centurion Scientific Lid Chichester, UK) at 10 000 rpm at 4 °C for 25 min. The supernatant was collected and washed following the same procedure. The supernatants were pooled.

For globulin analysis, 100 mL of 0.5 M NaCl (Sigma Aldrich®) were added to the above residue and shaken for 1 h under refrigeration. Subsequently, it was centrifuged (PrO-Research Centrifuge by Centurion Scientific Lid, Chichester, UK) at 10 000 rpm at 4 °C for 25 min, the supernatant was separated, and the residue was washed following the same procedure. The supernatants were pooled and the residue was used for the extraction of prolamins, for which 50 mL of 70 % ethanol (JTBaker®) were added,

shaken for 1 h under refrigeration, and centrifuged (Centrifuge PrO-Research by Centurion Scientific Lid, Chichester, United Kingdom) at 10 000 rpm for 25 min. The supernatant was collected, and the residue was washed following the same procedure. The supernatants were pooled and the residue was used for glutelin extraction. For the latter, 50 mL of 70 % sodium acetate 0.5 % mercaptoethanol 0.1 M ethanol solution (Sigma Aldrich®) was added to the previous residue and shaken for 1 h. The supernatant was separated and washed with 25 mL of the same solution pooling the supernatants.

Biscuit test (AACC Method 10-50.05)

The vegetable shortening was mixed with sugar, salt, and baking soda for 3 min. The Ramon seed flour was added and mixed for 2 min. Then, water was added and kneaded. Two 7 mm diameter glass rods were placed on a table, 20 cm apart, and the dough was placed in between. The dough was then rolled out with a rolling pin to form a uniform layer; six cookies were then cut with the same circular mold and baked at 220 °C for 10 min. Finally, the cookies were allowed to cool for 30 min. They were aligned continuously and the length of the six pieces together was measured; they were rotated 90° each and the operation was repeated. Finally, one was placed on top of the other, height was measured, the order of the cookies was changed, and a second reading was recorded. The measurements were averaged and divided by six to obtain the diameter and height of each cookie. To obtain the expansion factor, the diameter was divided by the height and multiplied by ten.

Statistical analysis

The Student's *t*-test was used to compare samples, and the difference between the means of the samples was calculated to determine that the data were statistically different, using the formula:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_c^2}{n_1} + \frac{S_c^2}{n_2}}}$$

with G.L.= 4, t= 2.78 and α= 0.05.

RESULTS AND DISCUSSION

Determination of proximal chemical analysis

The chemical composition (Table 1) of Ramon seed yielded a dietary fiber content of 43.99 %, i.e., 44 g of dietary fiber per 100 g of seed. In contrast, chia, quinoa, and amaranth seed contain 34, 15, and 11 % by weight of dietary fiber (USDA, 2023). This is relevant considering that the FAO and WHO expert committee issued a daily intake recommendation of 25 g of total dietary fiber per day for adults. In addition, a recent

Table 1. Proximal chemical analysis of Ramon tree (*Brosimum alicastrum* Swartz.) seed.

Component	Result (per 100 g)*
Moisture (g)	7.87
Ash (g)	3.41
Fat (g)	0.82
Protein (g)	13.60
Dietary fiber (g)	43.99
Total digestible carbohydrates (g)	30.31
Energy value (Kcal)	183.02

study reports that the most studied macronutrients for shaping the gut microbiota are those making up dietary fiber (Berding *et al*., 2021). It is important to emphasize that there has been an exponential increase in scientific research related to the impact of the gut microbiota on various aspects of human health, including brain health, in recent decades (Cryan *et al*., 2019).

