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Artículo de investigación

Obtaining and characterizing hass avocado seed starch *(persea americana 'hass)* by wet alkali treatment as a source of biodegradable material

Obtención y caracterización de almidón de semilla de aguacate hass *(persea americana 'hass)* mediante tratamiento por álcalis húmedo como fuente de material biodegradable

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Abstract

The agro-industrial waste from Hass Avocado Seeds (HAS) (Persea americana 'Hass) is an abundant residue that generates different by-products of high added value. The high starch macromolecule content makes them an interesting derivative with diverse functional applications. The starch extraction was performed using the wet alkaline method with sodium hydroxide (NaOH) and sodium metabisulfite ($Na_2S_2O_5$) treatment at low concentrations. The extraction yield of HAS starch was 14.93% at 35°C, presenting a type B structure with OH, C-H, O-H, C-O-C, C-OH, and C-C bonds, and a crystallinity of 38.63%. Particle size analysis revealed granules with an average size of 12.87±5.29 μm, which exhibited high water absorption properties, significant swelling power, and superior viscosity of 5.94±0.05 g gel/g sample, 6.05±0.05 g gel/g starch, and 1685.37 mPa·s, respectively. The native HAS starch showed a high thermal stability profile at a maximum temperature of 110.23°C and an enthalpy of 541.76 J/g, thanks to its improved structural configuration, which is superior to what has been reported for this botanical source in the literature. The starch extraction was successful even though it can be considered low yield; however, the use of this waste as a source of material for industrialization, both in food and non-food systems, can benefit the environment and food security.

Keywords: Agro-industrial Waste, Alkaline Process, Clean Technology, Eco-friendly Materials, Hass Avocado Seed Starch.

Resumen

El desecho agroindustrial Semilla de Aguacate Hass (SAH) (Persea americana 'Hass) es un residuo abundante que genera diferentes subproductos de alto valor agregado. El elevado contenido de la macromolécula de almidón las transforma en un derivado de interés con diversas aplicaciones funcionales. La extracción de almidón se realizó por el método álcalis en vía húmeda con hidróxido de sodio NaOH y tratamiento de metabisulfito de sodio ($Na_2S_2O_5$) a bajas concentraciones. El rendimiento de extracción del almidón de SAH fue del 14,93% a 35°C, presentando una estructura tipo B con enlaces OH, C-H, O-H, C-O-C, C-OH y C-C, y cristalinidad del 38,63%. El análisis granulométrico reveló gránulos con un tamaño medio de 12,87±5,29 μm, los cuales presentaron propiedades de alta absorción de agua, un elevado poder de hinchamiento, y una viscosidad superior de 5,94±0,05 g gel/g muestra, 6,05±0,05 g gel/g almidón y 1685,37 mPa·s, respectivamente. El almidón nativo de SAH evidenció un perfil de alta estabilidad térmica a la temperatura máxima de 110,23°C y entalpía de 541,76 J/g, gracias a su configuración estructural mejorada y superior a lo reportado para esta fuente botánica en la literatura. La extracción de almidón fue exitosa a pesar de que puede considerarse de bajo rendimiento, no obstante, el aprovechamiento de este desecho como fuente de material para la industrialización, tanto en sistemas alimentario como no alimentarios, puede beneficiar al medio ambiente y la seguridad alimentaria.

Palabras Clave: Residuos Agroindustriales, Proceso Alcalino, Tecnología Limpia, Materiales Eco-amigables, Almidón de Semilla de Aguacate Hass.

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1 Introduction

The agro-industrial waste of Hass avocado seed (HAS) *(Persea americana 'Hass)* is an abundant residue, because this fruit is commercialized worldwide prioritizing the consumption of the pulp by being valued as guacamole, puree, oils, soups, soaps, shampoos, cosmetics, etc., antagonistically to the non-application of the residue in the bioeconomy, representing a challenge for its elimination [1]. That is, an active source of phytochemicals has been dismissed in the functional application of food or cosmetics, in the generation of value and exploitation in proactivity to the modern demand for environmentally friendly food or non-food products [2].

