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# Determination of the nutritional and antioxidant properties of the Maya nut flour (*Brosimum alicastrum*) for the development of functional foods

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**Abstract:** In Mexico, the Ramon tree (*Brosimum alicastrum*) has historical relevance due to the nutritional importance it had within the Mayan culture. Its seed is used to produce flour for the preparation of various foods. The objective of this study was determinate nutritional properties and the antioxidant activity of the Maya nut flours for the production of functional foods. The different drying methods: natural (NF), dehydrater at 45°C (F45) and 60°C (F60). All Maya nut flours were sources of carbohydrates, protein and fiber, as well as potassium, calcium, iron, and zinc. F45 produced a whiter Maya nut meal ($L^*$= 82.44) compared to the other methods. The different drying methods used here did not affect the morphology of the native starch of the Maya nut flour, nor were important molecular-structural changes generated. However, it does affect the nutritional and functional quality of the flours, this effect is observed in C[illegible]t presented the highest content of total polyphenols (13.35 mgGAE/g) and antioxidant activity by DPPH tests (29.84 μM TEAC/g). Maya nut cookies have antioxidant activity and higher levels of fiber than commercial ones, resulting in a functional product, which could replace some products in the market due to its benefits.

**Keywords:** drying methods; Maya nut flour; physicochemical properties; antioxidant activity

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## 1. Introduction

According to historical data, the Mayas cultivated *Brosimun alicastrum* to use its seed as food in their diet, despite this, it is considered a little exploited plant with high economic potential. Currently, its wood, leaves, fruits, and latex have different uses, as well as a food source and in traditional medicine [1]. The Ramon tree produces a sweet-tasting fruit with a single seed. Also, the seed is known as the Maya nut [2]. These seeds, cooked or roasted, are edible and are consumed alone, mixed with corn, or sweetened with honey to prepare various foods and hot drinks [3].

The Maya nut and flour obtained are a source of protein, dietary fiber, micronutrients and functional compounds that can work as an enriching ingredient in foods with little nutritional value or for new formulations [4].

Scientific information on the nutritional composition of *B. alicastrum* seeds has shown contents of 10.49% fat, 5.21% crude fiber and 2.02% crude protein, in addition to minerals such as copper, potassium, iron, zinc and sodium [4, 5].

Recently, phenolic compounds (gallic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid and p-coumaric acid), which give the antioxidant capacity of Maya nut, have been identified [5]; therefore, these seeds can be considered a functional ingredient [6].

Different chemical, physicochemical or mechanical processes can affect these types of compounds, so in the production of flours, an important aspect to consider to maintain the nutritional quality is the drying process. Drying has been one of the options for preservation, reducing moisture content, the growth of microorganisms and minimizing the chemical deterioration of some foods. However, this process leads to physicochemical changes that influence the color, flavor, texture and overall quality of the dried product [7]. Moreover, roasting degrades phenols and thus decreases the antioxidant activity of a food. The lack of information on the effect of drying on the physicochemical and nutritional properties of Maya nut poses the necessity to carry out further studies.

Nowadays, with improvements in living standards and lifestyle, consumers no longer demand foods that only fulfil necessary nutrients, but desire foods with functional and nutraceutical properties [8]

Cookies may be the right product to meet health-promoting dietary demands [9]. Therefore, through the application of bioactive components, the production of novel cookies with enhanced nutritional value is the result of a growing interest.

The objective of this research was to assess the effect of drying methods on nutritional properties and antioxidant activities and to explore its functionality by developing cookies with a higher health impact due to their fiber and protein concentration, which is directly related to possible applications in foods with beneficial properties.

## 2. Materials and Methods

### *2.1. Obtaining the seeds*

The community of Nuevo Antonio, municipality of Venustiano Carranza, in the state of Chiapas in Mexico provided two types of seeds, which were naturally sun-dried seeds stored for six months and seeds collected in March 2021. Leaves or branches were discarded as well as spoiled seeds. The seed was husked and disinfected with a sodium hypochlorite solution at 200 ppm. In addition to the seeds mentioned above, Maya nut flour was purchased from Productos Orgánicos La Selva, from the Ruez catalog, produced in the community of Tres Garantias, Quintana Roo, Mexico.

### *2.2. Physical characteristics of the Maya nut*

#### 2.2.1 Measurement of seed dimensions

Physical measurements of Maya nut were determined as described by Joshi et al [10] with modifications. The weight of the seed was determinate on an OHAUS analytical balance with an accuracy of 0.001 g of 25 seeds from each group. Their main dimensions, length (L), width (W) and thickness (T), were measured using a vernier caliper. The determination of mean diameters (Da), geometric diameters (Dg) and sphericity (ψ) were performed by adopting the equations of Palilo et al. [11]:

$$D_a = \left(\frac{L + W + T}{3}\right) \quad (1)$$

$$D_g = (LWT)^{1/3} \quad (2)$$

$$\Psi = \left(\frac{D_g}{L}\right) * 100 \quad (3)$$

### *2.3. Drying and flour preparation*

Three methods of drying were compared to obtain Maya nut flour. The first method was natural drying [4]. The seeds were collected and left on rectangular platforms exposed to the sun for five days and stored in bottles until further use.

For the second and third drying methods, an electric food dehydrator (Excalibur 3926TB) was used at 45 °C for 27 h and at 60 °C for 24 h, respectively [12,13], the dried seeds were allowed to cool to 25 °C and stored in bottles until further use.

Flour preparation: The dry seeds were ground in a high-speed Multifunction GRINDER and sieved on a 100 mesh in Cole-Parmer equip. Finally, the flours were kept in airtight stand up pouch and stored in a dry place until further analysis.

The flours obtained were named as follows: natural dried flour (NF), dried at 45 ºC flour (F45), dried at 60 ºC flour (F60) and commercial flour (CF), made from seeds roasted at 180 °C for half an hour.

