Physicochemical and functional properties of starches from *Brosimum alicastrum* (Sw.) and *Zea mays* obtained by wet-extraction without chemical agents †

[Propiedades fisicoquímicas y funcionales del almidón de *Brosimum alicastrum* (Sw.) y *Zea mays* obtenidos mediante extracción húmeda sin agentes químicos]

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SUMMARY

Background: Starch is a polysaccharide widely used in the food industry and other applications due to its functional properties. It is commonly extracted from different sources, mainly grains and tubers. *Brosimum aliscastrum* Sw. tree is a non-conventional source that may represent a viable alternative that does not compete with resources for either human or animal consumption. **Objective:** To characterize the physicochemical, functional, and thermo-structural properties of starches isolated from ramon seed (*B. alicastrum*) and maize (*Zea mays*) employing a green wet-extraction method without chemical agents. **Methodology:** Flours from ramon seed (RS) and maize were subjected to soaking, washing, filtering, and centrifugation using only distilled water to isolate starches. **Results:** Extraction yields were 28.7% for ramon seed starch (RSS) and 36.33% for maize starch (MS). Chemical composition, particularly carbohydrate content (86% for RSS and 87% for MS), was comparable to values obtained by alkaline and acid methods. Amylose content was higher in MS (27.61%) compared to RSS (23.60%). **Implications:** The results suggest that green extraction demonstrated efficiency in recovering starches with properties comparable to those obtained through conventional chemical methods while also promoting the use of non-conventional sources such as RS. **Conclusion:** The isolation method employed enables the recovery of starches with yields and physicochemical properties similar to those obtained through conventional methods. The findings highlight the potential of RSS as an alternative source of starch for various applications.

Key words: sustainable processing; non-conventional starch sources; green extraction; starch properties; structural characterization.

RESUMEN

Antecedentes: El almidón es un polisacárido ampliamente utilizado en la industria alimentaria y en otras aplicaciones, debido a sus propiedades funcionales. Generalmente se obtiene de diferentes fuentes, principalmente granos y tubérculos. El árbol *Brosimun alicastrum* Sw., es una fuente no convencional que representa una alternativa viable sin competir con los recursos destinados al consumo humano y animal. **Objetivos:** Caracterizar las propiedades fisicoquímicas, funcionales y termo-estructurales de los almidones extraídos de la semilla de ramon (*B. alicastrum*) y del maíz (*Zea mays*) mediante un método de extracción húmeda sin el uso de agentes

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químicos. **Métodos:** Las harinas obtenidas de la semilla de ramón (SR) y del maíz se sometieron a un proceso de remojo, lavado, filtrado y centrifugado utilizando únicamente agua destilada para obtener el almidón de semilla de ramon (ARS) y el almidón de maíz (AM). **Resultados:** El rendimiento (28.7% del ASR y 36.33% del AM) y la composición química, especialmente los carbohidratos (86% y 87% para el ASR y AM, respectivamente), estuvieron dentro de los rangos esperados, comparados con extracciones alcalinas o ácidas. El contenido de amilosa fue mayor para el AM (27.61%) en comparación con el ASR (23.60%). **Implicaciones:** Los resultados sugieren que la extracción húmeda sin químicos es una alternativa viable para la obtención de almidones con propiedades comparables a las técnicas de extracción tradicionales, para aprovechar fuentes no convencionales como lo es la semilla de ramón. **Conclusión:** La extracción húmeda sin el uso de agentes químicos permitió extraer almidones con rendimientos y propiedades fisicoquímicas similares a los métodos tradicionales. Los resultados obtenidos destacaron el potencial del ASR como una fuente alternativa para diversas aplicaciones.
Palabras clave: procesamiento sustentable; fuentes no convencionales de almidón; extracción verde; propiedades del almidón; caracterización estructural.

INTRODUCTION

Brosimum alicastrum Swartz, commonly known as breadnut, Maya nut, ramon, ojoche, capomo or by its Mayan name *ox*, is a neotropical tree widely distributed in the Mesoamerican regions, along the southeastern part of Mexico through Central America and the Caribbean. In México, particularly in the Yucatán Peninsula, it is referred to as *ramon* and may reach heights of 15 to 22 m and 1 m in diameter. Due to its high carbohydrate content, its seed has traditionally been used as food in rural communities and as an energy source in monogastric animal diets. Evidence suggests that the Mayan people used these seeds as a staple food, especially during times of scarcity (Hernández-González, Vergara-Yoisura and Larqué-Saavedra, 2015; Carter and Northcutt, 2023; Losoya-Sifuentes *et al.*, 2023). The estimated seed production per hectare is 38.2 tons annually (in a plantation of 400 trees per hectare) (Olguin-Maciel *et al.*, 2017).

Ramon seed (RS) represents a promising underexploited resource due to its high carbohydrate content, high yield per hectare, and limited current utilization, making it potentially usable in the tropical and subtropical regions. Previous studies have shown that starch extracted from RS has a slightly lower amylose content compared to amylopectin, exhibits a C-type diffraction pattern and possesses a lower digestible energy value (2538.7 kcal/kg) than maize (Moo-Huchin *et al.*, 2015; Montfort-Grajales *et al.*, 2024), due to its internal structure and botanical origin (Pérez-Pacheco *et al.*, 2014; Pech-Cohuo *et al.*, 2021). For this reason, it is crucial to evaluate the physicochemical and functional properties of ramon seed starch (RSS) and compare them with those from conventional sources such as maize.

Starch is a polysaccharide in which plants store their carbohydrates, and it serves as a source of energy in animal and human nutrition. It consists of two molecules, amylose (20-30%) and amylopectin (70-80%); both of which are composed essentially of linear chains of glucose with α-(1,4) linkages. The main difference between them is the degree of branching, with α-(1,6) linkages present; amylose has less than 1%, while amylopectin is approximately 5%. Internally, starch granules are organized in concentric amorphous and semicrystalline growth rings, alternating from the center to the surface (BeMiller and Whistler, 2009; Wang *et al.*, 2022). Although starch is primarily extracted from grains and tubers, it may be obtained from fruits, seeds, and legumes. Interest in non-conventional starch sources has gained importance for implementation in food, papermaking, textile, adhesive, and pharmaceutical industries, and its incorporation into animal diets. This is associated with their low cost, availability, biodegradability, and the fact that they do not compete with conventional sources of energy for humans (Moo-Huchin *et al.*, 2020; Miao and Bemiller, 2023). In this regard, RS is a tropical, underexplored starch source scarcely studied that has shown its potential in the manufacture of biodegradable materials due to its physicochemical properties and high processing temperature (Pérez-Pacheco *et al.*, 2014).