This has been evidenced by the exponential growth of imports during the period from 2011 to 2018, amounting to 1.601.755 t and exports of 1.662.046 t worldwide [3], with around 918.531 hectares being allocated in more than 60 countries, given that production is not limited to a specific time of year [4]. The latter is especially important for the producing of thickeners, gelatins, stabilizers and biofilms, since the seed is made up of around 66.3% starch [5]. Just as per capita consumption has increased from 0.83 to 0.85 kg in 2020, a situation proportional to productive growth of around 7.2 million tons in 2019, highlighting Mexico in Latin America and Spain in the European Union (EU) as industry leaders, however, it is important to note that Mexico's production is more than a third of global consumer product [6].

The excessive accumulation of agro-industrial waste from the Hass avocado seeds has brought about negative changes in the air we breathe, land use, and the aquatic environment. It is also it is prone to being a vector of organism that transmit diseases that affect human health and the ecosystem. Utilizing this renewable source of starch-rich in polysaccharides can be easily achieved and free the environment from pollution. Currently, multiple studies and research have begun to be carried out related to the exploitation of naturally occurring starches, which are easy to obtain and process for the production of a useful raw material in thickeners, gelling agents and biodegradable films, becoming a profitable, useful and efficient input, alternative to conventional sources of starch [7].

In this sense, technologies for the utilization of Hass avocado seeds have focused their on the extraction of primarily phenols and polysaccharides. The starch from Hass avocado seeds has a composition generally according to the literature of 16-10% moisture content, ash content of 0.2-0.25% and amylopectin and amylose composition of approximately 70-75% and 25-30%, respectively. It also exhibits high solubility, low water absorption, low swelling power, and thickening, gelling, and stabilizing properties, essential for imparting viscosity, texture and consistency in various applications [8, 9].

In this order of ideas, the valorization of abundant and conventional sources of starch such as cassava, potato, corn, plantain, or other with industrial uses, being these a feeding mechanism, threatens food security, since the agricultural product required poses a challenge in terms of the volume of specific raw material of land, water and in the generation of the food that will be revalued, in addition to the fact that the starch to be processed needs in many cases large quantities of cassava, corn, potato, barley, lychee or other, with minimal results of "extraction of the order of 1.4 to 19%" [8, 10].

Consequently, the utilization and integration of Hass avocado seed waste into the bioeconomy benefits the conservation of commodities and final consumption destination, "contrary to the ecological burden, indirect changes in land use, and increased water expenditure that could result from a considerable rate of conversion of the agricultural product" [11], so, "reuse the 25% of Hass avocado waste" [12], disregarded at the drum in traditional valorization chains promotes bio-industrialization.

Therefore, in this research it was proposed to extract starch from the Hass avocado seed *(Persea americana 'Hass)* by alkaline treatment and to characterize the chemical, functional, thermal, structural and morphological properties of the polysaccharide. The starch was obtained from Hass avocado seeds by hydrolysis with sodium hydroxide (NaOH)-0.2% w/w and treatment with sodium metabisulfite ($Na_2S_2O_5$)-0.2% w/w. The native starch samples were characterized by Scanning Electron Microscopy (SEM) with which the morphology was identified. By X-ray Diffraction (XRD) the type of starch structure was determined. By Fourier Transform Infrared Spectroscopy (FTIR) the functional groups of the obtained starch were found. Through Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) the thermal behavior was studied. The functional properties evaluated were water absorption index, water solubility index, swelling power, gelatinization temperature, viscosity, pH, dry matter content, ash content and pulp. This information is useful for determining the technical and technological applications of Hass avocado seed starch processed at low concentrations in alkalis.

Figure 1: Process of wet alkaline starch extraction.

2 Methodology

2.1 Wet alkaline starch extraction

The starch was isolated according to the modified methodology of several authors [8, 9], [13-16]. For extract the starch 20 kg of Hass avocado seeds were used as study subject and the process was standardized in triplicate by 1000 g samples.

Figure 1 shows the process of wet alkaline starch extraction. First, the seeds were washed with tap water and the shell was removed, once washed they were grated into smaller pieces, which were submerged in a sodium metabisulfite solution $Na_2S_2O_5$-0.2% in a 1:2 ratio, for 3 min are crushed in the food processor in order to reduce the particle size by passing it through a 75 μm sieve. The solids were left to settle for a set time and the suspension was decanted to remove the supernatant. The wet solids were then mixed with a sodium hydroxide solution NaOH-0.2% for 1 h 30 min at a speed of 500 rpm in a 1:2 ratio, the homogenized suspension was allowed to settle again.