The chemical composition of Ramon seed was compared with that of quinoa, chia, and amaranth (de Almeida Costa *et al*., 2006). The seed of the Ramon tree has a high fiber content compared to the others (Figure 1). Dietary fiber consists mainly of hemicelluloses, pectins, oligosaccharides, and lignins, which resist digestion in the

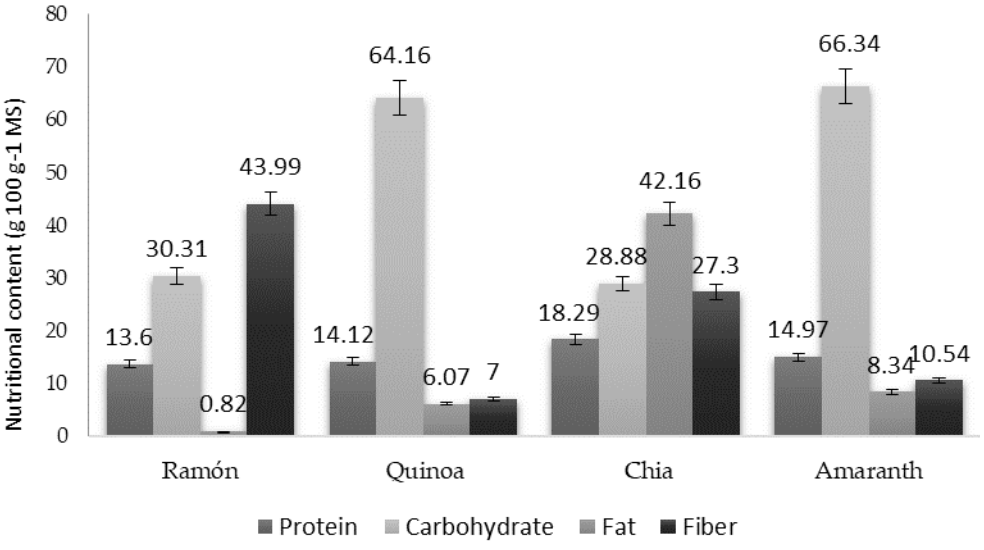

Figure 1. Comparison of the nutritional content of Ramon (*Brosimum alicastrum* Swartz.), quinoa (*Chenopodium quinoa* Willd.), Chia (*Salvia hispanica* L.), and amaranth (*Amaranthus hypochondriacus* L.) seeds.

small intestine, but are partially fermented in the large intestine, bringing with them beneficial health effects (Hughes and Swason, 1989; de Almeida Costa *et al*., 2006).

Ramon tree seed also has a relatively low-fat content compared to other seeds, its main fatty acids being linoleic, palmitic, linolenic, stearic, and eicosanoic acids (Losoya-Sifuentes *et al*., 2023). It is important to emphasize that Ramon seed has a similar carbohydrate content to chia but lower than quinoa and amaranth. Carbohydrates are among the main sources of energy for the human organism, including the brain and nervous system (Official Journal of the European Union, 2011). However, their excessive consumption is not advised since carbohydrates are stored as fat when these reserves are depleted.

Because the Mexican diet is high in carbohydrates, this quality is not among the most sought after in a healthy food product. Ramon seed has a similar protein content compared to the other seeds evaluated, expressed as total intakes (Figure 1). It was observed that Ramon seed has the lowest energy value (Figure 2) because it has the least amount of fat. This is not unfavorable because there is a consumer sector that demands food with low fat content. In addition, dietary lipids and fatty acids also shape the gut microbiota. For example, dietary intake of fat and fiber is associated with increased microbiota diversity in pregnant women (Roytio *et al*., 2017).

Determination of WHC

The WHC, defined as the amount of water remaining in the hydrated fiber after the application of an external force, was 2.08 g water g^{-1} Ramon seed. Amaranth had the best WHC value, followed by chia and quinoa (Figure 2). Because seeds are in their complete form, they can retain water to some extent thanks to the protein and soluble fiber content. The presence of fatty acids may also play a role in this property, as well as particle size (Parrot and Thrall, 1978).

In the present study, Ramon seed had a larger particle size than the other seeds, which could be one of the reasons why it incorporates less water. The lower WCH of Ramon seed has the advantage of being able to accelerate intestinal transit and prevent constipation, which contributes to reducing the contact time of potential carcinogens with the colon mucosa (Escudero-Álvarez and González-Sánchez, 2006). An increase in WHC causes a rise in stool volume, slowing intestinal transit speed. This is a coadjuvant to the fermentation capacity of fiber, causing the growth of microflora (Slavin, 2013).