Starch extraction methods depend on the botanical source, and since there is not a single universal method of extraction, the results obtained from each one will depend on the purpose for which starch is to be used. It has been reported that the isolation method affects yield, physicochemical, and functional properties (Estrada-León *et al.*, 2016; Singla *et al.*, 2020; Zhang *et al.*, 2020). Such is the case of only-water extraction methods, where authors have concluded that a high-purity starch was obtained (less than 1% of protein, lipids, and ash). Also, when comparing wet and dry milling extraction processes, differences have been found, resulting in higher damaged starch and a higher protein content in the latter (Kringel *et al.*, 2020).

Previous studies have shown that the extraction medium can significantly influence starch composition. For instance, Palacios-Fonseca *et al.* (2013), reported that the use of distilled water when extracting maize starch (MS) increased protein and lipid contents, while reducing ash and amylose levels, compared to alkaline (NaOH) extractions, with no significant differences in crystallinity. Estrada-León *et al.* (2016) noted that the parota (*Enterolobium cyclocarpum*) starch seed extraction using distilled water yielded higher protein and amylose contents, but lower crude fiber, ash and starch yield compared to the alkaline method. Generally, it has been observed that extracting starch with an alkaline NaOH solution results in a higher starch yield and low protein content (El Halal *et al.*, 2019). As for RSS, Pech-Cohuo *et al.* (2021) when comparing these two isolation methods (distilled water vs NaOH), no differences were found for yield, morphological, structural, or gelatinization properties; however, chemical compositions were not reported. The aim of this study was to evaluate the physicochemical, functional, and thermo-structural properties of native starches extracted from RS and maize using a green wet-extraction method without chemical agents, for eventually considering the potential use of RS as alternative starch source in animal feeding under tropical conditions.

MATERIALS AND METHODS

Obtention of flour

Twenty kg of ramon fruit were obtained from local producers at the Yucatan Peninsula, in the southeast of Mexico. Fruits were selected, and the testa was removed manually to obtain RS. In order to obtain ramon seed flour (RSF), the fruits were oven dried at 40 °C for 48 hours and milled (IKA ® MF-10) with a 0.5 mm sieve. The same process as milling was carried out for maize to obtain maize flour (MF). The resulting flours were stored hermetically at 4 °C for further analysis.

Starch isolation

Starch extraction was carried out following the technique described by Pech-Cohuo *et al.* (2021) with some modifications. Briefly, for each type of flour, 250 g were soaked in 500 mL of distilled water and left to rest for 24 h under refrigeration (4 °C). The distilled water used had a neutral pH (7.0), was colorless and odorless, and had no additives or contaminants, according to the safety data sheet (ECOPURA, 2018), supporting the chemical-free approach of this study.

The flour was homogenized in a blender (Oster-BLSTBPST-013) for 2 min and filtered using 150 and 45 µm mesh with 250 mL of distilled water. Recovered starch was centrifuged (Thermo Scientific SL 40R, Thermo Electron LED GmbH, Germany) at 4,500 rpm for 15 min at 25 °C, the resulting supernatant was decanted, and the protein layer on top was scraped off manually. The sediment was resuspended with 150 mL of distilled water, then it was left to sit for 16 h, and the supernatant was discarded. The suspension was centrifuged at 3,000 rpm for 15 min, decanting the supernatant and removing the protein layer again. Using the 45 µm mesh, it was screened again to remove impurities. Isolated starch was dried at 50 °C for 6 h in a convection oven, and samples were kept at 4 °C for further analysis. Starch yield (SY) of both RSS and CS was estimated using the following formula:

$$\text{SY (\%)} = \frac{\text{MS}}{\text{MF}} \text{x}100$$

Where:
MS is mass starch (g), and, MF is mass flour (g) of each input on dry basis.

Morphology of starch granules

For granule shape and size determination, a HY-2307 microscope camera (Shenzhen Hayear Electronics Co. Ltd.) mounted on a microscope (Olympus CH 25, Tokyo, Japan) was used, with the Touplite Software, version 1.0 for iOSMAC (Hangzhou ToupTek Photonics Co. Ltd.). Dry starch samples were placed on a slide and mounted under the microscope for observation at 40 and 100X.

Chemical composition

The proximal composition of RSF, CF, RSS, and CS was evaluated following the official AOAC procedures: fat (method 920.39), ash (method 923.03), crude fiber (method 962.09), moisture (method 925.09), and nitrogen content (method 954.01) using factor 6.25 to obtain crude protein content (AOAC, 1990). The total carbohydrate content was estimated as nitrogen-free extract (NFE) by difference (NFE = (fat + ash + crude fiber + protein) – 100).

Amylose content

Amylose content of extracted starch was determined according to Morrison and Laignelet (1983). An 80 mg sample of each starch was weighed in 20 mL tubes and then added to a magnetic agitator. Following, 10 mL of a DMSO-urea 6M (9:1) solution was added and heated for 15 min until the sample was homogenized. Tubes were then placed in an oven at 100 °C for one hour and allowed to cool at room temperature. A sample of 0.5 mL of each starch was taken and transferred to a 50 ml flask (in triplicate), and weights were registered. Then, 25 mL of distilled water and 1 mL of a I_2/IK (2 mg I_2/20 mg IK/mL) were added to each flask, and were gauged to 50 mL, stirred, and let rest for 15 min. Samples were read with a spectrophotometer at 635 nm (Thermo Scientific BioMate 3S UV/VIS, USA). The following equation was used to obtain the blue value and apparent amylose content:

$$\text{Blue value} = \frac{\text{absorbance x 100}}{\text{2 x g solution x mg starch}};$$

$$\text{Amylose (\%)} = \text{blue value x 28.414}$$

pH determination

A starch-water solution at 1% (w/v) was prepared at room temperature, and pH was determined using a PHM-295 potentiometer.