The solution formed on the starch surface was removed until a clear, impurity-free transparent suspension was observed, which was washed repeatedly with distilled water and citric acid until neutral pH. To avoid the formation of conglomerates, the sediment obtained was subjected to an ultrasonic bath in Ultrasonic Cleaner model CE-5600A, Super Dental from China for 8 min at a frequency of 50 Hz. Finally, the wet starch was dried in a Memmert model UF110 oven from Germany for 12 h at 35°C, ground, collected in Ziploc® bags and stored in a clean, hermetically sealed container. The yield of the extraction process was calculated using Ec. (1) where dry starch mass (g) is the weight of starch obtained after drying and raw material mass (g) is the weight of the avocado seed used in the extraction, and the presence of moisture in the starch was determined using the methodology proposed by AOAC, 2000 [13].

$$\%Yield = \frac{\text{dry starch mass (g)}}{\text{raw material mass (g)}} \times 100 \quad (1)$$

2.2 Characterization of HAS starch

2.2.1 Functional characterization

Some of the properties studied for the starch extracted from Hass avocado seeds include water absorption index (WAI), water solubility index (WSI), swelling power (SP), gelatinization temperature, viscosity, pH, dry matter content, ash content, and pulp. These properties were compared with a reference standard which in this case was corn starch. Corn starch was selected as a reference because it is one of the most commercially available and widely studied starches, with extensive technical documentation and standardized methodologies for the measurement of physicochemical properties. Although starches from tubers or roots could offer structural similarities for example B-type crystallinity, corn starch provides a well-established baseline for comparative purposes due to its accessibility and abundance of reference data.

The WAI, WSI, and SP were determined using the method proposed by the authors [17] and [18]. A solution is prepared with 1.25 g of starch and 50 mL of distilled water, which is heated in a water bath at 60°C and centrifuged for 30 min at 4900 rpm., the decanted supernatant is separated, and 10 mL are taken and to dry at 70°C for 12 h; these are known as insoluble precipitates. The indices are calculated according to Ec. (2), (3), and (4) shown below:

$$WAI: \frac{\text{weight of gel (g)}}{\text{sample weight (g)}} \quad (2)$$

$$WSI = \frac{\text{soluble weight (g)} \times \text{volume (mL)} \times 10}{\text{sample weight (g)}} \quad (3)$$

$$SP = \frac{\text{weight of gel (g)}}{\text{sample weight (g)-soluble weight (g)}} \quad (4)$$

The procedure for determining the Gelatinization Temperature (GT) is carried out according to the methodology described by [18]. First, 10 g of starch dissolved in distilled water are weighed to make 100 mL, then water is heated in a 250 mL beaker to 85°C and 50 mL of the suspension is taken in a 100 mL beaker. The beaker with the sample is then placed in the 85°C water and the starch suspension is continuously stirred with a thermometer until a paste forms and the temperature remains stable for a few seconds, thus recording the GT.

The viscosity was measured on a SNB-2 speed controlled viscometer, Shanghai Dobetter Instrument & Equipment Co., Ltd of China, with Searle system, precision of 2% and rotating spindle L2, applying the technique suggested by the International Starch Institute (ISI), 2002, in the determination of viscosities for Brookfield [18]. The viscosities of both the corn starch and the starch extracted from the HAS were obtained at a concentration of 5% of the suspension, at an average temperature of 26.4±0.49°C and 10 rpm.

The pH is measured with a high-precision CPN pH meter, an advance model from China, in order to establish the acidity or alkalinity of the starch sample, which are recommended according to the authors and are in the pH range of 4.5 and 7.0. [19].