### *2.4. Proximal Composition of Maya nut flours*

The nutritional analysis of the Maya nut flours was conducted as stated by the following Association of Official Analytical Chemists [14] fat (AOAC 945.16), ash (AOAC 920.181), crude fiber (AOAC 962.09), and total protein (total nitrogen∗6.25) (AOAC 978.02). Then, the carbohydrates were quantified by the difference.

### *2.5 Mineral Content*

X-ray fluorescence spectrometry (XRF) is a non-destructive analytical technique used to obtain elemental information on different types of materials. 1 g of the flours were placed in the energy dispersive X-ray fluorescence spectrometer (ED-XRF) (Epsilon 1, Malvern Panalytical, Madrid, Spain) to determine the mineral content of each flour. The mineral content analysis was performed using the Omnian® software in a period of 20 min per sample.

### *2.6. Techno-Functional properties of Maya nut flours*

#### 2.6.1 Water activity

The water activity of all the flours was measured using a water activity meter (AquaLab series 3 Decagon devices) at 24.7 ±1°C.

#### 2.6.2 Water and oil holding capacity

Measured functional properties included water-holding capacity (WHC) and oil-holding capacity (OHC). A gravimetric method determined the capacity to absorb water or oil from the flours. 0.1 g of each flour was weighed and poured into a 50 ml Falcon centrifuge tube; after that, was added 10 mL of distilled water or corn oil. The suspensions were vigorously mixed using a Vortex stirrer and allowed to stand at room temperature (25°C) for 24 hours. Samples for water or oil absorption were centrifuged at 3000 rpm for 20 min. The supernatant was decanted, and the sediment was reweighed. The weight change was expressed as percentage of water or oil absorption based on the weight of the original sample, calculated based on the following equation [15]:

$$WAC/OAC\ (\%) = \left(\frac{W_2 - W_1}{W_1}\right) \times 100\% \quad (3)$$

Where WAC/OAC is the water/oil absorption capacity of the flours (%), $W_1$ and $W_2$ are the initial and final weight of the sample (g).

### *2.7. Color of Maya nut flours*

The Maya nut flours were poured into Petri dishes and a 10 mm thick layer was formed. A colorimeter (3nh NR110 Precision Colorimeter) measures the $L^*$, $a^*$ and $b^*$ pa-

rameters of the International Commission on Illumination (CIE) of all samples. Corresponding *L** value (lightness of color from zero (black) to 100 (white); $a*$ value (degree of redness (0–60) or greenness (0 to −60); and $b*$ values (yellowness (0–60) or blueness (0 to −60) were measured for all the samples. Tree readings were taken at different locations on the surface of the samples to obtain the mean values of *L*, a*, and b*.*

### *2.8. Morphological characteristics of Maya nut flours*

A tabletop scanning electron microscope was used to observe the microstructure of Maya nut flour (TM3000, Hitachi, Ltd., Tokyo, Japan). The images were taken at an acceleration voltage of 15 kV and a magnification of 2000.

### *2.9. X-Ray diffraction of Maya nut flours*

The X-ray diffractometer (Panalytical Empyrean, Netherlands) generated a diffractogram with an interval of 2θ angles ranged from 7° to 50 °C, at a rate of 4.3°/min operating at a power of 40 kV/30 mA.

### *2.10. ATR-FTIR Spectroscopy*

A PerkinElmer Frontier spectrometer with a universal attenuated total reflection (ATR) determined the main functional groups in the flour and worked in in transmittance mode with a resolution of 4 cm-1 in the region of 4000 to 600 cm-1 at room temperature.

### *2.11. Total polyphenol content and antioxidant activity*

#### 2.11.1. Extraction

The extraction of total polyphenols and antioxidant components were performed in the dark at room temperature, and 100 mg of sample was weighed and mixed with 5 ml of ethanol/water (1:1 v/v) according to Moo-Huchin et al. [6]. The suspension was allowed to stand for 24 hours in the dark. Subsequently, the samples were placed in ultrasound bath equipment at 25 °C for 10 min. The extracts were then centrifuged at 4500 g at 25 °C for 10 min and the supernatant was recovered and stored in the refrigerator, hidden from light, until analysis [16].

#### 2.11.2. Total phenolic content

The content of total polyphenols was estimated using the Folin-Ciocalteu procedure according to the method suggested by Goiris et al [17] with modifications. 200 µL of the obtained extracts (2.10.1) were mixed with 1.5 mL of Folin Ciocalteu reagent (previously diluted ten times with distilled water) and allowed to stand at room temperature for 5 minutes. Then 1.5 ml of sodium bicarbonate solution (60 g L-1) was added. After a 90 min incubation at room temperature, the absorbance at 750 nm was measured in a spectrometer (Epoch™ microplate, Biotek, Winooski, VT, USA). A standard curve of gallic acid was prepared from 0 to 400 ppm in 50% methanol. Phenolic content was expressed in gallic acid equivalents per g of sample (mg GAE/g).

#### 2.11.3. Evaluation of antioxidant activity

The antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS±), and ferric reducing antioxidant power (FRAP) methods, described by González et al., [16] with slight modifications. In each test, trolox was used as a reference, and the results obtained were expressed as Trolox equivalents per gram of flour.

The DPPH assay was performed as follows, a 60 μM solution of DPPH reagent was prepared with methanol. Then, 7 μL of each extract was taken with 193 μL of DPPH solution at the desired absorbance (0.7 ± 0.02 ) and kept in the dark for 30 min. The absorbance was determined at 517 nm.

To determine antioxidant activity by ABTS, the reagent was prepared. ABTS solution at 7mM concentration was prepared with ethanol and mixed with 2.45mM $K_2S_2O_8$ (1:1 v/v). This ABTS solution was allowed to stand for 16 hours in the dark at room temperature (25 °C ± 2). This ABTS solution was diluted with ethanol to an absorbance of 0.7 ± 0.02 at 734 nm. 5 μL of each sample and 95 μL of ABTS± solution was placed in a microplate. Solutions rested for 1 min before reading at 734 nm.