Color parameters

Using a portable colorimeter CR30 (Hangzhou Color Spectrum Technology Co., LTD), three samples of each starch were selected and placed in a Petri dish for evaluation of the L (Lightness), a* (red-green), and b* (yellow-blue) parameters. Then, they were used to obtain hue angle (h°) using the following formula, where Tan-1 is the inverse tangent:

$$\text{h}° = \text{Tan} - 1\frac{\text{b}*}{\text{a}*}$$

Functional properties

Solubility, swelling power, and water absorption capacity of RSS and CS were determined following the technique described by Pérez-Pacheco *et al.* (2014) with slight modifications. A 2% (w/v) starch suspension was prepared for each input in a previously weighed 15 mL tube. All tubes were kept in agitation at constant temperature (70, 80, 90 °C) in a warm bath for 30 min. The supernatant was discarded, and the sample was dried at 105 °C in a crucible until constant weight. Swollen granules were weighted, and functional properties were calculated as follows:

$$\text{Solubility (\%)} = \frac{\text{WSS}}{\text{WS}}\text{x100}$$

$$\text{Swelling power}\left(\frac{\text{g}}{\text{g}}\right) = \frac{\text{WG}}{\text{WS} - \text{WSS}}$$

$$\text{Water absorption capacity}\left(\frac{\text{g}}{\text{g}}\right) = \frac{\text{WG}}{\text{WS}};$$

Where:
WSS is the weight of the solid soluble,
WS is the weight of the sample, and
WG is the weight of the gel.

Differential scanning calorimetry (DSC)

Starch gelatinization parameters were estimated with a DSC-6 (Perkin Elmer Corp., Norwalk, CT) following the Moo-Huchin *et al.* (2020) methodology. Approximately one mg of starch sample was weighed and placed in an aluminium pan, and 3 µL of water was added. Pans were heated from 30 to 110 °C with an increased temperature of 10 °C/min, while the sample chamber was flushed with dry nitrogen to avoid moisture condensation. As a reference, an empty aluminum pan was used. Temperatures [onset (To), peak (Tp), and conclusion (Tc)], enthalpy of gelatinization (ΔH_{gel}), gelatinization temperature range (GEL_{TR}), and peak height index (PHI) were determined. The enthalpy of gelatinization (ΔH_{gel}), expressed as Joules per g of starch (dry weight) (J/g), was calculated by integrating the area between the thermograph and the baseline under the peak.

X-ray diffraction pattern (XRD)

Samples were analyzed with an X-ray diffractometer (Bruker D-8 Advance), equipped with a copper anode X-ray tube operating at 40 kV and 30 mA, with a size step of 0.002° and an exposure time of 0.5 s (Pech-Cohuo *et al.*, 2021).

Statistical analysis

A completely randomized design with three replicates per sample was employed for each evaluated parameter. A one-way analysis of variance (ANOVA) was conducted to evaluate the effect of the extraction method (independent variable) on starch physicochemical, functional and thermo-structural properties (dependent variable). The assumptions of normality and homogeneity of variances were assessed and when significant differences were found ($p \leq 0.05$), a Tukey test was used to assess differences between treatment means. All analyses were carried out using the statistical software package Minitab 2022 (Minitab, 2022). Results are expressed as mean ± SD (standard deviation).

RESULTS AND DISCUSSION

Morphology of starch granules

RSS granules appeared to be oval-circular shaped, meanwhile CS granules were polygonal. Shapes of both starches were found to be similar to those reported by Pérez-Pacheco *et al.* (2014). As for other native starch sources such as parota (*Enterolobium cyclocarpum*), potato (*Solanum tuberosum*), yucca (*Manihot esculenta*), and velvet bean (*Mucuna pruriens*), their granules have been described as round-oval, similarly to the RSS granules found in the current experiment (Betancur-Ancona *et al.*, 2002; Singla *et al.*, 2020). CS granules had a similar polygonal shape as previously reported for rice (Betancur-Ancona *et al.*, 2002).

Regarding size, RSS exhibited an average granule size of 18.1 µm (ranging from 8 to 25 µm), while CS was 24.5 µm (ranging from 19 to 36 µm). These values were found to be higher than the results from Pérez-Pacheco *et al.* (2014), for the same starches, where RSS averaged 10.8 µm, and CS 15 µm. It has been reported that cereal starches possess a larger granule size than granules from tuber or seed starches. Granule shape is associated with the botanical source of the starch and the type of crystalline structure (Cornejo-Ramírez *et al.*, 2018).

Yield and chemical composition

Yield (dry basis) of RSS was calculated from seeds without testa, resulting in 28.37±1.96%, which is lower than the value for CS (36.33±2.57%) (p=0.0005). Pech-Cohuo *et al.* (2021) and Pérez-Pacheco *et al.* (2014) found a similar yield of starch for RSS using the same extraction method. In contrast, Palacios-Fonseca *et al.* (2013) reported a higher yield for CS (46%) using the distilled water-only method, while the highest yield was found with the use of the alkaline isolation method (51.03%).

RSF showed higher levels of crude protein, crude fiber, ash, and ether extract (12.57%, 1.61%, 3.32%, 2.39%, respectively) when compared to CF (7.45%, 0.24%, 1.03%, 1.49%, respectively). Only moisture and nitrogen-free extract (NFE) content were estimated to be higher for CF (11.20 and 78.59%, respectively) in comparison to RSF (9.01 and 71.1%, respectively). The chemical composition of RSS and CS is presented in Table 1. As for RSS, only moisture (12.76%) and ash (0.20%) contents were found to be higher than those from MS (10.71% and 0.15%, respectively), even though ash content was found to be statistically similar (p>0.05). Regarding CS, values for NFE (87.09%) and in a lesser extent, crude protein, crude fiber, and ether extract exhibited higher values (1.30, 0.01, 0.73%, respectively), compared with RSS (86.09, 0.56, 0.00, 0.39%, respectively), which were statistically different (p<0.05). The higher protein levels in CS are a result of the presence of zein, a water insoluble protein (Shukla and Cheryan, 2001).