The dry matter content Ec. (5) was determined using the ISO 11465:1993 technique [20], this involved weighing the empty crucible (P1), then weighing the crucible with a 10 g starch sample (P2) and placing the crucible with the starch sample in an oven at 80°C for 24 h, the crucibles with the dry starch are left in a desiccator until obtaining constant weight (30-45 min) (P3). That is, P1 is the weight of the empty crucible (g), P2 is the weight of the crucible with the wet starch sample before drying (g) and P3 is the weight of the crucible with the starch after drying to constant weight (g).

$$\%Drymatter = \frac{P3 - P1}{P2 - P1} \times 100 \quad (5)$$

The ash content was determined using Ec. (6) and according to the analytical method proposed by AOAC, 2000 [18]. The measurement is carried out by placing a starch sample in a porcelain crucible and incinerating it at 550°C for three and a half hours, the ashes are left in a desiccator until a constant weight is achieved, and the weight of the crucible with the ashes is recorded.

$$\%Ash = \frac{\text{weight of the ashes (g)}}{\text{weight of sample (g)}} \times 100 \quad (6)$$

The analysis of the pulp content Ec. (7) consisted of boiling a 2 g starch sample (P3) for one hour in 100 mL of 0.4 %v/v hydrochloric acid. The liquid was then filtered in a previously weighed filter crucible fitted with filter paper (P1), then washed with hot water and dried at 105°C to constant weight (P2) [18]. That is, P1 is the weight of the empty filter crucible with filter paper (g), P2 is the weight of the filter crucible with the retained and dried pulp (g) and P3 is the initial weight of the starch sample used for the analysis (g).

$$\%Pulp = \frac{P2 - P1}{P3 \times 100} \tag{7}$$

2.2.2 Morphological characterization by scanning electron microscopy (SEM)

By means of Scanning Electron Microscopy (SEM), a 10-millimeter nanolithography system, secondary detection under vacuum and coating of the samples with graphite to prevent the accumulation of electrical charge in the starch during the analysis, the surface morphology. The particle size distribution of the extracted starch was determined using the free software Image Tool® version 3.0. Additionally, elemental chemical analysis was performed by means of Energy Dispersion of X-rays (EDS) in a geometric area. The equipment used for the SEM and EDS study in this study was a JEOL microscope, model JSM 6490LV from Japan.

2.2.3 Structural characterization by X-ray diffraction (XRD)

The crystallographic study pattern was performed by X-ray Diffraction (XRD), using a BRUKER D8 ADVANCE diffractometer from Germany with DaVinci geometry under the following conditions: voltage (40 kV), current (40 mA), divergence slit (0.6 mm), primary Soller slit (2.5°), secondary Soller slit (2.5°), step size (0.02035° 2Theta). The phases present in the samples were identified using the Expert HighScore 3.0 software by comparing the diffraction patterns with standard reference data. The degree of crystallinity of the diffractogram was performed with Origin software version 8.5 applying the integration method of the area under the curve of the crystallite and the amorphous region (8), where Ia corresponds to the amorphous region and Ic to the crystalline region [21].

$$\%\text{Relative crystallinity} = \frac{I_c}{I_a + I_c} \times 100 \tag{8}$$

2.2.4 Chemical characterization by fourier-transform infrared spectroscopy (FTIR)

To analyze and characterize the functional groups of HAS starch, Fourier Transform Infrared Spectroscopy (FTIR) is used with the support of the IRPrestige-21 spectrometer SHIMADZU brand of Japan. The sample was taken with an average of 10 scans recorded at a resolution of 4 cm^{-1} in the range of 400-4500 cm^{-1}.

2.2.5 Weight loss determination by thermogravimetric analysis (TGA)

The weight losses were evaluated for native starch, using Thermal Analysis equipment of the TA Instruments brand, model TGA/DTA 5500 from the United States, under ambient conditions up to 600°C with a heating ramp of 10 °C/min in air atmosphere and standard flow of 25 mL/min.

2.2.6 Determination of thermal transition by differential scanning calorimetry (DSC)

The thermal transition phases were studied in native starch, using the Thermal Analysis equipment of the TA Instruments brand, model DSC550 from the United States, in initial conditions of 40°C up to 250°C with a heating ramp of 10 °C/min in air atmosphere and standard flow of 25 mL/min.

3 Results and Discussion

3.1 Extraction of starch via wet alkaline process from HAS

The average starch extracted after the standardization of the process was 14.93%, excluding the seed shell, which accounted for 4.63±3.72% according to the applied procedure. This result can be attributed to factors such as the extraction method configuration, seed variety, precipitation time, and the low concentrations of NaOH and $Na_2S_2O_5$ [7, 13, 14]. Consequently, it can be stated that the extraction yield in this research is in the range of 4 to 15%, which is consistent with the data reported in the literature, which include percentages of 4.50% [22], 5.01% [9], 10.28% [8], 10.30% [15], and 11.38% [13] for the Hass avocado seed.

