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PROXIMAL, THERMAL, AND STRUCTURAL CHARACTERIZATION OF STARCH EXTRACTED FROM TWO VARIETIES OF WHITE SORGHUM CULTIVATED IN MEXICO

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ABSTRACT

Sorghum (*Sorghum bicolor*) is a starch source that may contain tannins in red varieties, so white grains are preferred as a better alternative. The hypothesis of this study was that white sorghum starch grown in Mexico has the necessary properties for industrial use. The objective was to evaluate the chemical, thermal and structural characteristics of starch extracted from white sorghum var. Mazatlan-16, and RB-Paloma. Protein, moisture, ash, fiber, fat, water absorption rate, and swelling capacity were evaluated. Thermal properties were determined by calorimetry and viscosity analysis; structural properties by Fourier transform infrared spectroscopy and X-ray diffraction. One-way analysis of variance and Student's t-test ($p \le 0.05$) were performed. The starch extracted from Mazatlán-16 grain contained the highest moisture (5.47 %), protein (0.72 %) and crude fiber values, and no significant differences ($p \le 0.05$) were found for ash and fat content in both starch varieties. The water absorption rate and swelling power was higher for RB-Paloma variety starch ($p \le 0.05$). Regarding thermal properties, the maximum viscosity temperature was 80.55 and 89 °C for RB-Paloma and Mazatlan-16 starch, respectively; the peak gelatinization temperature was higher for Mazatlan-16 (72.28 °C) and showed differences ($p \le 0.05$) with RB-Paloma. Structural analysis revealed that the starches of both varieties correspond to the A-type crystalline pattern, with the presence of OH, CH in stretching, H_2O and CH_2 groups. The starches extracted from the two varieties showed properties suitable for use as a viable alternative in the food industry.

Keywords: *Sorghum bicolor*, chemical composition, analysis, utilization.

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is one of the five most important cereals worldwide, belonging to the Poaceae family, along with millet (*Panicum miliaceum*) and wheat (*Triticum aestivum* L). This cereal is an important food source in Africa, where it originated, and Asia, with a reported *per capita* consumption of 18 and 2.1 kg, respectively (Bhagavatula *et al*., 2013). Among 20 sorghum species, *S. bicolor* stands out in human food, being used in products such as tortillas, pastas, cookies, alcoholic beverages, and porridges (Treviño-Salinas *et al*., 2021). In Mexico, it is primarily used as cattle feed and to produce bioethanol; however, its nutritional properties are now becoming well-known and it has been considered as a substitute or accompaniment for other cereals (Adebo, 2020).

The color of sorghum kernels is determined by the phenolic compounds and tannins found in the testa and pericarp. This, in turn, is determined by the genotype and can include red, cinnamon, black, purple, yellow or white grains (Dykes *et al*., 2005). Despite their beneficial effects, these metabolites can cause gastric mucosal irritation or act as anti-nutritional compounds that reduce the availability and digestibility of carbohydrates, proteins or minerals at high concentrations (Tasie and Gebreyes, 2020). For this reason, white sorghum varieties are preferred for human consumption, since they have no testa.

White sorghum contains protein, lipids, carbohydrates, ash and fiber levels comparable to corn, including unsaturated fatty acids (Treviño-Salinas *et al*., 2021). Like all cereals, the main component is starch, which is an important source of energy and nutrients in both human and animal diets. However, this is not the only potential use of white grain sorghum, it has also been reported that the starch can absorb and retain tannins if the grain contains them (Beta *et al*., 2001).

White sorghum starch has a variety of applications. For example, the formulation of gluten-free pastas with different percentages of sorghum starch in place of rice flour has been evaluated; the authors reported that using 15 % sorghum starch in pasta resulted in a high-fiber, slow-digesting pasta, without affecting sensory properties (Cervini *et al.*, 2021). In addition to its use in food, starch is used in industry as a thickening agent, emulsifier and raw material for beverages, among other things. Such applications are dependent on the amylose to amylopectin ratio, which determines the structural and functional properties of starch (Mohamad Yazid *et al*., 2018).