When we compare results of RSS with those reported by Pérez-Pacheco *et al.* (2014), in which an alkaline extraction method was used, moisture (7.49%) and crude protein content (0.12%) were found to be lower compared to those obtained in this experiment. Meanwhile, crude fiber, ash, ether extract, and NFE (1.27, 0.47, 0.47, and 90.16%, respectively) presented higher values. Regarding CS, Pérez-Pacheco *et al.* (2014), using a reagent grade corn starch from Sigma-Aldrich, found lower values for all the proximal composition (8.14, 0.03, 0.49, and 0.02% for moisture, crude protein, ether extract, and ash, respectively), except crude fiber (1.24%). This indicates that the isolation method employed in this experiment resulted in a starch with low fiber, ash, and lipid content, ensuing an efficient extraction method of starch.

Table 1. Yield and chemical composition of ramon seed (*Brosimum alicastrum* Sw.) and maize (*Zea mays*) starches (%)

	Ramon seed starch	Maize starch
Yield	28.37±1.96 [a]	36.33±2.57 [b]
Moisture	12.76±0.47 [a]	10.71±0.40 [b]
Crude protein	0.56±0.04 [a]	1.30±0.04 [b]
Crude fiber	0.00 [a]	0.01±0.00 [b]
Ash	0.20±0.00 [a]	0.15±0.19 [a]
Ether extract	0.39±0.23 [a]	0.73±0.00 [b]
NFE	86.09±0.50 [a]	87.09±0.26 [b]

Values expressed as mean ± standard deviation (n=3).
NFE: nitrogen free extract
Different letters in the same row indicate statistical differences (p≤0.05)

Other authors have extracted starch using a water-only method, and they agree with the results found in the current experiment. For example, parota starch (Singla *et al.*, 2020), where moisture, ash, crude fiber, and lipid levels were found to be lower than acid and alkaline isolation methods. Meanwhile, CS isolated by Palacios-Fonseca *et al.* (2013), employing the same water-only isolation method a lower ash content was reported. As for the protein content, both authors reported higher values when the water-only extraction method was used. The method of extraction may be the principal influence in these differences, since an alkaline method helps to separate and solubilize protein (Estrada-León *et al.*, 2016).

Physicochemical properties

Physicochemical properties of RSS and CS are shown in Table 2. Apparent amylose content for RSS was lower (23.60%) than CS (27.61%) (p<0.05). On the other hand, amylopectin values were higher for CS than RSS (76.39 vs 72.38%, respectively, p<0.05). When comparing with results from Pérez-Pacheco *et al.* (2014), where an alkaline extraction method was used, RSS had a higher amylose content than the one obtained for this experiment; meanwhile, CS had similar values. Palacios-Fonseca *et al.* (2013), observed that the amylose content of CS decreased when using only a water extraction method, whereas alkaline isolation increased it up to 30.81%. This trend is also evident in cases where alkaline isolation methods have yielded higher amylose content (27.33-30.81%) for CS (Palacios-Fonseca *et al.*, 2013; Pérez-Pacheco *et al.*, 2014). This leads us to assume that the extraction method affects the apparent amylose content, as the current technique employed determines it by measuring lipid-complexes with amylose, influenced by the ether extract content, and yielding. It should be noted that values for apparent amylose for RSS had not been determined using a distilled water-only method before.

Table 2. Physicochemical characteristics of ramon seed (*Brosimum alicastrum* Sw.) and maize (*Zea mays*) starch.

Parameter	Ramon seed starch	Maize starch
Starch yield (%)	28.37±1.96 [a]	36.33±2.57 [b]
Amylose (%)	23.60±0.44 [a]	27.61±0.48 [b]
Amylopectin (%)	76.39±0.44 [a]	72.38±0.48 [b]
Amylose/amylopectin ratio	0.30±0.0	0.38±0.0
pH	6.04±0.05 [a]	4.20±0.17 [b]
L*	86.77±0.63 [a]	91.62±0.99 [b]
a*	2.28±0.12 [a]	-0.16±0.13 [b]
b*	15.22±0.65 [a]	6.07±0.05 [b]
Hue angle	81.49±0.15 [a]	91.52±1.20 [b]

Values expressed as mean ± standard deviation (n=3).
Values based on weight of seed without testa.
Different letters in the same row indicate statistically differences ($p \leq 0.05$)
L, lightness; a, red-green axis; b, yellow-blue axis.

As both starches from this experiment were isolated by steeping in distilled water, pH values were unaffected by an external alkaline or acidic solution. However, they resulted in pH values of 6.04 for RSS and 4.20 for CS ($p<0.05$). These values were found to be different compared with those reported by Pérez-Pacheco *et al.* (2014), where RSS presented a pH value of 9.1 and CS a value of 5.9 due to the isolation method employed (alkaline steeping with NaOH).

Colorimetric results show lower lightness L* value (86.77) for RSS compared with CS (91.62) ($p=0.0021$). Meanwhile, RSS obtained by Pérez-Pacheco *et al.* (2014) showed similar results for lightness L* (86.0) but CS exhibited a higher lightness L* value (95.8), which means that RSS and CS starches obtained from this experiment presented more impurities and a darker color. The values for a*, b* and hue angle of RSS and CS were different in all three parameters ($p=0.0001$, $p=0.0001$, $p=0.0001$, respectively). RSS obtained a higher value of a* and b* (2.28 and 15.22, respectively) compared to CS (-0.16 and 6.07, respectively), but lower hue angle (81.49 and 91.52, respectively), which indicates that RSS presented a redness-yellowness color, as for CS it was a yellow and white color. Other authors for the same starches have reported less redness- yellowness color for both RSS and CS starches (Pérez-Pacheco *et al.*, 2014).