Determination of SC

This swelling study, which measures the volume occupied by a sample when immersed in an excess of water relative to its actual weight, yielded a swelling capacity of 1.62 mL of water g^{-1} of dry Ramon seed. The swelling capacity of Ramon seed is lower than that of chia and amaranth (Figure 2). Our study suggests that the high soluble fiber content of chia causes water uptake via osmotic action and the formation of gel-like colloids, which significantly increases its volume. Amaranth, on the other hand, contains 6.1 % of total dietary fiber, of which 2.5 % corresponds to soluble fiber and

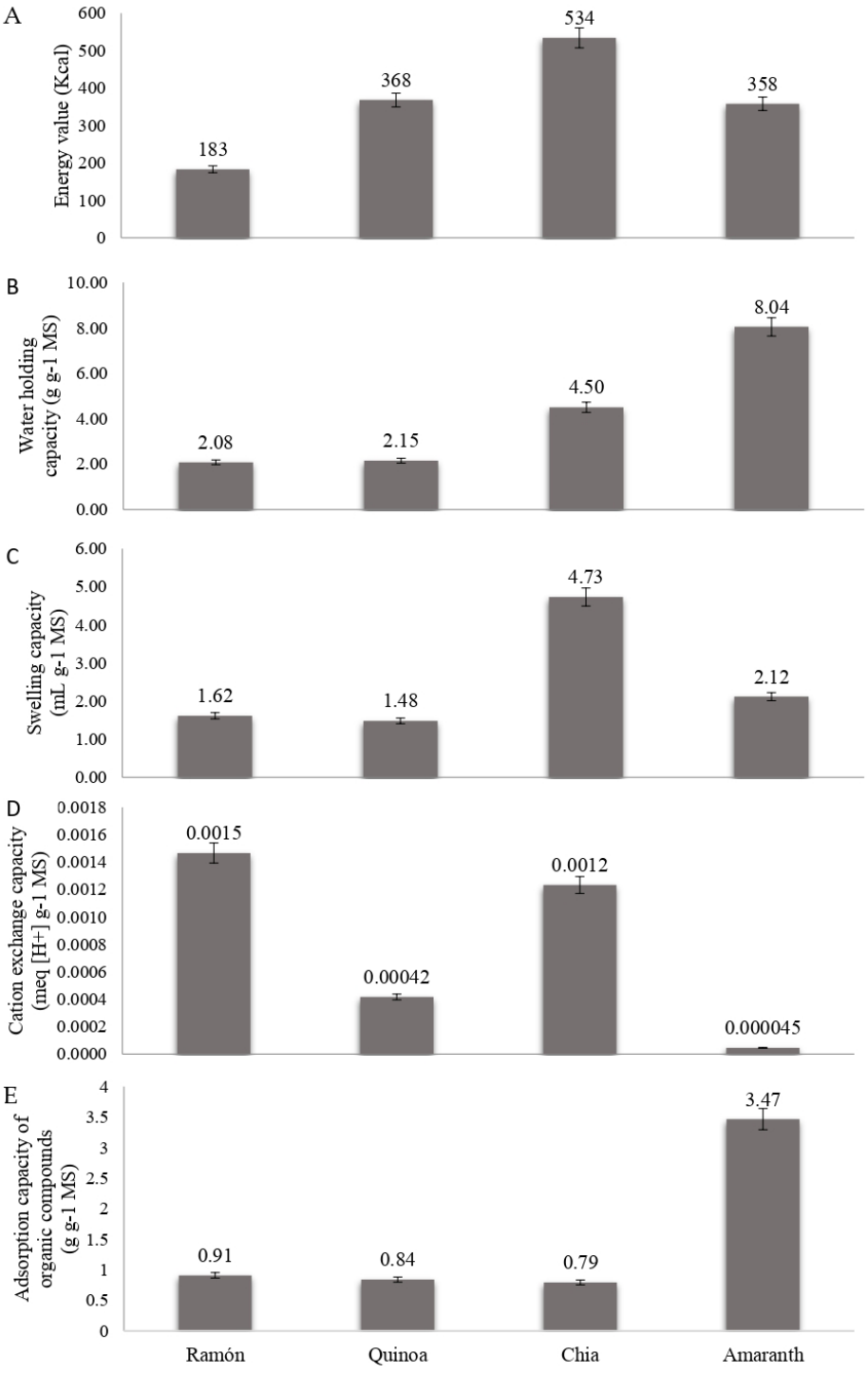

Figure 2. Comparison of Ramon (*Brosimum alicastrum* Swartz.), quinoa (*Chenopodium quinoa* Willd.), Chia (*Salvia hispanica* L.), and amaranth (*Amaranthus hypochondriacus* L.) seeds. A: energy value; B: water holding capacity; C: swelling capacity; D: cation exchange capacity; E: adsorption capacity of organic compounds.

3.6 % to insoluble fiber (CIAD, 2017). In this sense, it has been observed that insoluble fiber can exhibit better swelling, probably due to an increase in its porosity. The seeds, by increasing their volume, bring a signal of satiety, which can be beneficial to people in the process of losing weight. The latter has no direct nutritional consequences.

Determination of CEC

The amount of minerals absorbed at the gastrointestinal tract level (CEC) for the measurement of the potential of the fiber to form insoluble complexes with inorganic ions, representing the number of cations that can be retained (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}). A CEC of 0.0015 meq [H+] g^{-1} was obtained for dry Ramon seed. Based on the results (Figure 2D), Ramon seed has the highest CEC value, which is directly related to the composition of materials, such as pectins and the presence of residual nitrogen (charged molecules that can interact with cations). The higher the CEC, the greater the retention of nutrients, which indicates an increase in the fecal extraction of some minerals and electrolytes of nutritional importance.