On the other hand, in the research of [7] a yield higher than 20% was reported, specifically reaching 24.20%. This increase in yield is due to the prolongation of the precipitation time for up to 24 hours. That is, the increase in yield is proportional to the precipitation time. Therefore, to optimize the procedure used in this study, the precipitation time should be considered as an additional variable alongside those described in the extraction method. This is because the sedimentation time was completed only when the precipitated starch was observed, which could vary between the iterations for obtaining the polysaccharide.

In the case of other agro-industrial wastes such as mango seeds, the extraction varies between 74.56 and 81.87% [23]. In durian seeds, the yield reached 7.53% [24]. On the other hand, plantain peels have yields ranging from 16 to 48% of dry starch [25], while banana peels can provide up to 70% of raw starch [26]. The variability in yield from agro-industrial wastes is influenced by factors such as granular size, species, origin and place of collection, conditions that complicate the extraction of the starch granule [23, 24]. This suggests that soil composition can also directly influence the extraction results.

In line with the above, the variety of the avocado seed species significantly affects the yield. The extraction results for the Mill avocado seed show yields of 19.54% [14] and 42.20% [27]. This finding is confirmed in the study conducted by [28], who found differences in the chemical composition, morphology and functional properties of avocado seed starch in the Criollo, Fuerte and Hass species. In this sense, the yield

obtained in our research is within the expected range and can be considered positive, not only due to the conditions of the wet alkali method, but also due to the Hass variety, which can "be made up of up to 70% starch". [19].

3.2 Physicochemical and functional properties of HAS starch

The replication unit in the determination of the physicochemical and functional properties is n ≤ 3 for each parameter applying a single-factor variance analysis ANOVA and if there was a difference, a Tukey comparison test was performed for averages (p = 0.05) using free Excel software. Some physicochemical properties of the extracted starch are discussed in Table 1.

Table 1: Physicochemical properties of HAS starch. Average values for n = 2 and n = 3(*) repetitions followed by different letters represent statistically significant differences between columns with Tukey's test (p < 0.05) standard deviation (SD).

Property	Unit	HAS starch	Corn starch
WAI	g gel/g sample	5.94 ± 0.05^{a}	5.64 ± 0.01^{b}
WSI	%	3.69 ± 0.89^{a}	2.68 ± 0.15^{a}
SP	g gel/g starch	6.05 ± 0.05^{a}	5.71 ± 0.01^{b}
GT	°C	67.30 ± 0.57^{a}	69.10 ± 1.13^{a}
Viscosity	mPa·s	$1685.37^{a*}\pm84.36$	$772.17^{b*}\pm31.89$
pH		6.17 ± 0.03^{a}	5.77 ± 0.01^{b}
Dry matter content	%	86.10 ± 0.01^{a}	90.00 ± 0.01^{b}
Ash content	%	50.35 ± 0.07^{a}	0.90 ± 0.01^{b}
Pulp content	%	0.88 ± 0.04^{a}	0.00 ± 0.01^{b}

The WAI is higher than that reported in the literature for starches from the botanical source HAS. According to [8], the WAI was 1.17 g gel/g sample; in [14] a value of 0.334±0.014 g gel/g sample was recorded; in [9], the index varied between 3.66-5.54 g gel/g sample; and in the research of [30], the WAI ranged between 3 to 5.5 g gel/g sample, depending on the extraction solvent used.

This functional phenomenon is explained by the increase in branched sites in the amylopectin molecule, which facilitates greater water retention, the degree of coupling of hydrogen bonds in the polymer chains and an increase in the leaching and solubility of amylose when crystalline destructuring occurs [29]. These changes are associated with an increase in gelatinization, that is, the growth in the number of hydrogen and water bonds [14]. Likewise, significant differences were found (p < 0.05) in the behavior of WAI for corn starch, which could be attributed to a higher content of lipids, proteins, and the influence of the vegetal origin of starch, as pointed out by the authors [29].