In the case of the FRAP assay, the FRAP solution was obtained by mixing TPTZ (2,4,6-Tripyridyl-s-triazine) at 10 mM, $FeCl_3$ (20 mM) and acetate buffer (0.3 M, pH 3.6) in the proportions of 1:1:10 v/v/v. Sample solutions (10 μL) were added to the microplate, followed by 290 μL of the FRAP solution. Subsequently, the samples were incubated in the dark at 37 °C for 15 min. The absorbance was measured at 593 nm.

### *2.12. Development of Maya nut Cookies*

Natural dried flour (NF) was used for the development of the formulations because it has better nutritional and functional properties.

The ingredients (Table 1) were incorporated for 10 min at medium speed in a Blazer mixer to obtain a homogeneous mixture. The dough was rolled out to 3 mm thickness, and cut into 5 cm diameter circles. Bake at 180 °C for 15 min in a San-Son HCX PLUS3 convection oven. Cool to 35 °C and pack.

**Table 1.** Formulation of Maya nut cookies

| Ingredients | Quantities (g) | | | |
|---|---|---|---|---|
| | **F0** | **F20** | **F40** | **F60** |
| *Maya nut flour* | 0 | 20 | 40 | 60 |
| *Wheat flour* | 100 | 80 | 60 | 40 |
| Milk | 45 | 45 | 45 | 45 |
| Butter | 30 | 30 | 30 | 30 |
| Honey | 11 | 11 | 11 | 11 |
| Egg | 6 | 6 | 6 | 6 |
| Walnut | 3 | 3 | 3 | 3 |
| Cranberries | 3 | 3 | 3 | 3 |

### *2.13. Statistical analysis*

All experiments were carried out at least in triplicate and results were reported as the average ± standard deviation (SD). The statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's test with a significance level of 5 %, using INFOSTAT software version 2018 for Windows (Córdoba, Argentina).

## 3. Results and Discussion

### *3.1. Physical characteristics of the Maya nut*

The seeds correspond to dicotyledonous seeds, as seen in fig. 1, the seeds are small and have spherical shape, covered by a fine yellowish peel. The seeds are dark brown and light green on the inside. The minimum, maximum and mean of Maya nut length, width, thickness, weight, geometric mean diameter, and sphericity are presented in Table 1.



**Figure 1.** Ramon tree seeds that is also called Mayan nut.

**Table 2.** Physical characteristics of fresh Maya nut.

| Parameter | Mean | Minimum | Maximum |
|---|---|---|---|
| **Weight (g)** | 1.71 ± 0.27 | 1.27 | 2.20 |
| **Length (mm)** | 18.01±1.05 | 15.20 | 20.00 |
| **Width (mm)** | 13.00 ± 0.10 | 11.01 | 15.10 |
| **Thickness (mm)** | 17.34 ± 0.81 | 14.10 | 19.40 |
| **Mean diameter** | 16.83 ± 0.82 | 15.83 | 17.47 |
| **Geometric mean diameter (mm)** | 16.62 ± 1.20 | 15.71 | 17.27 |
| **Sphericity (%)** | 90.83 ± 3.99 | 86.35 | 93.88 |

*Brosimum alicastrum* grows in areas with average annual temperatures of 18 to 27 °C, with annual rainfall of 600 mm (Tamaulipas) and up to 4,000 mm (Chiapas and Tabasco) [18] making it an interesting alternative for the development of new beneficial products.

*3.2. Proximal composition of Maya nut flours.*

After drying and grinding the Maya nut, the NF, F45, F60 and CF flours were obtained. The moisture content and chemical composition of the developed flours are shown in Table 2.

**Table 2.** Nutrimental composition and mineral content of Maya nut flours.

| Parameter | NF | F45 | F60 | CF |
|---|---|---|---|---|
| **Moisture (%)** | 9.32 ± 0.19$^{b}$ | 6.05 ± 0.36$^{a}$ | 5.92 ± 0.45$^{a}$ | 6.51 ± 0.29$^{a}$ |
| **Ashes (g/100 g)** | 3.64 ± 0.11$^{b}$ | 3.08 ± 0.01$^{a}$ | 3.25 ± 0.29$^{ab}$ | 3.13 ± 0.02$^{a}$ |
| **Protein (g/100 g)** | 6.35 ± 0.44$^{c}$ | 4.48 ± 0.07$^{a}$ | 4.93 ± 0.40$^{ab}$ | 5.63 ± 0.36$^{b}$ |
| **Fat (g/100 g)** | 1.26 ± 0.11$^{a}$ | 1.52 ± 0.27$^{a}$ | 1.24 ± 0.07$^{a}$ | 1.62 ± 0.73$^{a}$ |
| **Total Carbohydrates (g/100 g)** | 73.83 ± 0.23$^{a}$ | 84.87 ± 0.11$^{c}$ | 84.67 ± 1.06$^{bc}$ | 83.10 ± 0.59$^{b}$ |
| **Crude Fiber (g/100 g)** | 6.75 ± 0.29$^{a}$ | 5.30 ± 0.19$^{b}$ | 5.49 ± 0.16$^{b}$ | 4.17 ± 0.46$^{c}$ |
| **Energy (Kcal)** | 354.46 ± 0.43$^{a}$ | 371.07 ± 2.78$^{b}$ | 369.52 ± 2.75$^{b}$ | 369.51 ± 4.55$^{b}$ |
| Mineral content (mg/100g) | | | | |
| **Potassium** | 2608.81± 0.10 $^{d}$ | 2104.01 ± 0.10$^{a}$ | 2252.31 ± 0.02$^{c}$ | 2185.46 ± 0.03$^{b}$ |
| **Calcium** | 829.08 ± 0.02$^{b}$ | 797.35 ± 0.16$^{b}$ | 818.27 ± 0.05$^{ab}$ | 755. 15 ± 0.01$^{a}$ |
| **Iron** | 60.23 ± 0.10$^{b}$ | 15.56 ± 0.07 $^{a}$ | 18.47 ± 0.10$^{a}$ | 16.18 ± 0.02$^{a}$ |
| **Zinc** | 10.09 ± 0.03$^{b}$ | 6.01 ± 0.20$^{a}$ | 7.89 ± 0.35$^{a}$ | 11.67 ± 0.35$^{b}$ |