Functional properties

When exposed to high temperatures, starch granules undergo changes in their crystalline and amorphous arrangement, their hydrogen bonds are replaced by water, and granules begin to hydrate. This process is called swelling power, and depending on its botanical source, amylose-amylopectin ratio, presence of lipid-starch complexes, granule size, and presence of pores and water channels, it can affect functional properties such as water holding capacity and solubility index. These properties are influenced by the type of polymorphic structures present in starch, which are related to the amount of amorphous and crystalline areas found in starch granules, and temperature of gelatinization (Moorthy, 2002; Cornejo-Ramírez *et al.*, 2018). As can be seen in Figure 1, solubility was lower for RSS (3.62%) at 70 °C compared to CS (5.78%). The same was observed for the swelling power (Figure 2) and water absorption capacity (Figure 3) at the same temperature (3.08 and 2.96 g water/g starch for RSS and 7.27 and 6.86 g water/g starch for CS). However, in the interval from 70 °C to 80 °C, values for these properties were inverted, and RSS reached higher values than CS. At 90 °C, both starches reached their higher solubility (18.14 and 7.43% for RSS and CS, respectively), swelling power (18.03 and 14.31 g water/g starch for RSS and CS, respectively), and water absorption capacity (14.76 and 13.28 g water/g starch for RSS and CS, respectively).

These differences may be attributed to the higher temperature of gelatinization present in RSS and its lower-amylose content, which makes the granule more thermostable at lower temperatures retarding swelling and granule disruption during processing (Chi *et al.*, 2021). Also, smaller granules have a higher swelling power capacity and solubility, which could explain the results found for RSS (Cornejo-Ramírez *et al.*, 2018; Moo-Huchin *et al.*, 2020).

Results for water absorption capacity and swelling power are in accordance with those reported by Pérez-Pacheco *et al.* (2014), as values for RSS were lower at 70 °C and by 80 °C they surpassed CS values. Due to the different extraction methods used by those authors and this experiment, and because factors such as amylopectin and lipid content affect their hydrothermal behavior, they could be involved in differences found in the solubility index for RSS at 70 °C.

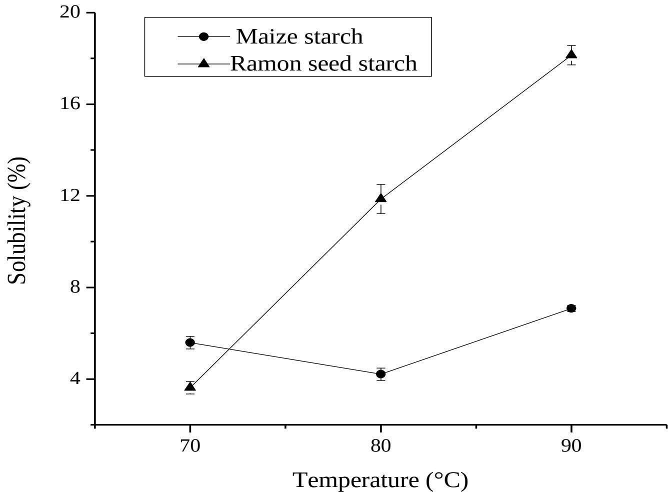

Figure 1. Solubility (%) of ramon seed and maize starches at 70, 80 and 90 °C.

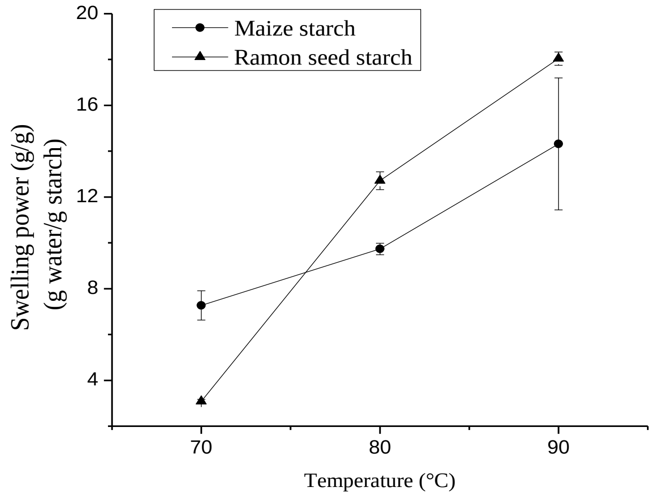

Figure 2. Swelling power pattern of ramon seed and maize starches at 70, 80 and 90 °C.

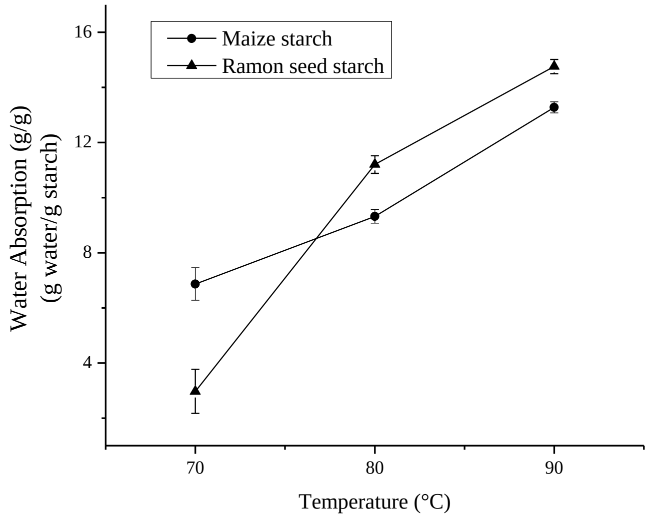

Figure 3. Water absorption capacity of ramon seed and maize starches at 70, 80 and 90 °C

Differential scanning calorimetry (DSC)

Using DSC, the gelatinization parameters, such as temperatures (onset, T_o; peak, T_p; and conclusion T_c), enthalpy of gelatinization (ΔH_{gel}), gelatinization temperature range (GEL_{TR}), and peak height index (PHI) for RSS and CS were analyzed and are shown in Table 3.

Table 3. Thermal properties of ramon seed (*Brosimum alicastrum* Sw.) and maize (*Zea mays*) starches.