Research in the last decade has pointed to a wide range of applications for this polymer, including energy generation, packaging, 3D printing, and even regenerative medicine. To that end, starch modification has been proposed in order to enable a broader range of applications (Liu *et al*., 2019). Corn is the most common source of starch; however, it is important to search for new, cost-effective and easily accessible sources, such as agro-industrial residues or other grains. White sorghum is an alternative for native starch extraction because it can grow in arid environments, alkaline soils, or soils with low organic matter load, where corn cannot.

Sorghum grain has a starch content of approximately 70 %, making it a viable source of starch. However, in order to determine potential applications, the chemical and structural properties must be evaluated. The remaining protein, fat, ash and fiber content affects the swelling properties of starch. On the other hand, structural properties determine the fundamental thermal properties in the industry, which is why the gelatinization temperature, viscosity and water absorption capacity must be evaluated (Udachan *et al.*, 2012). The hypothesis of this study is that starch extracted from white sorghum has properties suitable for use in the food industry. Therefore, the objective was to evaluate the structural, thermal and chemical properties of starch extracted from two varieties of white sorghum grown in Mexico.

MATERIALS AND METHODS

Raw material

White sorghum grains of the RB-Paloma and Mazatlan-16 varieties were used, which were developed under the "Sorghum Genetic Improvement" program of the Institute of Forestry, Agricultural and Livestock Research (INIFAP), at the Rio Bravo Experimental Field (Tamaulipas) and Culiacan Valley Experimental Field (Sinaloa). The grains were donated by INIFAP- Rio Bravo Experimental Field, located in Rio Bravo, Tamaulipas, Mexico. The agricultural soil of Rio Bravo is xerosol (arid), the climate is semi-dry and semi-warm with little rainfall throughout the year, and the temperature ranges between 18 and 38 °C during planting (April) and harvest (August) (Government of Tamaulipas, 2020).

Starch production and conditions from sorghum grain flour

The grains were dried at 60 °C for 48 h. For starch extraction, a grain mill (BI-DTOOL 3000A, China) was used, followed by sieving with a No. 20 mesh (850 mm) and a No. 40 mesh (420 mm). Once the flour was obtained, NaOH 0.1 % was added and the mixture was left to stand for 18 h. The wet flour was triturated in a home blender for two minutes, the obtained suspension was filtered on a No. 100 mesh (0.149 mm) followed by a No. 200 (0.074 mm). The filtrate of the above mixture was washed with distilled water until it was clear. The obtained suspension was centrifuged at 4034 *g* for 10 minutes (Optima XL-100L Beckman Coulter, USA) and the supernatant was discarded.

The isolated starch was neutralized with 1.0 M HCl to pH 6.5, washed three times with distilled water, and centrifuged again under the same conditions. Finally, the extracted starch was dried in a recirculating air oven (CE5F ShelLab®, USA) at 55 °C for 24 h, ground and sieved again, and stored at 4 °C in hermetically sealed plastic containers until proximal, thermal and structural analysis (Wang and Wang, 2001).

Proximal chemical analysis

The AACC (American Association of Cereal Chemists) official methods were used to determine proximal starch content, and all determinations were made in triplicate: crude fiber (32-10.01), protein by the Kjeldahl method (46-13.01), ash by combustion (08-03.01), moisture by oven drying (44-15.02), and lipids by the Soxhlet method (30-10.01). Carbohydrate content was determined by difference.

IAA and swelling capacity

To determine the water absorption index (IAA), 10 mL of distilled water were added to 1 g of starch extracted from the corresponding variety, shaken vigorously, and subjected to centrifugation for 15 min at 4034 *g*; the supernatant was decanted into a pre-weighed porcelain capsule. The AAI was calculated as a weight percentage of the original sample using the dry weight of the dried solid supernatants. The results are expressed as percentage of grams of water retained per gram of sample.

The swelling power was determined with the method proposed by Subramanian *et al*. (1994), using 100 mg of sample mixed with 2 mL of distilled water and exposing the mixture to different temperatures (40, 50, 60 and 70 °C) for 4 h. The mixture was then centrifuged at 8232 g for 15 min, the supernatant was decanted, and the wet sediment was weighed. The swelling power was expressed as the ratio between the weight of the wet sediment and the initial weight of the dry starch. In both cases an analytical balance accurate to 0.0001 g (Denver Instrument P-114.1 Analytical Balance, 110, USA) was used; the analyses were performed in triplicate.