Mean ±SD. All values are presented on dry weight basis; NF- natural flour, F45- flour drying at 45° C, F60- Flour drying at 60° C y CF-commercial flour.
Differents letters indicate significant difference ($p$<0.05).
[a] Total carbohydrate were determined by difference method.

The moisture of the flours was 9.32% para NF, while for F45 (6.05%) and F60 (5.92%) the moisture decreased, which means that the drying method directly affects this parameter. Low moisture values could result in a stable product for long-term storage with impact on quality improvement and stability of the product [19].

The ash content of the flours was highest for NF (3.64 ± 0.11g/100 g), while the lowest was found in F45 (3.08 ± 0.01 g/100 g). The highest protein value was also recorded for NF (6.35 ± 0.44 g/100 g). The lowest protein content was for flour F45 (4.48 ± 0.07 g/100 g). There were no significant differences between crude fat values, from 1.24 to 1.62 g/100 g. The highest carbohydrates value was for F45 (84.87 ± 0.11 g/100 g), higher than CF.

These results obtained on the composition of the Maya nut of all the flours differ from those of other authors. Subiria-Cueto et al., [4] reported a higher protein content (11.5 ± 0.39%), and lower values of fat (0.6 ± 0.00 %), total carbohydrates (71.2 ± 0.56%) and crude fiber (3.4 ± 0.13%) than those found in this investigation. In comparison, Pérez-Pacheco et al.[13] reported lower values for protein (2.02 ± 0.06%) and higher values for crude fat (10.49 ± 1.67%). To date, few investigations have reported the chemical and nutritional composition of Maya nut, but our results demonstrate the importance of the drying process to which the Maya nut is subjected. It is clearly observed that natural drying increases the protein concentration.

Traditionally in some regions of Mexico, Maya nut flour has been mixed with other flours, such as corn flour [20]. In this way, foods such as tortillas have been prepared, which have been shown to be a nutritionally enriched product [4]. This is because cereals such as corn and wheat are deficient in essential amino acids and especially in dietary fiber [21].

In addition, the results of an analysis of the mineral content by means of XRF in Maya nut flour are reported for the first time. At least 15 minerals were detected in the samples evaluated but the most abundant minerals are presented in Table 2. Mayan nut flours contained mainly potassium (2608.81± 0.10 mg/100g) and calcium (829.08 ± 0.02 mg/100g), which are two of the most important macrominerals in the daily diet [22] the iron was the third most abundant (60.23 ± 0.10 mg/100). Other essential elements found in the samples were zinc, manganese, sulfur, chlorine and copper. Subiría et al., recorded a lower content of potassium, iron, zinc and sodium, surely this is due to the different techniques used in the determination of these minerals [4].

### *3.3. Techno-Functional properties*

Table 3 shows that the water activity (Aw) of the Maya nut was affected by the drying treatment to which it was subjected, with the natural flour having the highest Aw 0.416 ± 0.01, and as the drying temperature increased, this Aw decreased, water content and Aw are two important factors affecting food safety [19].

The water absorption capacity (WAC) and oil absorption capacity (OAC) of Maya nut flours are also presented in Table 3. The observed WAC values ranged from 189.39 ± 10.35% for F45 to 220.32 ± 7.58% for NF flour.

It was shown that all Maya nut flours presented higher WAC (189.39 to 220%) than whole wheat flour (103.36%), amaranth (136.64%), soybean flour (202.26%), lupine flour (155.17%) and corn starch (106.05%) reported by Liu et al., [23]. This ability of flour to absorb water is primarily due to its composition, mainly the content of hydrophilic compounds, such as carbohydrates, proteins, and dietary fiber [24].

OAC is another critical property in ingredients intended to be incorporated into formulations with a high percentage of fat, to facilitate emulsification [25]. The lowest OHC values were found in the flours dried at F45 and F60 (153.22 ± 3.08% and 157.27 ± 7.94 &, respectively), compared to flour NF (173.34 ± 6.46%) and CF (185 ± 4.96%). These values were higher than the OHC of wheat flour (146.00 ± 8.94%) recorded by Chandra et al [25]. Adding components with higher WHC and OHC values to food formulations can contribute to improving product properties, such as viscosity, stability and texture [26].

**Table 3.** Techno-Functional properties of Maya nut flours.

| Parameter | NF | F45 | F60 | CF |
|---|---|---|---|---|
| WAC (%) | 220.32 ± 7.58$^{b}$ | 189.39 ± 10.35$^{a}$ | 204.05 ± 4.02$^{ab}$ | 203.101 ± 6.07$^{ab}$ |
| OAC (%) | 173.34 ± 6.46$^{b}$ | 153.22 ± 3.082$^{a}$ | 157.27 ± 7.94$^{a}$ | 185.08 ± 4.96$^{b}$ |
| Water activity (Aw) | 0.416 ± 0.01 | 0.320 ± 0.01 | 0.280 ± 0.01 | 0. 380 ± 0.01 |

Mean ±SD. NF- natural flour, F45- flour drying at 45 °C, F60- Flour drying at 60 °C y CF-commercial flour. WAC (water absorption capacity), OAC (oil absorption capacity). Differents letters indicate significant difference ($p$<0.05).