Parameter	Ramon seed starch	Maize starch
T_o (°C)	77.41±1.24 ^{a}	66.81±1.29 ^{b}
T_p (°C)	80.38±0.71 ^{a}	73.14±0.20 ^{b}
T_c (°C)	84.53±0.45 ^{a}	77.06±0.21 ^{b}
ΔH_{gel} (J/g)	9.83±0.78 ^{a}	11.57±0.32 ^{a}
GEL_{TR} (°C)	5.94	12.66
PHI (J/g °C)	3.38	2.74

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH_{gel}, enthalpy of gelatinization; GEL_{TR}, gelatinization range $2(T_p - T_o)$; PHI, peak height index $\Delta H_{gel}/(T_p - T_o)$.
Values expressed as mean ± standard deviation.
Different letters in the same row indicate statistically differences ($p \leq 0.05$)

When starch is heated in the presence of water above the gelatinization temperature, the crystalline matrix begins melting, the molecular order is lost, and starch solubilization takes place. Gelatinized starch contains more fragile structures and is more susceptible to enzyme attack than native starch. Gelatinization of starch is associated with the breakdown of starch semi-crystalline structures during heating, especially the crystalline lamellae (Chi *et al.*, 2021; Donmez *et al.*, 2021).

Based on the results, thermal properties (To, Tp, Tc) of RSS had higher ($p<0.05$) values (77.41, 80.38, and 84.53 °C, respectively) compared to CS (66.81, 73.14, and 77.06 °C, respectively). Meanwhile, the enthalpy of gelatinization, which represents the energy required to disrupt the molecular arrangement within the granule, particularly in the crystalline region (9.83 J/g and 11.57 J/g, for RSS and CS, respectively), were not statistically different ($p>0.05$).

Comparing with results from Pérez-Pacheco *et al.* (2014), using an alkaline extraction method, RSS showed a lower T_o (75 °C) but a higher T_p (83 °C) and T_c (95 °C), and for CS, values for T_o and T_p (64 and 71.08 °C, respectively) were lower and T_c (80 °C) was higher. As for results from Pech-Cohuo *et al.* (2021), for RSS, where both an alkaline and a distilled water-only extraction method were used, all gelatinization temperatures were found to be lower.

When compared with other conventional starch sources, RSS and CS from this experiment had higher T_p compared to rice (61.53 °C) (De Souza *et al.*, 2016), potato (67 °C) (Ratnayake and Jackson, 2007), and cassava (69.5 °C) (Ratnayake and Jackson, 2007). It has been stated that a high gelatinization temperature indicates a higher amount of energy needed to gelatinize starch, as can be seen for RSS and CS. This could be due to differences in granule size, internal arrangement of starch fractions, and also because of variation in amylose content.

Gelatinization enthalpy results are in accordance with those reported by Pech-Cohuo *et al.* (2021), for RSS, as they indicated 10.58 J/g, and Palacios-Fonseca *et al.* (2013) mentioned 10.93 J/g for CS using the same water-only extraction method. PHI values are parameters used to determine the homogeneity and uniformity of the gelatinization; in this experiment they were found to be lower for CS (2.74) than for RSS (3.38). Meanwhile, as values for GEL_{TR} are higher for CS (12.66 °C), its higher amount of crystalline proportion needed more energy to melt compared with RSS (5.94 °C).

X-Ray diffraction pattern (XRD)

The X-ray diffraction patterns of RSS and CS are shown in Figure 4. RSS exhibited the highest XRD peaks for the 2θ- angles at 10°, 11°, 15°, 17°, 18° and 23°, corresponding to a C-type pattern, meanwhile, CS peaks were found at 2θ - 15°, 17°, 18° and 23°, which belong to an A-type polymorph pattern according to Junejo *et al.* (2022). As for the crystalline percentage, RSS had lower values (38.29%), when compared with CS (43.72%).

Results found in this experiment for the type of XRD pattern are in agreement with those reported by Moo-Huchin *et al.* (2015), where RSS presents a C-type diffraction pattern and CS an A-type. According to its peaks in the 2θ angles, there can be A, B and C-type diffraction patterns. A-type starches are found in cereals and have two strong diffraction peaks at 2θ- 15° and 23° and a doublet around 2θ- 17° and 18°. B-type starches can be found in tubers and high amylose starches and presents a characteristic peak at 2θ- 5.6°, a high peak at 2θ- 17° and small peaks at 2θ- 15°, 22° and 24°. C-type starches are found in legume seeds and rhizomes, and its diffraction pattern has a combination of both A- and B-type crystallinity (Pech-Cohuo *et al.*, 2021).

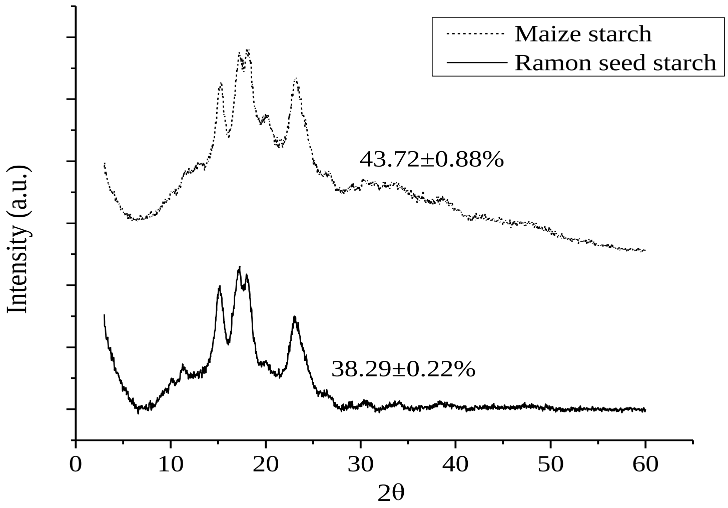

Figure 4. X-ray diffraction patterns of ramon seed and maize starches. Intensity is expressed in arbitrary units (a.u.).

When comparing the crystallinity values mentioned by Moo-Huchin *et al.* (2015), it was found that RSS had higher (30.56%) values when compared to CS (26.68%); nevertheless, both starches presented lower values compared with those obtained in this experiment. Palacios-Fonseca *et al.* (2013) stated that a positive correlation was observed between the crystallinity and the amylose content, suggesting that higher amylose content rises crystallinity values which was substantiated due to the high amylose and crystallinity values of CS (27.61 and 43.72%, respectively) compared with RS (23.60 and 38.29%, respectively) found in this experiment (Table 2), when compared with results from Moo-Huchin *et al.* (2020). It is believed that the extraction method did not influence the XRD pattern of both starches, however it had an effect on the amylose content, which correlates positively with the crystallinity values as mentioned before (Palacios-Fonseca *et al.*, 2013).