Viscosity and gelatinization profile

The following heating and cooling cycles were programmed into an RVA-4500 rapid viscosity analyzer (Perten Instruments, Australia): heating at 50 °C for 1 min, followed by heating 1.5 °C per minute until 95 °C was reached, held at this temperature for 5 min, and cooled to 50 °C for 7.7 min (Alvis *et al*., 2008). The following parameters were determined: the temperature of first viscosity (viscosity values are reported in centipois), which refers to the increase by at least 25 cp over a period of 20 s; peak viscosity (the maximum viscosity reached); the peak time (time at which the peak viscosity occurred); final viscosity (viscosity at the end of the test after cooling); viscosity decomposition (peak viscosity holding force); and receding viscosity (final viscosity - holding force).

Differential Scanning Calorimetry (DSC)

Thermal properties of isolated sorghum starch were determined using a DSC 822E differential scanning calorimeter (Mettler Toledo, USA) calibrated with native standard (T0 = 156.6 °C, ΔH = 28.4 J g^{-1}). The starch was hydrated in distilled water with a 1:4 ratio. Then, 40 μL were taken and placed in aluminum crucibles; finally, they were evaluated in the temperature range of 30 to 95 °C with a heating rate of 5 °C min^{-1} (Palacios-Fonseca *et al*., 2013).

X-ray diffraction (XDR)

The pulverized starch grains were packed inside a 30 × 30 mm aluminum mount to obtain X-ray diffraction patterns with an Equinox 2000 equipment (Inel, France) and the method by Navarro-Cortez *et al*. (2016). The equipment has a Cu $K\lambda$ radiation line (1 to 1.5406 °) and a power difference of 30 kV with a current density of 20 mA. The packed samples recorded a diffraction angle for the 2θ beam from 5 to 35 ° with 0.1 ° intervals by probing for 8 s per degree, as well as a counting time per step of 15 min.

Fourier Transform Infrared Spectroscopy (FTIR)

Starch samples were pulverized with a mortar to a particle size of 230 µm and 2 mg of sample of each variety's sample mixed with KBr were weighed; the mixture was placed in a press to form a crystalline tablet, which was read in a spectrophotometer (Spectrum 3 FT-IR Spectrometer, Perkin Elmer, England). Each sample was analyzed with 10 scans from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. Spectra baseline was corrected and normalized by Spelwin32 - Spectroscopy (Navarro-Cortez *et al*., 2016).

All measurements (chemical, thermal and structural) were made in triplicate, and the mean and standard deviation were calculated. A one-way analysis of variance was performed using SPSS version 22.0 software (SPSS Inc., Chicago, IL, USA), followed by Student's t test ($p \leq 0.05$). Graphs were constructed in Excel 2010 (Microsoft®, USA).

RESULTS AND DISCUSSION

Proximal content

The results of starch proximal analysis of RB-Paloma and Mazatlan-16 varieties show differences ($p \leq 0.05$) in moisture, protein, fiber and carbohydrate parameters (Table 1). These analyses are used to assess the purity of the extracted starch as well as its potential stability; for example, moisture content is an important parameter to ensure the microbiological stability of a raw material. The higher the moisture content, the greater the risk of microbial growth, which leads to changes in structure and quality. In starches extracted from sorghum varieties, the percentage of this parameter was less than 6 %; Arrazola-Paternina *et al.* (2020) analyzed starches isolated from yellow and purple sweet potatoes (*Ipomoea batatas*) and found a higher moisture content (10.9 %) than that recorded in this study. The results suggest that there is less risk of microbial attack, and thus the structural stability of the starch is maintained for a longer period of time.

For protein content, sorghum starch var. Mazatlan-16 had the highest value (0.72 %). This may be due to the presence of insoluble type proteins associated with starch granules that were not completely isolated during the extraction process (Kaur *et al*., 2018); however, the content found is lower than that reported for corn (0.88 %) (Ahmad *et al*., 1999). Although the proteins present in the starch granule are bound to the internal matrix by hydrogen and disulfide bonds, they can be entirely isolated by using an alkaline solution during starch soaking (Contreras-Pérez *et al*., 2018). On the other hand, the presence of lipids in the sorghum starches analyzed was 0.60 to 0.69 %

Table 1. Proximal chemical composition (%) of starch extracted from two varieties of white grain sorghum.