Determination of ACOC

This property refers to the ability of Ramon seed fiber to retain and bind organic molecules such as phosphoproteins and phospholipids as they pass through the intestine. These molecules can be trapped or bound to the active sites of the fiber. In this study, Ramon dry basis seed had an ACOC of 0.91 g oil g^{-1}, indicating a binding capacity similar to that of quinoa and chia but lower than amaranth (Figure 2E). Fiber acts as a binding agent and transporter of molecules such as bile acids, carcinogenic molecules, and mutagenic agents, so low values of this property could be considered nutritionally healthy. Recently, the potential for glucose and cholesterol uptake from algal dietary fiber has been studied (Wang *et al*., 2022).

Statistical analysis

Analysis of variance was performed between the physical and chemical properties of the seeds under study (Table 2), showing that Ramon seed had no significant

Table 2. Significant differences in properties of Ramon tree (*Brosimum alicastrum* Swartz.) seed.

Capacity:	Comparative significant difference *			
	Ramon	Quinoa	Chia	Amaranth
Water holding	Y	Y	X	X
Swelling	Y	Y	X	X
Cation exchange	Y	X	Y	X
Absorption of organic compounds	Y	Y	X	X

*Means with the same letter X or Y per column are not statistically different (α = 0.05). Chia (*Salvia hispanica* L.), quinoa (*Chenopodium quinoa* Willd.), and amaranth (*Amaranthus hypochondriacus* L.).

difference when compared to quinoa in three physical and chemical properties: WHC, SC, and ACOC. Chia, on the other hand, had no significant difference in one property: CEC. In the case of amaranth, there was a significant difference for all the physical and chemical properties evaluated.

Total phenols content

The total phenol content was 154 mg gallic acid 100 g^{-1} of Ramon seed. Compared to the other seeds (Table 3), it has more phenols than quinoa and amaranth.

Table 3. Antioxidant capacity of Ramon (*Brosimum alicastrum* Swartz.), Chia (*Salvia hispanica* L.), quinoa (*Chenopodium quinoa* Willd.), and amaranth (*Amaranthus hypochondriacus* L.) seeds.