Starch structure is related to its enzymatic hydrolysis, A-type starch tends to have higher enzyme susceptibility due to the “weak points” within crystalline structures attributed to the shorter chains of amylopectin present. However, slowly digestible starch (SDS) is found in A-type crystalline structures (Lee and Moon, 2015). Meanwhile, B- and C-type starches show high and intermediate resistance to enzymatic hydrolysis, respectively, due to the well-defined polymorph structures. This type of starch diffraction patterns (B- and C-type), have been shown to have higher amount of resistant starch (RS) and SDS than A-type ones. This is also correlated with a higher proportion of long chains in the amylopectin molecule, increasing the number of hydrogen bonds among chains and producing higher enzymatic resistance (Shrestha *et al.*, 2012; Chi *et al.*, 2021; Magallanes-Cruz *et al.*, 2023).

CONCLUSIONS

RSS and CS starches obtained using a water-only extraction method were found to be different in their physicochemical characteristics, probably because of their distinct botanical sources. However, values for the chemical composition were within the expected range for the isolation method used. The extraction method employed did not affect both starches' functional, thermal, and structural properties. Further studies are required to determine whether the extraction method influences its enzymatic hydrolysis susceptibility. Additionally, future research should explore the potential for upscaling this green extraction approach and evaluate its applicability to other non-conventional starch sources.

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REFERENCES

AOAC, 1990. *Official Methods of Analysis of the Official Analytical Chemists*. 15th ed. Arlington, VA:AOAC.

BeMiller, J. and Whistler, R., 2009. Starch: Chemistry and Technology, 3ed. Ed. Elsevier.

Betancur-Ancona, David.A., Chel-Guerrero, L.A., Bello-Pérez, L.A. and Dávila-Ortiz, G., 2002. Isolation of Velvet Bean (Mucuna pruriens) Starch: Physicochemical and Functional Properties. *Starch/Stärke*, 54, pp.303–309. https://doi.org/10.1002/1521-379X(200207)54:7<303::AID-STAR303>3.0.CO;2-2

Carter, C.T. and Northcutt, J.K., 2023. Raw or roasted Brosimum alicastrum seed powder as a nutritional ingredient in composite sugar-snap cookies. *Cereal Chemistry*, pp.1–11. https://doi.org/10.1002/cche.10661

Chi, C., Li, X., Huang, S., Chen, L., Zhang, Y., Li, L. and Miao, S., 2021. Basic principles in starch multi-scale structuration to mitigate digestibility: A review. *Trends in Food Science and Technology*, 109, pp.154–168. https://doi.org/10.1016/j.tifs.2021.01.024

Cornejo-Ramírez, Y.I., Martínez-Cruz, O., Del Toro-Sánchez, C.L., Wong-Corral, F.J., Borboa-Flores, J. and Cinco-Moroyoqui, J., 2018. The structural characteristics of starches and their functional properties. *CyTA - Journal of Food*, 16(1), pp.1003–1017. https://doi.org/10.1080/19476337.2018.1518343

Donmez, D., Pinho, L., Patel, B., Desam, P. and Campanella, O.H., 2021. Characterization of starch–water interactions and their effects on two key functional properties: starch gelatinization and retrogradation. *Current Opinion in Food Science*, 39, pp.103–109. https://doi.org/10.1016/j.cofs.2020.12.018

Ecopura, 2018. *Technical data sheet: distilled water REDECOPURA*. [online] REDECOPURA México. Available at: https://www.redecopura.com.mx/ [Accessed 16 Jul. 2025].

Estrada-León, R.J., Moo-Huchin, V.M., Ríos-Soberanis, C.R., Betancur-Ancona, D., May-Hernández, L.H., Carrillo-Sánchez, F.A., Cervantes-Uc, J.M. and Pérez-Pacheco, E., 2016. The effect of isolation method on properties of parota (*Enterolobium cyclocarpum*) starch. *Food Hydrocolloids*, 57, pp.1–9. https://doi.org/10.1016/j.foodhyd.2016.01.008

El Halal, S.L.M., Kringel, D.H., Zavareze, E. da R. and Dias, A.R.G., 2019. Methods for Extracting Cereal Starches from Different Sources: A Review. *Starch/Stärke*, 71(11–12). https://doi.org/10.1002/star.201900128

Hernández-González, O., Vergara-Yoisura, S. and Larqué-Saavedra, A., 2015. Studies on the productivity of Brosimum alicastrum a tropical tree used for animal feed in the Yucatan Peninsula. *Wulfenia Journal*, 22(7), pp.206–217

Junejo, S.A., Flanagan, B.M., Zhang, B. and Dhital, S., 2022. Starch structure and nutritional functionality – Past revelations and future prospects. *Carbohydrate Polymers*, 277. https://doi.org/10.1016/j.carbpol.2021.118837

Kringel, D.H., El Halal, S.L.M., Zavareze, E. da R. and Dias, A.R.G., 2020. Methods for the Extraction of Roots, Tubers, Pulses, Pseudocereals, and Other Unconventional Starches Sources: A Review. Starch/Staerke, 72(11–12). https://doi.org/10.1002/star.201900234

Lee, C.J. and Moon, T.W., 2015. Structural characteristics of slowly digestible starch and resistant starch isolated from heat – moisture treated waxy potato starch. *Carbohydrate Polymers*, 125, pp.200–205. https://doi.org/10.1016/j.carbpol.2015.02.035

Losoya-Sifuentes, C., Pinto-Jimenez, K., Cruz, M., Rodriguez-Jasso, R.M., Ruiz, H.A., Loredo-Treviño, A., López-Badillo, C.M. and Belmares, R., 2023. Determination of Nutritional and Antioxidant Properties of Maya Nut Flour (Brosimum alicastrum) for Development of Functional Foods. *Foods*, 12(7). https://doi.org/10.3390/foods12071398

Magallanes-Cruz, P.A., Duque-Buitrago, L.F. and del Rocío Martínez-Ruiz, N., 2023. Native and modified starches from underutilized seeds: Characteristics, functional properties and potential applications. *Food Research International,* 169, p.112875. https://doi.org/10.1016/J.FOODRES.2023.112875