Variety	Moisture	Protein	Ash	Fat	Raw fiber	Carbohydrates
Mazatlán-16	5.47±0.04a	0.72±0.09a	0.22±0.06a	0.69±0.01a	2.67±0.06a	90.62±0.21b
RB-Paloma	4.04±0.1b	0.58±0.05b	0.17±0.04a	0.60±0.19a	1.01±0.19b	93.54±0.09a

a,b Different letters in the same column indicate statistical differences ($p \leq 0.05$).

(Table 1), higher than that reported in yam (*Dioscorea* spp.) (0.1 to 0.42 %) and maize (*Zea mays*) (0.20 %) (Elmi-Sharlina *et al*., 2016). The crude fiber content was higher than that reported for corn starch (0.24 %) (Ahmad *et al*., 1999).

The ash content was also determined, as starches must contain less than 0.5 % ash for industrial use. This criterion was met by isolates of both white sorghum varieties (0.17 to 0.22 %) (Table 1). Yam starch had a higher ash content (0.88 %) than sorghum starch (Elmi-Sharlina *et al*., 2016). Differences in the proximal content of sorghum starch compared to other sources could be related to its origin, isolation and growing conditions (Elmi-Sharlina *et al*., 2016). The low protein and fiber content suggests that an adequate starch extraction process was performed, which is important as the presence of these components in high concentrations interferes with the thermal properties and potential uses of starch in food (Kumar *et al*., 2019).

Thermal properties

Starch isolated from the RB-Paloma variety presented a higher index ($p \leq 0.05$) of water absorption (IAA) (Table 2); starches with higher water absorption have larger granule sizes and the presence of hydrophilic groups, which together increase water retention (Martinez *et al*., 2015). In Italy, white sorghum starch with an IAA of 89 % was reported (Giuberti *et al*., 2019), which is lower than the value found in this study. The IAA is a measure of the degradation of intra- and intermolecular hydrogen bonds in starch, which lead to the formation of hydroxyl groups that react with the water molecules present (Ye *et al*., 2018).

Swelling capacity is a useful measure to evaluate the water absorption capacity and expansion power of the starch granule, as well as fractional exudation in relation to temperature and time. Swelling is directly related to amylopectin content; long chains of this polysaccharide increase this property (Li and Yeh, 2001). The results show that the RB-Paloma variety has a higher swelling power ($p \leq 0.05$). In both starches, this property increases as temperature rises (Table 3), obtaining a higher malleability of the starch molecule.

In contrast to the aforementioned, amylose has a lower swelling power since it absorbs less water than amylopectin, allowing for slow swelling while maintaining

Table 2. Water absorption index (IAA) of starches from Mazatlan-16 and RB Paloma varieties.

Variety	IAA[†] (%)
Maztlán-16	129±04b
RB-Paloma	167±13a

[†] The mean and standard deviation (±) of three replicates. a,b Different letters in the same column indicate statistical differences ($p \leq 0.05$).

Table 3. Swelling capacity (g g^{-1}) of starches isolated from white sorghum grain at different temperatures.

Variety	1 h (40 °C)	2 h (50 °C)	3 h (60 °C)	4 h (70 °C)
Mazatlán-16	2.44±0.3a	2.53±0.3a	2.87±0.02b	3.85±0.47b
Paloma-RB	1.96±0.3b	2.45±0.3a	3.54±0.0a	4.65±0.23a

a,b Different letters in the same column indicate statistical differences ($p \leq 0.05$).

viscosity (Contreras-Pérez *et al*., 2018). With the results obtained, it is suggested that Mazatlan-16 variety starch has a higher amylopectin content which facilitates higher water entrapment in the granule (Ye *et al*., 2018).

Regarding viscosity, it was found that the maximum temperature for the isolated sorghum starch varieties was 81 °C for RB-Paloma and 89 °C for Mazatlan-16 (Figure 1). The rapid viscosity analyzer (RVA) determines the temperature and swelling time of starch granules. When the heating stage begins, there is an increase in viscosity due to the swelling of the starch granules, the breaking of hydrogen bonds, and progressive hydration (Montoya *et al*., 2012).