Seed	Ramon	Quinoa	Amaranth	Chia
Total phenols* (mg gallic acid 100 g^{-1} seed)	154	159	35	854
Antioxidant capacity (mg Trolox g^{-1} seed)	0.88	0.91	0.66	24.71

It is necessary to evaluate the phenolic content in order to determine their antioxidant capacity. However, this method only evaluates the total phenolic compound content and not the antioxidant efficiency. In addition, numerous environmental factors such as light, maturity, and preservation can affect total polyphenol content (Quiñones *et al.*, 2012).

Flavonoid content and antioxidant capacity

Flavonoids are the most common phenolic compounds in nature and have the highest antioxidant activity (Pietta, 2000). Catechin was used to perform the calibration curve, yielding a flavonoid content of 72.14 mg of catechin per 100 g of sample on a dry basis. Flavonoid consumption helps promote health; a high flavonoid intake correlates with a lower risk of cardiovascular disease (Pietta, 2000; Lolito and Frei, 2006).

Flavonoids decrease cholesterol and oxidize low-density lipoprotein (LDL) rates due to their antioxidant properties as strong transition metal chelators and hydrogen donors through hydroxyl groups, thus preventing the formation of reactive oxygen species at the cellular level (Quiñones *et al*., 2012). This has an impact on inflammatory processes and even helps to reduce carcinogenic processes.

When comparing the antioxidant capacity of Ramon seed to quinoa, amaranth, and chia, it can be observed that Ramon seed has a higher antioxidant capacity than amaranth (Table 3). Ramon and quinoa seeds have similar phenol content, which suggests that this could explain the similarity of their antioxidant characteristics, since in most cases these two properties are related. This is supported by the case of chia, which has a much higher phenol content than the rest.

In the results obtained over the inhibition of the stable radical DPPH, Ramon seed showed an antioxidant activity of IC_{50} = 1.602 mg mL^{-1}, indicating a linear relationship between the inhibition capacity and the sample concentration. However, the most remarkable finding in this study was obtained with the leaf of the Ramon tree having an IC_{50} = 0.618 mg mL^{-1}, demonstrating that the leaf obtained a higher value than the seed in terms of DPPH radical inhibition, even higher than Trolox (1.751 mg Trolox g^{-1}). This can be attributed to the fact that the leaf contains phenols with a high molecular weight and an antioxidant capacity up to 20 times stronger than vitamin E (Padilla *et al*., 2008). This fact may allow promoting the use of this tree´s leaves for functional food beverages.

Storage proteins

The concentration of storage proteins in Ramon seed was determined based on their functional properties. Ramon seed (Table 4) is rich in albumins and globulins, known to be easily digestible (Venskutonis and Kraujalis, 2013). Albumin is a complete protein with high biological value that contains all nine essential amino acids (leucine, lysine, isoleucine, methionine, phenylalanine, threonine, valine, tryptophan, and histidine). Globulins contribute to the nutritional quality of grains. In terms of functional properties, globulins have higher protein solubility, emulsifying capacity, and oil absorption capacity, whereas albumins have better water absorption capacity and foaming capacity for product preparation (Sánchez-Mendoza *et al*., 2017). Ramon seed, on the other hand, contains a low concentration of prolamins and glutelins, as well as a low concentration of extracted soluble protein (Rosa *et al*., 2000).

Table 4. Storage protein concentrations in Ramon tree (*Brosimum alicastrum* Swartz.) seed.

Protein	mg of protein g^{-1} of seed
Albumins	0.91
Globulins	0.65
Prolamins	0.04
Glutelins	0.02

Expansion factor in food test by AACC Method 10-50D

Ramon seeds whole wheat flour was used to evaluate its performance in the production of biscuit products (gluten-free), and the expansion factor obtained was 62.8. A high-quality biscuit flour is associated with expansion factor values close to 100 because of its ability to incorporate a large amount of gas and retain it as the protein settles during baking. However, one of the major issues associated with gluten-free bakery products is low nutritional quality due to lower vitamin, mineral, and dietary fiber

content (Mohammadi *et al*., 2022). This can be solved by adding suitable ingredients in the formulation, such as the alternative of chestnut flour for all of its nutritional properties, as it has a high fiber content and an important vitamin content such as vitamin C, vitamin E, and vitamins of the B group (Pastrana, 2010).