The results for the WSI do not show significant differences between the botanical sources studied, being lower than those reported by [19] who obtained an WSI of 9.80% and [29] at 5.22%. This divergence could be related to factors such as amylose/amylopectin, polymeric chain length, molecular weight distribution, lipid, protein and soluble carbohydrate content of starch, as proposed by these authors [19, 29]. Although these parameters were not determined in our study, it is plausible that they influence the differences observed. Therefore, these sources do not have the desirable attribute to be used as thickening, gelling or stabilizing agents in the food industry [31].

Consequently, it can be said that processed HAS starch has low solubility and is therefore more resistant to swelling, has greater intergranular strength, and also has the quality to hinder the diffusion of amylose easily outside the granules, enjoys a lower formation of soluble short-chain amylose and a greater amount of hydrogen bonds [32, 33].

The evidence obtained in this study shows that the extracted starch has a higher swelling power (SP) compared to that reported by [8], who recorded a value of 1.50 g gel/g starch. It is also higher than that described by [31], who reported values lower than 2.00 g gel/g starch, and to the authors [29], who obtained swelling data between 3.57 to 3.68 g gel/g starch. However, similar results were found to those reported by [30] with a SP lower than 6.00 g gel/g starch, and to that of [19], whose evidence exceeds by 1.25 g gel/g starch. These differences in the SP can be attributed to the variability in the starch sources, the experimental extraction conditions and molecular structure of the polysaccharide, which highlights the importance of contextualizing the values obtained.

The limited SP is due to the composition of the starch species, particularly the amylose-lipid ratio, and the presence of a considerable number of crystals formed by long-chain amylopectin, which affects the crystalline phase at the granular level [8], which in turn increases the water retention rate and limits the swelling caused by amylose, thus reducing the hydration capacity [29].

In relation to the GT, it is relevant to mention that no significant differences were observed, that is, the origin of the starch did not influence the results of the test. In contrast to what was previously stated, the HAS starch exhibited a remarkable gelatinization temperature profile and excellent behavior in film formation. According to [34], the temperature scale is related to the variation in the amylose content and to a greater thermal stability, likewise, it indicates that the starches of different potato varieties showed a paste formation temperature between 62 to 66°C, differing by 1.30°C to the HAS starch. This difference is due to the relationship between amylose and amylopectin, because the latter affects the critical stability and the granular structure of the starch.

In the case of starches from HAS, the high gelatinization temperatures with peaks between 62 and 75°C, as reported by [30], are explained by the content of crystalline fractions associated with amylopectin, because more energy is required to break the glycosidic bonds, which increases the melting temperature, a fact that justifies the thermal behavior of the starch synthesized in this research, by agreeing on a gelatinization temperature of 67.30±0.57°C which is located in this range (p < 0.05).

The viscosity of the extracted HAS starch is higher by 913.20 mPa·s compared to the reference standard and exceeds the limit of 1500 mPa·s referenced by [18] for cassava starches. It should also be noted that there are significant differences in the viscosities between the botanical sources HAS and corn from the Tukey test statistic (p < 0.05).

With this in mind, the starch from HAS extracted at low concentrations in humid alkali reached a viscosity higher than that reported by [7, 8, 14, 30], who reported values of 1400

mPa·s, 1273 mPa·s, 1662 mPa·s, and 1196 mPa·s, respectively. This may indicate a favorable molecular reassociation behavior, indicating that "starch isolated from HAS is more stable under mechanical, heating and cooling processes, compared to corn or cassava starch" [30], however, this type of starch has not shown a high tendency to retrogradation, since this will depend on the combination of associative forces in the crystalline phase.

The pH of HAS starch is within the recommended limits for this property, around 4.5 to 7.0 [19], in accordance with starches extracted from various plant sources such as achira starch (Canna indica) with a pH of 5.91, cassava with 4.33 [35], platan in the range of 2.03 to 2.62 [36] and potato between 5.6 to 6.5 [37, 38]. The importance of determining the degree of acidity lies in its direct effect on gelation [35], since the pH tends to increase due to acid fermentation, a process that favors the growth of fungi and leads to the release of ammonia (NH_3) [38]. It is also important to distinguish that at pH values lower than 5 or higher than 7, the gelatinization temperature is reduced [39], therefore, depending on the pH level, the degree of intergranular ordering of the starch can be reduced, and thus the loss of a higher gelatinization temperature.