The biochemical characteristics of starch, such as amylose and amylopectin content, influence viscosity and gelatinization properties, as these are compounds present in cereals that determine their final use in baking or food manufacturing. Therefore, it is important to determine the conditions of granule breakage and retrogradation for their effect on viscosity, as they are closely related to texture and stability (Contreras-Pérez *et al*., 2018; Ye *et al*., 2018). Cereal starches tend to be defined by a high amylose

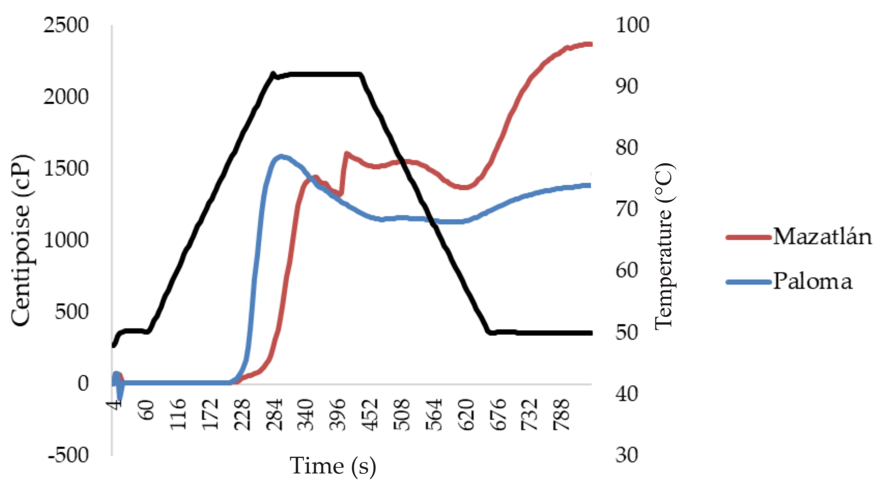

Figure 1. Viscosity properties of starch paste isolated from white sorghum grain Mazatlan-16 and RB-Paloma varieties.

content, which limits the properties of amylopectin due to the formation of helical complexes with other lipid molecules; this results in low viscosity and an opaque paste as temperature increases (Srichuwong, 2017).

Regarding the gelatinization capacity of the two-sorghum starch isolate varieties, it was found that the initial temperature ranged from 67 to 68 °C, and the final temperature from 79 to 80 °C (Table 4). Ehtiati *et al*. (2017) reported for dual-purpose sorghums (lines KDFGS1, KDFGS6, KDFGS9, and KDFGS20) initial temperature properties of 67 °C, peak temperature of 72 °C, and final temperature of 77 °C, indicating that the RB-Paloma variety is within the normal range for sorghum granules. These starch properties could be attributed to the relative crystallinity and amylase content of the grain. The values found are comparable to those found in sorghum starch sold in Rome, Italy, with an initial and peak temperature of 66.6 and 71.5 °C, respectively (Giuberti *et al*., 2019).

Table 4. Gelatinization capacity of white sorghum starches.

Variety	Ti[†]	Tp[¶]	Tf[§]	I[Þ]
Mazatlán-16	68.99±0.6a	75.28±0.4a	80.52±0.7a	11.53±0.47
RB-Paloma	67.09±0.7b	72.48±0.03b	79.46±0.3b	12.37±0.34

[†]Ti: initial temperature; [¶]Tp: peak temperature; [§]Tf: final temperature; [Þ]I: temperature interval. a,b Different letters in the same column indicate statistical differences ($p \leq 0.05$).

The thermal properties of starches are related to granule size, fractures and amylose content; in addition, gelatinization is affected by the presence of proteins, salts or fats in the starch (Donmez *et al*., 2021). Although the Mazatlan-16 variety has a higher water absorption capacity, its protein, fiber and fat content interfere with its gelatinization capacity. It is known that at higher gelatinization temperatures, there is a higher degree of ordering in the starch granule, requiring a higher cooking temperature if applied to food (Salgado-Ordosgoitia *et al*., 2019).