CONCLUSIONS

This work successfully explored the use of Ramon tree seed (*Brosimum alicastrum* Swartz.), an emblematic seed of the Mayan culture, as a functional food. The results showed that Ramon seed is comparable to other functional seeds available on the international functional food market. The physical, chemical, and nutritional properties identified in the seed of the Ramon tree (*Brosimum alicastrum*) allowed us to recognize its potential in the elaboration of beneficial food products due to its high nutritional value. The high fiber and protein content, in addition to its low-fat content and high antioxidant capacity, allowed identifying future applications of Ramon seed as a functional food in Mexico.

The scope of these results will depend on future research that could focus on studies on gut microbiota and its intrinsic relationship with health, as well as on food safety for Mexico and Latin America for this seed. Finally, although the present study focused on the Ramon seed, it should be noted that studies on the leaf revealed an antioxidant capacity superior to that of Trolox under the conditions studied. In this sense, the tree's leaf could be used as an antioxidant in herbal tea beverages.

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REFERENCES

AACC International. 2010. Approved methods of analysis (11th Edition). Cereals and Grains Association: St. Paul, MN, USA. https://www.cerealsgrains.org/resources/Methods/About/Pages/default.aspx (Retrieved: April 2023).

Alvídrez-Morales A, González-Martínez B, Jiménez-Salas Z. 2002. Tendencias en la producción de alimentos: alimentos funcionales. Revista Salud Pública y Nutrición 3 (1): 1–7.

Ananey-Obiria D, Matthews L, Azahrani MH, Ibrahim SA, Galanakis SM, Tahergorabi R. 2018. Application of protein-based edible coatings for fat uptake reduction in deep-fat fried foods with an emphasis on muscle food proteins. Trends in Food Science and Technology 80: 167–174. https://doi.org/10.1016/j.tifs.2018.08.012

AOAC International. 2005. Official method of Analysis (18th Edition) Association of Officiating Analytical Chemists: Washington, DC, USA. https://www.aoac.org/about-aoac-international (Retrieved: April 2023).

Berding K, Vlckova K, Marx W, Schellekens H, Stanton C, Clarke G, Jacka F, Dinan TG, Cryan JF. 2021. Diet and the microbiota–gut–brain axis: sowing the seeds of good mental health. Advances in Nutrition 12 (4): 1239–1285. https://doi.org/10.1093/advances/nmaa181

CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad). 2020. Información de especies de árboles de Berg, C. (1972). *Brosimum alicastrum* Sw. subsp.

alicastrum C.C. Berg. http://www.conabio.gob.mx/conocimiento/info_especies/arboles/doctos/47-morac1m.pdf (Retrieved: April 2023).

de Almeida Costa GE, da Silva Queiroz-Monici K, Pissini Machado Reis SM, de Oliveira AC. 2006. Chemical composition, dietary fiber and resistant starch contents or raw and cooked pea, common bean, chickpea and lentil legumes. Food Chemistry 94 (3): 327–330. https://doi.org/10.1016/j.foodchem.2004.11.020

Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, Codagnone MG, Cussotto S, Fulling C, Golubeva AV, Fulling C *et al*. 2019. The microbiota-gut-brain axis. Physiological Reviews 99 (4): 1877–2013. https://doi.org/10.1152/physrev.00018.2018

Cuadrado C, Sanchiz Á, Linacero R. 2021. Nut allergenicity: effect of food processing. Allergies 1 (3): 150–162. https://doi.org/10.3390/allergies1030014

Donovan J, Rutsaert P, Domínguez C, Peña M. 2022. Capacities of local maize seed enterprises in Mexico: Implications for seed systems development. Food Security 14 (2): 509–529. https://doi.org/10.1007/s12571-021-01247-8

Official Journal of the European Union. 2011. Regulation (EU) No 1169/2011 of the European Parliament and of the Council on the provision of food information to consumers. Strasbourg, France. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:304:0018:0063:en:PDF (Retrieved: April 2023).