The dry matter, with a content of 86.10±0.01% means that the starch is harmless and free of microorganisms, complying with the limits recommended in the technical guide for starches [18].

The ash content showed low levels of contamination; therefore, it is possible to affirm that the starch presents good quality conditions, however, it is superior to that reported in the literature due to a higher mineral content in the HAS starch. In the case of starches extracted from the Hass variety avocado seed, the ash content has been 0.10% [19], 0.16% [13], 0.02% [8], 0.23% [7], 0.33% [14] and even 0.00% [3], figures that differ from that achieved by [40], with an ash level of 1.64%.

For HAS starch, the pulp content is 0.88±0.04%, which contrasts significantly with corn starch, since the latter did not show the presence of cellular tissue. This allows us to interpret that the pulp content is variable between the different starch sources ($p < 0.05$). The higher amount of fiber in HAS starch can be mainly attributed to the lack of fine or ultra-fine sieving after the extraction of starch powder. In contrast, "corn starch is finely sieved in order to be used as a raw material in the production of cereals, tortilla flours, dairy products, among other food products" [41].

The presence of 0.88% pulp meaning residual fibers could affect final viscosity, gel clarity, and the occurrence of syneresis or water release, especially in food or pharmaceutical applications. Therefore, finer sieving is essential for industrial-scale development [42].

3.3 Morphological characterization of HAS starch

Figure 2 shows the image obtained by Scanning Electron Microscopy (SEM), in which the morphology, distribution, and size of the starch granules are analyzed. Different particles of HAS starch free of impurities are observed, which confirms the effectiveness of the extraction method. Likewise, it is confirmed that these granules correspond to the polysaccharide starch, since its characteristic composition is that of carbon and oxygen atoms shown through X-ray Energy Dispersion (EDS) (see Figure 2B). The HAS starch granules have diverse shapes, among which the most notable are circular (see Figure 2AC), ovoid (see Figure 2AB), polygonal (see Figure 2AA), elongated rod-shaped, and ellipsoidal (see Figure 2AD and AE). In addition, they have "a smooth surface, characteristic of their vegetable origin, which also confirms the purity of the starch" [14, 43]. This smooth surface is due to the absence of pores, although small holes are usually found at their ends.

The particle size distribution of HAS starch it is characterized by 85.04% of particles with sizes between 7 to 19 μm, 10.11% from 21 to 43 μm and 4.85% in the range of 1 to 5 μm, with an average size of 12.87±5.29 μm. This shows a length behavior greater than that reported in this class of starches by [29], who identified sizes between 7 to 18 μm, as indicated by [7] with a distribution of 5 to 35 μm, and the average diameter of 21 μm reported by [4]. It is also compared with the granular size of 10.7 to 8.5 μm and the range of 10 to 22 μm described by [43]. According to [19], a larger particle size turns out to be beneficial in preventing the formation of conglomerates and in improving surface fluidity, so a smaller amount of energy is required for the molecules to group together.

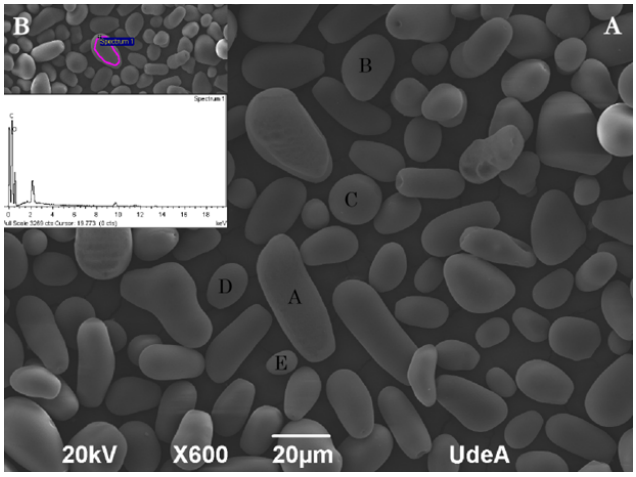

Figure 2: A) SEM micrograph of HAS starch morphology. B) Chemical composition analysis by X-ray Energy Dispersive Spectroscopy (EDS).