Miao, M. and Bemiller, J.N., 2023. Enzymatic Approaches for Structuring Starch to Improve Functionality. *Annual Review of Food Science and Technology*, 14, pp.271–295. https://doi.org/10.1146/annurev-food-072122

Minitab, 2022. *Minitab Statistical Software. V.21* USA. Disponible en: http://www.minitab.com/en-US/products/minitab/

Montfort-Grajales, S., Sarmiento-Franco, L.A., Urtecho-Novelo, R. and Sandoval-Castro, C.A., 2024. Estimation of metabolizable and digestible energy of ramon (*Brosimum alicastrum* Swartz) seed in broilers under tropical conditions. *Tropical and Subtropical Agroecosystems*, 27(2). https://doi.org/10.56369/tsaes.4775

Moo-Huchin, V.M., Ac-Chim, D.M., Chim-Chi, Y.A., Ríos-Soberanis, C.R., Ramos, G., Yee-Madeira, H.T., Ortiz-Fernández, A., Estrada-León, R.J. and Pérez-Pacheco, E., 2020. Huaya (Melicoccus bijugatus) seed flour as a new source of starch: physicochemical, morphological, thermal and functional characterization. *Journal of Food Measurement and Characterization*, 14(6), pp.3299–3309. https://doi.org/10.1007/s11694-020-00573-3

Moo-Huchin, V.M., Cabrera-Sierra, M.J., Estrada-León, R.J., Ríos-Soberanis, C.R., Betancur-Ancona, D., Chel-Guerrero, L., Ortiz-Fernández, A., Estrada-Mota, I.A. and Pérez-Pacheco, E., 2015. Determination of some physicochemical and rheological characteristics of starch obtained from Brosimum alicastrum Swartz seeds. *Food Hydrocolloids*, 45, pp.48–54. https://doi.org/10.1016/j.foodhyd.2014.11.009

Moorthy, S.N., 2002. Physicochemical and Functional Properties of Tropical Tuber Starches: A Review. *Starch/Stärke*, pp.559–592. https://doi.org/10.1002/1521-379X(200212)54:12<559::AID-STAR2222559>3.0.CO;2-F

Morrison, W.R. and Laignelet, B., 1983. An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. *Journal of Cereal Science*, 1(1), pp.9–20. https://doi.org/10.1016/S0733-5210(83)80004-6

Olguin-Maciel, E., Larqué-Saavedra, A., Pérez-Brito, D., Barahona-Pérez, L.F., Alzate-Gaviria, L., Toledano-Thompson, T., Lappe-Oliveras, P.E., Huchin-Poot, E.G. and Tapia-Tussell, R., 2017. Brosimum alicastrum as a novel starch source for bioethanol production. *Energies*, 10(10), pp.1574. https://doi.org/10.3390/en10101574

Palacios-Fonseca, A.J., Castro-Rosas, J., Gómez-Aldapa, C.A., Tovar-Benítez, T., Millán-Malo, B.M., Del Real, A. and Rodríguez-García, M.E., 2013. Effect of the alkaline and acid treatments on the physicochemical properties of corn starch. *CYTA - Journal of Food,* 11(SUPPL.1), pp.67–74.

https://doi.org/10.1080/19476337.2012.761651

Pech-Cohuo, S.C., Hernandez-Colula, J., Gonzalez-Canche, N.G., Salgado-Transito, I., Uribe-Calderon, J., Cervantes-Uc, J.M., Cuevas-Bernardino, J.C., Ayora-Talavera, T. and Pacheco, N., 2021. Starch from Ramon seed (Brosimum alicastrum) obtained by two extraction methods. *MRS Advances*, 6(38), pp.875–880. https://doi.org/10.1557/s43580-021-00134-w

Pérez-Pacheco, E., Moo-Huchin, V.M., Estrada-León, R.J., Ortiz-Fernández, A., May-Hernández, L.H., Ríos-Soberanis, C.R. and Betancur-Ancona, D., 2014. Isolation and characterization of starch obtained from Brosimum alicastrum Swarts Seeds. *Carbohydrate Polymers*, 101(1), pp.920–927. https://doi.org/10.1016/j.carbpol.2013.10.012

Ratnayake, W.S. and Jackson, D.S., 2007. A new insight into the gelatinization process of native starches. *Carbohydrate Polymers*, 67(4), pp.511–529. https://doi.org/10.1016/j.carbpol.2006.06.025

Shrestha, A.K., Blazek, J., Flanagan, B.M., Dhital, S., Larroque, O., Morell, M.K., Gilbert, E.P. and Gidley, M.J., 2012. Molecular, mesoscopic and microscopic structure evolution during amylase digestion of maize starch granules. *Carbohydrate Polymers*, 90(1), pp.23–33. https://doi.org/10.1016/j.carbpol.2012.04.041

Shukla, R. and Cheryan, M., 2001. Zein: the industrial protein from corn. *Industrial Crops and Products*, 13(3), pp.171–192. https://doi.org/10.1016/S0926-6690(00)00064-9

Singla, D., Singh, A., Dhull, S.B., Kumar, P., Malik, T. and Kumar, P., 2020. Taro starch: Isolation, morphology, modification and novel applications concern - A review. *International Journal of Biological Macromolecules,* 163, pp.1283–1290. https://doi.org/10.1016/j.ijbiomac.2020.07.093

De Souza, D., Sbardelotto, A.F., Ziegler, D.R., Marczak, L.D.F. and Tessaro, I.C., 2016. Characterization of rice starch and protein obtained by a fast alkaline extraction method. *Food Chemistry*, 191, pp.36–44. https://doi.org/10.1016/j.foodchem.2015.03.032

Wang, Y., Ral, J.-P., Saulnier, L. and Kansou, K., 2022. How Does Starch Structure Impact Amylolysis? Review of Current Strategies for Starch Digestibility Study. *Foods,* 11(9), p.1223. https://doi.org/10.3390/foods11091223

Zhang, Z., Saleh, A.S.M., Wu, H., Gou, M., Liu, Y., Jing, L., Zhao, K., Su, C., Zhang, B. and Li, W., 2020. Effect of Starch Isolation Method on Structural and Physicochemical Properties of Acorn Kernel Starch. *Starch/Staerke*, 72(1–2). https://doi.org/10.1002/star.201900122