Escudero-Álvarez E, González-Sánchez P. 2006. La fibra dietética. Nutrición Hospitalaria 21 (2): 61–72.

FAO (Organización de las Naciones Unidas para la Alimentación y la Agricultura), IFAD (Fondo Internacional de Desarrollo Agrícola), UNICEF (Fondo de las Naciones Unidas para la Infancia), WFP (Programa Mundial de Alimentos), WHO (Organización Mundial de la Salud). 2021. The state of food security and nutrition in the world 2021. Rome, Italy. https://doi.org/10.4060/cb4474en

Rosa MJS, Ferreira RB, Texeira AR. 2000. Storage Proteins from *Lathyrus sativus* Seeds. Journal of Agricultural and Food Chemistry 48 (11): 5432–5439. https://doi.org/10.1021/jf000447r

Fukushima D. 1991. Structures of plant storage protein and their function. Food Reviews International 7 (3): 353–381. https://doi.org/10.1080/87559129109540916

Hughes JS, Swason BJ. 1989. Soluble and insoluble dietary fiber in cooked common bean (*Phaseolus vulgaris*) seeds. Food Microstructure 8 (1): 15–21.

Kumar A, Agarwal DK, Reddy YM, Chintagunta AD, Saritha KV, Pal G, Kumar SPJ. 2019. Nutraceuticals derived from seed storage proteins: Implications for health wellness Biocatalysis and Agricultural Biotechnology 17: 710–719 https://doi.org/10.1016/j.bcab.2019.01.044

Krisnawati DI, Alimansur M, Atmojo DS, Rahmawati EQ, Rahayu D, Susilowati E, Kuo TR. 2022. Food allergies: immunosensors and management. Applied Sciences 12 (5): 2393. https://doi.org/10.3390/app12052393

Lolito BS, Frei B. 2006. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: Cause, consequence, or epiphenomenon. Free Radical Biology and Medicine 41 (12): 1727–1746. https://doi.org/10.1016/j.freeradbiomed.2006.04.033

Losoya-Sifuentes C, Pinto-Jimenez K, Cruz M, Rodríguez-Jasso RM, Ruiz HA, Loredo-Treviño A, López-Badillo CA, Belmares R. 2023. Determination of nutritional and antioxidant properties of maya nut flour (*Brosimum alicastrum*) for development of functional foods. Foods 12 (7): 1398. https://doi.org/10.3390/foods12071398

Mcconell AA, Eastwood MA, Mitchel WD. 1974. Physical characteristics of vegetables foodstuffs that could influence bowel function. Journal of Science Food and Agriculture 25 (12): 1457–1464. https://doi.org/10.1002/jsfa.2740251205

Mohammadi M, Khorshidian N, Yousefi M, Khaneghah AM. 2022. Physicochemical, rheological, and sensory properties of gluten-free cookie produced by flour of chestnut, date seed, and modified starch. Journal of Food Quality 2022: 1–10. https://doi.org/10.1155/2022/5159084

Munekata PES, Gullón B, Pateiro M, Tomasevic I, Domínguez R, Lorenzo JM. 2020. Natural antioxidants from seeds and their application in meat products. Antioxidants 9 (9): 815. https://doi.org/10.3390/antiox9090815

Olmos E, Román-García I, Reguera M, Mestanza C, Fernández-García N. 2022. An update on the nutritional profiles of quinoa (*Chenopodium quinoa* Willd.), amaranth (*Amaranthus* spp.), and chia (*Salvia hispanica* L.), three key species with the potential to contribute to food security worldwide. JSFA Reports 2 (2): 591–602. https://doi.org/10.1002/jsf2.93

Padilla FC, Rincón AM, Bou-Rached R. 2008. Contenido de polifenoles y actividad antioxidante de varias semillas y nueces. Archivos Latinoamericanos de Nutrición 58 (3): 303–308.