The results obtained in this study (Table 4) are consistent with what has been reported in isolated corn grain starch, with initial temperatures ranging from 65 to 67 °C, peak temperatures between 70 and 71 °C, and final temperatures at 75 and 77.75 °C (Paraginski *et al*., 2018). Therefore, the starches obtained from the two sorghum varieties can be used in the production of pasta (with low gelatinization temperatures), extruded foods (high gelatinization temperatures), and industrial fermentations (Donmez *et al*., 2021).

Structural properties

In the DRX analysis, the Mazatlán-16 variety reported peaks at 2θ of 17.91, 20.17, 23.40 and 26.83 °, while the RB-Paloma variety showed a pattern with peaks at 2θ of 17.91, 20, 23 and 26.76 °; the peaks presented in the diffractogram correspond to the crystalline pattern type A (Guo *et al*., 2019) (Figure 2). The results found are similar to those reported for corn starch; Navarro-Cortez *et al*. (2016) found the presence of an A-type structure in corn starches and observed signals at 15, 18 and 22 °.

Type A starch is constituted of a compressed double helix of amylose and amylopectin, with homogeneous internal strengths, making it more resistant to enzymatic attack (Alves de Melo-Neto *et al*., 2017). It has been suggested that because sorghum has higher resistant starch content than corn, it can be used as a functional compound with various benefits to the human health (Giuberti *et al*., 2019). The mechanical force generated during the milling process has been reported to fade hydrogen bonds, which have the ability to generate bonds in the side chains of the groups present in amylopectin, causing crystallinity to decrease (Li *et al*., 2020); nonetheless, this was not observed in the starches analyzed in this study as the milling was adequate.

After performing the FTIR analysis of the two starch moieties, a broad peak between 3600 and 3300 cm^{-1} was found, indicating the presence of a stretching hydroxyl group (Figure 3). The starch peaks had values between 3000 and 2800 cm^{-1} (Table 5), which is attributed to the stretching of the -CH group of the glucose unit (Kaur *et al*., 2018). The presence of water is essential for the maintenance of the structure and its molecular regeneration. The two varieties showed a band at 1656 cm^{-1} caused by the starch molecule (Jagadeesan *et al*., 2020), where bound water can be found forming hydrogen bonds in the crystal structure and free water responsible for the hydration of the starch molecule.

In a study by Jafari *et al*. (2017) with sorghum from Iran, the presence of an amide group in the region 1600 to 1700 cm^{-1} was observed, which may be due to the presence of proteins. A similar peak is observed in the starch of the Mazatlan-16 variety, which coincides with a higher protein content (0.72 %) obtained from this variety compared to RB-Paloma (0.58 %). The starch analyzed in this study had peaks from 1460 to 1371

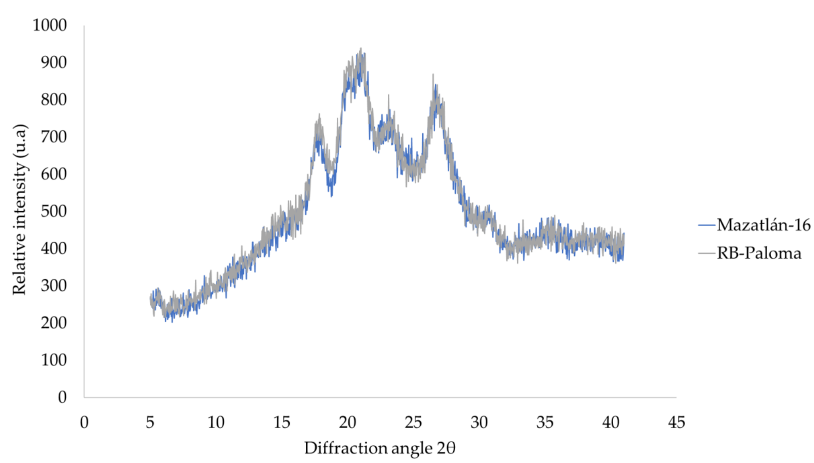

Figure 2. Diffractograms of starch extracted from white sorghum varieties Mazatlan-16 and RB-Paloma.