### *3.4. Color*

Table 4 shows the results of the color of the flours obtained. The color of the flours was significantly affected by the drying treatments to which the Maya nut were subjected. For example, the flours obtained from unroasted seeds were lighter and with fewer reddish and yellowish pigments than the flour developed from roasted seeds or commercial flour (CF). The values of $L^*$ (lightness) decreased from 82.44 in the F45 flour to 58.12 in the CF flour, the darkest. The redness (+value $a^*$) and the yellowness (+value $b^*$) of the CF sample were significantly higher (13.40 and 27.36) than all the other flours, being F45 and F60, the ones that presented the lowest value in these parameters and without significant differences between them (2.04,17.70 and 3.38,17.46, respectively). A darkening of flours obtained from roasted grains and cereals has been observed before [27]. The higher values of a* and $b^*$ in CF flour could be attributed to the Maillard reaction caused by chemical reactions between sugars and protein in the Maya nut during roasting [28], for this reason it is better to use temperature-controlled drying which does not affect the color parameters. In addition, the Maillard reaction generates brown-colored compounds, such as melanoidins, which could also affect the antioxidant properties of foods exposed to high temperatures, since it has been observed that toasting increases the antioxidant capacity of foods [29].

**Table 4.** Color parameter of Maya nut flours.

| Parameter | NF | F45 | F60 | CF |
|---|---|---|---|---|
| $L^*$ | 68.09 ± 1.33$^{b}$ | 82.44 ± 0.27$^{d}$ | 75.73 ± 2.20$^{c}$ | 58.12 ± 1.08$^{a}$ |
| $a^*$ | 6.41 ± 0.38$^{b}$ | 2.04 ± 0.05$^{a}$ | 3.38 ± 1.06$^{a}$ | 13.30 ± 0.12$^{c}$ |
| $b^*$ | 19.07 ± 0.72$^{b}$ | 17.60 ±0.04$^{a}$ | 17.46 ± 0.76$^{a}$ | 27.36 ± 0.19$^{c}$ |

Mean ±SD. NF- natural flour, F45- flour drying at 45 °C, F60- Flour drying at 60 °C y CF-commercial flour. Differents letters indicate significant difference ($p$<0.05).

### *3.5. Morphological characteristics*

Figure 2 shows the scanning electron micrographs of the Maya nut flours: NF (a), F45 (b), F60 (c), and CF (d). The particles observed in all the flours were round, oval and irregular, it follows that these particles correspond to starch granules. These particles were expected to be observed since Maya nut flour has been reported to have a high starch content (61%) [13]. These particles were observed independent and exposed, contrary to those observed by Olguin-Maciel et al., [30] who observed starch granules trapped in networks of protein or fibers. Large agglomerations were not observed either and the surfaces are smooth and uniform, with some presence of proteins.

The size of the granules was heterogeneous, with an average diameter ranging from 7 to 10 μm. Lindeboom, Chang, and Tyler [31] categorized the size of starch granules as large (>25 μm), medium (10-25 μm), small (5-10 μm), and very small (<5 μm). The size of the granules found in this study are classified as small. The size of the granules was similar

to those reported in Maya nut flour and Maya nut starch by Pech-Cohuo et al [32] and Pérez-Pacheco et al., [13].

The temperatures during the drying of the seeds did not seem to affect the structure of the amylose, which would have caused it to alter and transform the oval structure of the starch granule into an amorphous structure or that would implode [33]. The presence of gels is not observed, which reveals that the drying methods did not cause the gelatinization of the starch present in flours of Maya nut[34].

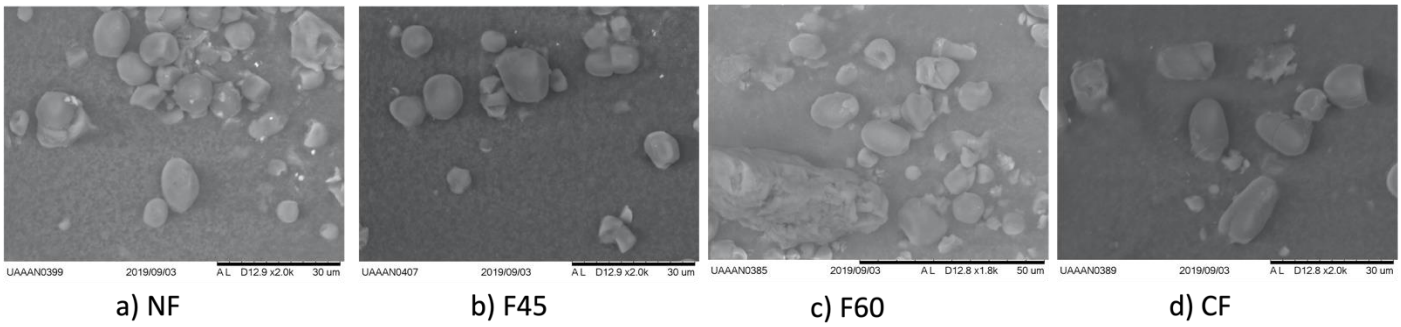



**Figure 2.** Scanning electron micrographs of a) NF, sun-dried flour, b) F45, flour dryer at 45 °C, c) F60, flour dryer at 60 °C d) CF, commercial flour.

### *3.6. X-ray diffraction analysis*

Figure 3 shows the XRD patterns of Maya nut flours obtained from seeds dried at different temperatures and conditions. In plants, starch is stored as a discrete semicrystalline granule and consists of two main components: linear amylose and highly branched amylopectin. According to their X-ray diffraction (XRD) patterns, there are starch polymorphs of type A (mainly in cereal starches), B (found in tubers, and C (A-type and B-type polymorphs) [35]. All Maya nut flours exhibited a starch with type C crystals. In Maya nut flours, the XRD peaks with the highest intensity for the 2θ angles were 15°, 17°, 18° and 23°, and minor peaks were also observed at 20° and 26°. The drying temperatures in the seeds did not alter the flour´s crystallinity structure, but it did reduce the diffraction intensity, as shown by the shape and size of the crystalline peaks [19].