Parrot ME, Thrall BE. 1978. Functional properties of various fibers: physical properties. Journal of Food Science 43 (3): 759–766. https://doi.org/10.1111/j.1365-2621.1978.tb02412.x

Pastrana L. 2010. Estudio acerca de las posibilidades de diversificación de los productos de la castaña y la vid. Proyecto "Diversificación de los recursos agrícolas y ganaderos". GDR Sil Bibei Navea: Ourense, España. 39 p.

Peters CM, Pardo-Tejeda E. 1982. *Brosimum alicastrum* (Moraceae): Uses and potential in Mexico. Economic Botany 36 (2): 166–175. https://doi.org/10.1007/BF02858712

CIAD (Centro de Investigación en Alimentación y Desarrollo). 2017. El amaranto, una alternativa saludable. Centro de Investigación en Alimentación y Desarrollo. Culiacán, México. https://www.ciad.mx/notas/item/1609-el-amaranto-una-alternativa-saludable (Retrieved: April 2023).

Pietta PG. 2000. Flavonoids as antioxidants. Journal of Natural Products 63 (7): 1035–1042. https://doi.org/10.1021/np9904509

Puleston DE. 1982. The role of Ramon in Maya subsistence. *In* Flannery K. (ed.). Maya subsistence: Studies in memory of Dennis E. Puleston. Academic Press: New York, NY, USA. 368 p. https://www.elsevier.com/books/maya-subsistence/flannery/978-0-12-259780-0 (Retrieved: April 2023).

Quiñones M, Miguel M, Aleixandre A. 2012. Los polifenoles, compuestos de origen natural con efectos saludables sobre el sistema cardiovascular. Nutrición Hospitalaria 27 (1): 76–89.

Robertson JA, de Monredon FD, Dysseler P, Guillon F, Amado R, Thibault JF. 2000. Hydration properties of dietary fibre and resistant starch: a European collaborative study. LWT - Food Science and Technology 33 (2): 72–79. https://doi.org/10.1006/fstl.1999.0595

Roytio H, Mokkala K, Vahlberg T, Laitinen K. 2017. Dietary intake of fat and fibre according to reference values relates to higher gut microbiota richness in overweight pregnant women. British Journal of Nutrition 118 (5): 343–352. https://doi.org/10.1017/s0007114517002100

Sánchez-Mendoza NA, Ruiz-Ruiz JC, Dávila-Ortiz G Jiménez-Martínez C. 2017. Propiedades tecnofuncionales y biológicas de harina, aislado y fracciones proteicas mayoritarias de semillas de Inga paterno. CyTA - Journal of Food 15 (3): 400–408 https://doi.org/10.1080/19476337.2017.1286522

Shewry PR, Napler JA, Tatham AS. 1995. Seed storage proteins: structures and boisyntesis. The Plant Cell 7 (7): 945–954. https://doi.org/10.1105/tpc.7.7.945

Slavin J. 2013. Fiber and prebiotics: Mechanisms and health benefits. Nutrients 5 (4): 1417–1435. https://doi.org/10.3390/nu5041417

USDA (U.S. Department of Agriculture). 2023. Agricultural Research Service, Food Data Central. U.S. Department of Agriculture. Washington, DC, USA. https://fdc.nal.usda.gov/fdc-app.html#/food-details/169414/nutrients (Retrieved: April 2023).

Venskutonis PR, Kraujalis P. 2013. Nutritional components of amaranth seeds and vegetables: A review on composition, properties, and uses. Comprehensive Reviews in Food Science and Food Safety 12 (4): 381–412. https://doi.org/10.1111/1541-4337.12021

Wang X, Zhang L, Qin L, Wang Y, Chen F, Qu C, Miao J. 2022. Physicochemical properties of the soluble dietary fiber from *Laminaria japonica* and its role in the regulation of type 2 diabetes mice. Nutrients 14 (2): 329. https://doi.org/10.3390/nu14020329

Zeb A. 2020. Concept, mechanism, and applications of phenolic antioxidants in foods, Journal of Food Biochemistry 44 (9): e13394. https://doi.org/10.1111/jfbc.13394