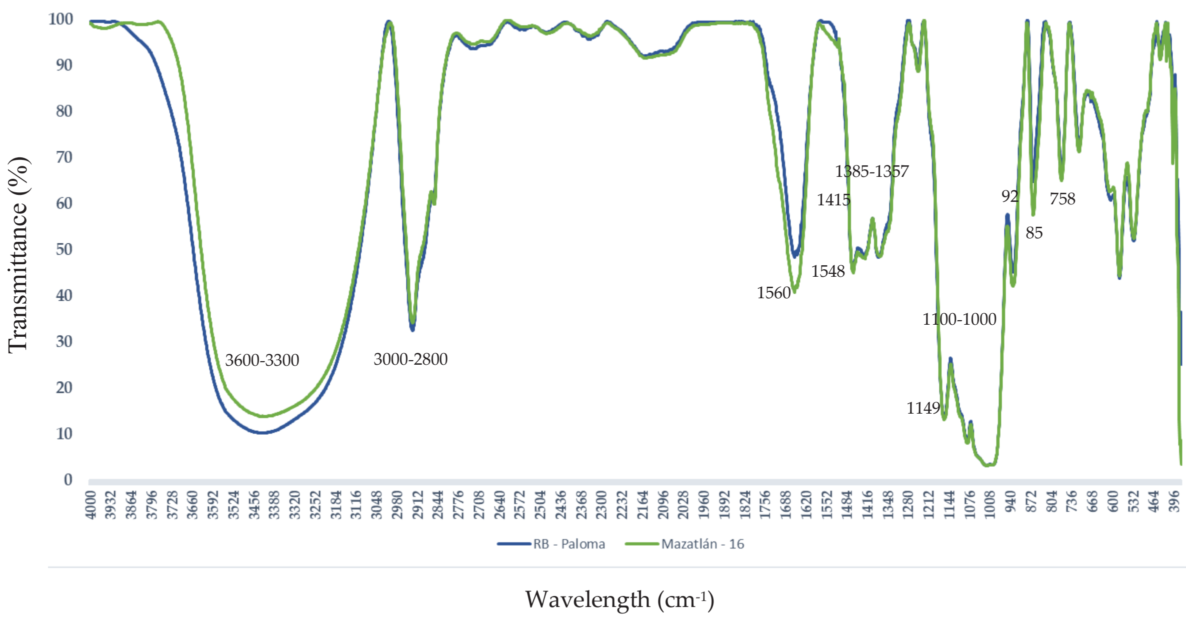

Figure 3. Infrared spectrum of starch isolated from white sorghum varieties Mazatlan-16 and RB-Paloma in FTIR analysis.

Table 5. Functional groups present in starch extracted from the two varieties of white sorghum grain.

Functional group	Wave number (µm)	Mazatlán-16	RB-Paloma
O-H (stretching)	3600-3300	3415	3424
C-H (stretching)	3000-2800	2928, 2854	2926
H-O-H	1650	1656	1656
CH_2	1458	1460	1460
CH_2	1415	1421	1421
C-H	1385-1357	1371	1377
C-O-C	1149	1158	1158
C-O	1100-1000	1081,1017	1081,1017
C-O-C	920, 856, 758	929, 862, 768	929, 862, 768

cm^{-1}, as well as peaks at 1158 cm^{-1}, which are related to the stretching vibration of the CH and COH groups (Kaur *et al*., 2018). These findings, combined with DRX analysis, confirmed the presence of a type A starch, which is typical of cereals, and indicate that the extraction process was adequate and that the polysaccharide's crystalline structure of the was not harmed.

CONCLUSIONS

The starch obtained was of sufficient purity for industrial use, as demonstrated by its low content of protein, fiber, ash, and fat, confirming the suitability of the extraction procedure used. The starch of the two white sorghum varieties showed high water absorption capacity and high swelling power. X-ray analysis and Fourier transform infrared spectroscopy confirmed the crystalline structure of a type A starch, which is common in cereals and has an adequate amylose content. Therefore, the starch obtained from Mazatlan-16 and RB-Paloma varieties has the chemical, thermal and functional properties to be used in the food industry as a thickening agent, a partial substitute for flours in gluten-free pastas, extruded foods, or as an agent for microbial fermentation.

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