Hay Moo-Huchin et al., [36] mention that the crystallinity is exclusively associated with the packing of the amylopectin double helices, while the amorphous regions represent mainly amylose.

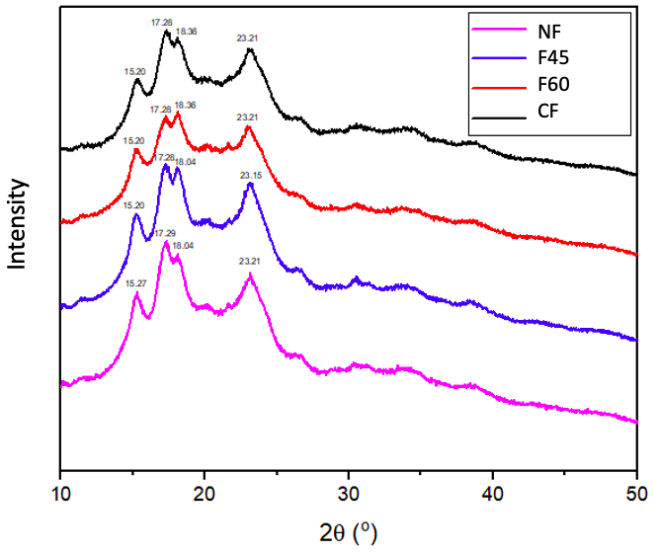


**Figure 3.** X-ray diffraction (XRD) diagrams of Maya nut flours under different drying treatments. NF- natural flour, F45- flour drying at 45 °C, F60- Flour drying at 60 °C y CF-commercial flour.

### *3.7. ATR-FTIR spectroscopy*

The effect of the different drying methods on the changes in the molecular properties of the obtained flours was determined by means of a spectroscopic analysis of FTIR bands. The signals showed similarity in the position of the bands obtained between the spectra of the flours samples, so the drying method of the seeds did not affect the molecular structure of the flours and did not generate different compounds in them. The FTIR spectra show an absorption band at 3260 cm − 1 corresponding to the stretching vibrations of the hydroxyl groups (–OH) and that contribute to the inter and intramolecular interactions of the hydroxyl group OH–; which is a particular characteristic of the starch structure. An intense band at 2920 cm − 1 is attributable to stretching vibrations of the C–H bonds [37].

Water absorbed by starch is observed between at 1620 cm−1. The bands at 1400 and 1300 cm−1 correspond to C–H bonds. The region known as characteristic starch "fingerprint" peaks are observed at 1150 and 1056 1019 cm − 1, attributed to the vibrations of the C – O – C bonds of glucose, and bands at 928, 927, 856, 758 and 678 cm − 1, corresponding to the pyranose ring [32].

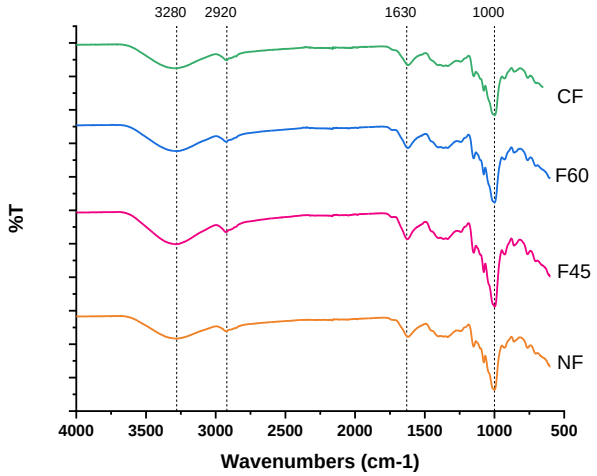



**Figure 4.** FTIR spectra of Maya nut flour. under different drying treatments. NF- natural flour, F45- flour drying at 45 °C, F60- Flour drying at 60 °C y CF-commercial flour.

### *3.8. Total Phenolic content and antioxidant activity*

Due to the importance of seeking new sources of antioxidants for their multiple health benefits and as natural preservatives in food [38], the total phenolic content an antioxidant activity of the flours obtained from Maya nut was studied. The total amount of polyphenols in the flours was significantly affected by the drying method used in the Maya nut (Table 5). CF shows a significantly higher TPC (13.35 ± 0.80 mg GAE/g), and the lowest was F60 (11.61 ± 0.79 mg GAE/g). This content agrees with the most recent data recorded for TPC in Maya nut (1230.12 ± 10.61 mg GAE/100) [6]. The first polyphenolic compound identified in the Maya nut were epicatechin, hydroxybenzoates, gallic, p-hydroxybenzoic, and vanillic acids, and hydroxycinnamates, caffeic and p-coumaric acids. These polyphenolic compounds may contribute to the antioxidant activity of the seed [5].

Significant differences were observed between treatments in antioxidant activity (Table 5). In the DPPH and FRAP assay, CF was the sample with the highest antioxidant capacity (16.87±0.63 and 29.84±0.22 μM TEAC/g respectively). In contrast, the ABTS test showed few significant differences, with NF having the highest antioxidant capacity

(72.89 ± 1.71). Phenolic compounds are the main antioxidant and radical scavenging components in Maya nut so far identified. The level of total polyphenol content increased after roasting (CF) in this current investigation; therefore, the antioxidant activity was also increased. Another possible cause of the increase in antioxidant activity could be that Maillard compounds have been generated during the roasting of the seeds, which could contribute to the increase in antioxidant activity. [29].

**Table 5.** Total Phenolic content and antioxidant activity of Maya nut flours.

| Parameter | NF | F45 | F60 | CF |
|---|---|---|---|---|
| TPC(mg GAE/g) | 12.20 ± 0.86$^{ab}$ | 12.78 ± 0.81$^{b}$ | 11.61 ± 0.79 $^{a}$ | 13.35 ± 0.80$^{c}$ |
| DPPH (μM TEAC/g) | 4.78±1.10$^{b}$ | 8.49±0.99$^{c}$ | 2.04±0.47$^{a}$ | 16.87±0.63$^{d}$ |
| ABTS (μM TEAC/g) | 72.89 ± 1.71$^{b}$ | 69.66 ± 2.88$^{ab}$ | 68.18 ± 3.66$^{a}$ | 68.79 ± 3.46$^{a}$ |
| FRAP (μM TEAC/g) | 9.76±0.63$^{b}$ | 10.95 ±0.44$^{c}$ | 7.032 ±0.08$^{a}$ | 29.84±0.22$^{d}$ |

Mean ±SD. TPC—total phenolic compounds NF- natural flour, F45- flour drying at 45 °C, F60-Flour drying at 60 °C y CF-commercial flour. Differents letters indicate significant difference ($p$<0.05).

The commercial sample (CF) has a higher content of polyphenols, therefore the antioxidant activity is increased, showing the highest values in this study. This may be due to the fact that the selected sample was treated and proceeded under different conditions than those of this analysis, in addition, that its geographical location of sample collection is different. The Maya nut collected from the state of Chiapas and that was evaluated under the three drying conditions presents a behavior that is affected by increasing the drying temperature. In the case of TPC, even when significant differences are shown, the values are similar, presenting the lowest value in the sample treated at 60 °C (F60), which was 11.61 ± 0.79 mg GAE/g, while at 45 °C a increase to 12.78 ± 0.86 mg GAE/g.

In the case of antioxidant activity by the DPPH method, it can be seen how the increase in AA when using drying at 45 °C is up to 4 times with a value of 8.49 ±0.99 μM TEAC/g while the F60 presents 2.04 ±0.47 μM TEAC/g AA. The effect of antioxidants on DPPH radical scavenging is explained by their ability to donate hydrogen. The odd electron of the nitrogen atom in DPPH is reduced by receiving a hydrogen atom from the antioxidants to the corresponding hydrazine. This assay has been widely used to investigate the antioxidant capacity of a wide variety of complex matrices. Can be used to examine both hydrophilic and lipophilic antioxidants [39] .

In this study, the antioxidant capacity of Maya nut flours was also investigated with the ABTS assay. ABTS is generated by reacting a strong oxidizing agent (eg, potassium permanganate or potassium persulfate) with the ABTS salt. The reduction of the blue-green ABTS radical by hydrogen donating antioxidants is measured by the suppression of its characteristic long-wave absorption spectrum. ABTS•+ is soluble in both water and organic solvents, which enables the antioxidant capacity of both hydrophilic and lipophilic compounds to be determined with the same basic methodology [40] . Using the ABTS technique, the difference in terms of AA is not significant since there is a value in F60 of 68.18 ± 3.66 μM TEAC/g and in F45 69.66 ± 2.88 μM TEAC/g, while in the FRAP technique it is observed the increase up to 30% of AA, being the highest F45 with 10.95 ±0.44 μM TEAC/g. To expand the research on the antioxidant capacity of Maya nut flour, the iron reducing antioxidant power test (FRAP), which is based on the reduction of a colorless complex of $Fe^{3+}$-TPTZ to intense blue $Fe^{2+}$-TPTZ once it interacts with a potential antioxidant [41].

### *3.9. Nutritional characterization of Maya nut cookies*

Figure 6 shows the results obtained from the characterization of the Maya nut cookies developed in this study.

The difference between the use of a commercial flour (CF) and a natural flour (NF) for the elaboration of cookies presents an increase in the amount of fiber in the cookies that were added 60% of Maya nut, this is due to the fiber of the Maya nut. With the substitution of non-conventional flours for the production of functional cookies, the amount of fiber improves and favors the amount of available carbohydrates that can contribute to a more adequate energy supply in the body and increase health benefits [42].

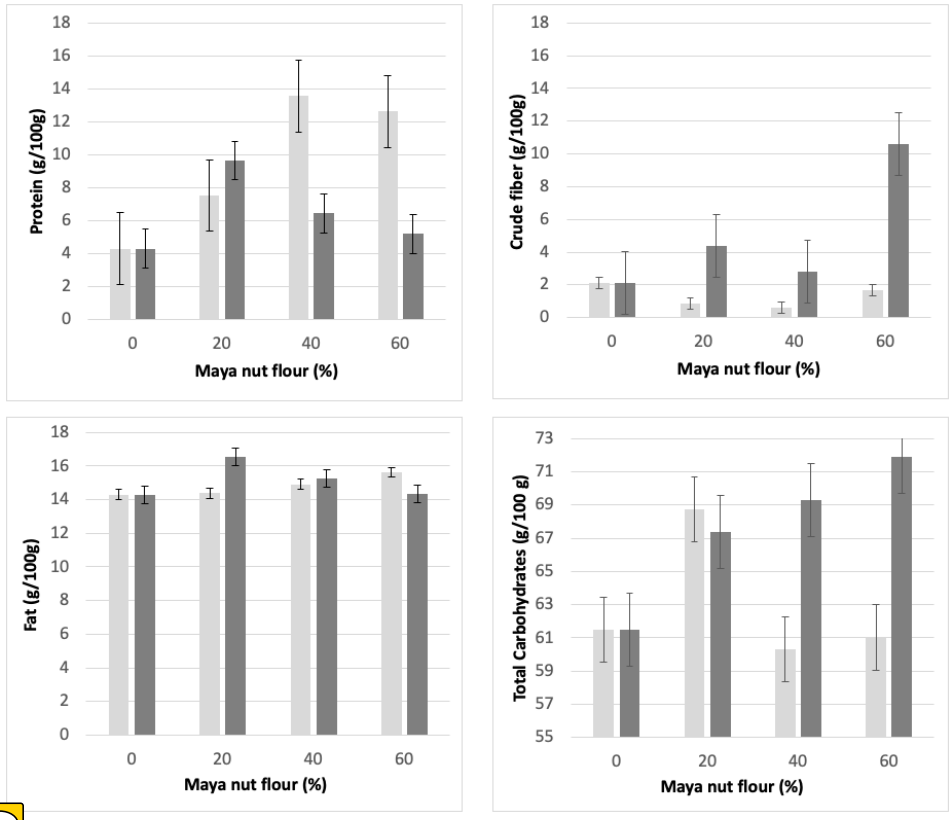



**Figure 6.** Important nutritional components in Maya nut cookies. □) NF, natural flour and ■) CF, commercial flour. Maya nut flour: 0%, 20%, 40% and 60%.

On the other hand, the protein content is directly affected (13.5 g/100g) as the flour concentration increases from 0% to 60% in NF cookies. But for CF this concentration decreases with increasing concentration (5 g/100g), the protein could be affected by the treatment given to the commercial sample since thermal processes can directly affect the protein.

The concentration of carbohydrates presents a significant increase as the amount of maya nut flour increases, this can be observed in the use of commercial flour, obtaining the highest content (71.9 g/100g) in the substitution of 60%; while when using natural flour the highest value of total carbohydrates is presented in the substitution of 20% of maya nut flour (68.4 g/100g).

Fat content in the cookies substituted with maya nut flour NF presents a constant increase depending on the substitution concentration, so the highest concentration was observed in the 60% substitution. Having an opposite effect when using CF in the substitution of the cookies, having higher fat concentration in the 20% concentration.

Therefore, this factor has a great importance in the elaboration of the doughs for the elaboration of cookies, mainly in the rheological factors, so the presence of the greater amount of fats helps to the better handling and elaboration of the cookies.

Henrique-Belmiro et al [43] evaluated cookies formulated with coffee residues where they mention that the partial substitution of flour does not cause changes in the dimensions and techno-functional properties of the cookies due to the low level of incorporation of residues (3 and 6%). While in the case of the substitutions that we are using in the present investigation they use very high concentrations around 60%, reason why for this case if it is affected the properties of manipulation of the mass.

### *3.9. Antioxidant activity of Maya nut cookies*

The previous analysis of antioxidant activity of each flour showed in the DPPH assay that CF had a higher antioxidant capacity (16.87±0.63 µM TEAC/g), but in the ABTS assay NF had a higher antioxidant capacity (72.89 ± 1.71).

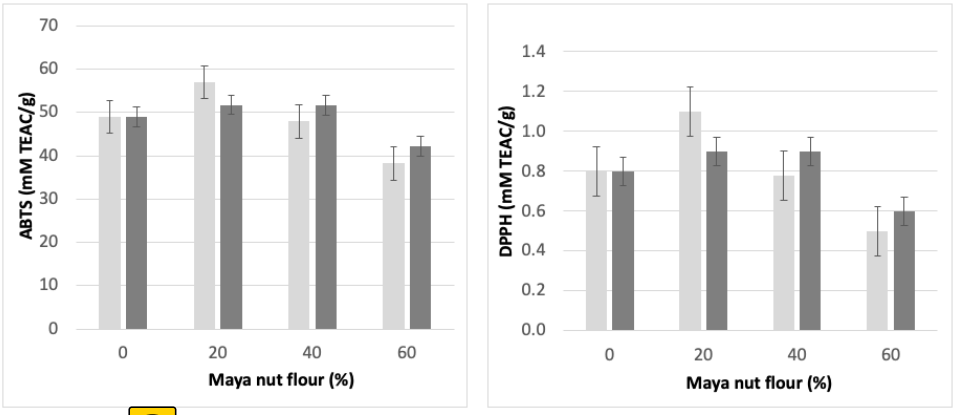



**Figure 7.** Antioxidant activity in Maya nut cookies by ABTS and DPPH radical. □) NF, natural flour and ■) CF, commercial flour. Maya nut flour: 0%, 20%, 40% and 60%.

On the other hand, Figure 7 shows it was determined that there is a difference but not significant between cookies containing no Maya nut flour (72.18 µM TEAC/g) compared to cookies containing Maya nut flours at 20% and 40% both flours (NF and CF) in the ABTS assay. Likewise, the determination of antioxidant activity with the DPPH radical shows a behavior similar to that of ABTS in all the fromulations and without significant differences.

The decrease in antioxidant activity after the preparation of Maya nut cookies may be mainly due to a degradation caused by the temperature (180 °C) used during the baking process of the cookies, since these compounds are more unstable under these conditions [44]. (Zhang, Chen, Li, Pei, & Liang, 2010).

## 4. Conclusions

Maya nut flour proved to be an enriching product for food formulations since it can positively influence the nutritional profile and improve its functional characteristics. According to the analysis methods used in this study, few differences were observed in the physicochemical characteristics of the Maya nut flours obtained since there was little impact on the structural and molecular characteristics of the seeds. However, the results of this work demonstrated that the drying methods used here for the development of Maya nut meal significantly affected the content of total polyphenols and the antioxidant activity. Therefore, a cookie rich in antioxidants that help stop or limit the damage caused by free radicals in the body and prevent them from causing damage to other cells, helping to improve the quality of life, can be obtained. In addition, expanding knowledge about un-

dervalued and little-used products such as Maya nut and evaluating the physical, chemical, nutritional, and functional characteristics of ingredients other than conventional ones opens up the opportunity to consider them in the development of new food formulations.

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**Data Availability Statement:** The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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