In this context, the observed particle size is similar to that reported for potato, cassava and corn starches, which can reach diameters of up to 43.10 μm [44, 45]. This larger granule size influences physicochemical properties such as viscosity, swelling power and water solubility. As the granule is larger, its swelling capacity increases proportionally, which in turn increases viscosity. Therefore, the results are consistent with reports in the literature and demonstrate coherence with the physicochemical properties of the starch extracted in this research, which exhibits a higher swelling power and viscosity compared to other HAS starches [46, 47].

However, it is important to note that, in terms of Feret diam-

eter, the particle size distribution mostly fits into a medium size, between 2.20 μm at the lower limit, 43.24 μm at the upper limit and an average of 12.87 μm, which is lower than the averages reported in the literature, since these range from 17.8 μm to 24.60 μm [4, 14, 27, 29]. This fact justifies the classification of HAS starch with a medium sizing, since one third of the particles have lengths between 15 to 43 μm.

Figure 3 shows the frequency distribution of the key morphological parameters, area and roundness, obtained through SEM image analysis. Regarding the spatial distribution of starch granule areas, an average size of 192.88±122.53 μm² was observed. Specifically, 20.18% of the granules had areas smaller than 100μm², 67.54% ranged between 100 and 300μm², and 12.28% exceeded 300μm². It should be noted that the minimum area recorded was 49.35 μm² and the maximum was 768.77 μm² (see Figure 3A).

To better understand the morphologies of starch granules, which can be circular, ovoid, polygonal, elongated or ellipsoidal, it is necessary to base ourselves on the concept of circularity. In this way, it is inferred that the most abundant granules called ellipsoids and ovoids, present a circularity of 0.80 to 0.90 corresponding to 51.75% of the total. The circular granules, with a circularity between 0.92 to 1.00, constitute 12.28%, while the elongated ones, with values of 0.70 to 0.75 represent 35.09%, and the polygonal ones, with a circularity of 0.62 to 0.65, 0.88% (see Figure 3B).

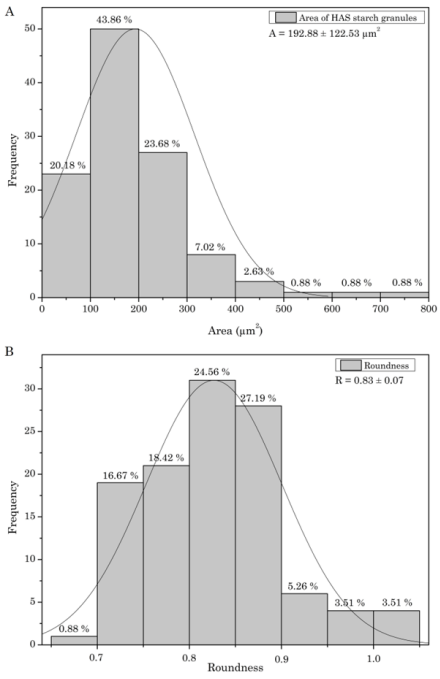

Figure 3: A) Granulometric frequency distribution of area for HAS starch B) Granulometric frequency distribution of roundness for HAS starch.

3.4 Structural characterization of HAS starch

The results of the XRD analysis allowed to identify the presence of Amylose $(C_6H_{10}O_5)$ x, α-Amylose dihydrate $(C_{18}H_{30}O_{15} \cdot 2H_2O)$ and a mixture of Amylose-amylopectin $(C_{21}H_{20}O_9)$. Figure 4 shows the characteristic diffraction pattern of Hass avocado seed starch, which allows the identification of its crystallinity type. Likewise, they are distinguished in 4 Å. corresponding to the angle 17.14°, a high coincidence with potato starch. The analysis was carried out by comparing the diffraction patterns of the HAS starch sample with those recorded in the PDF-ICDD databases, specifically PDF cards No. 00-031-1536, 00-062-1691 and 00-063-1339. The crystallinity pattern obtained corresponds to type B, with notable intensity peaks at 17.14° and 22.12° (high), 14.90° (medium) and 19.34° (low) in the spectrum measured at angle 2θ (see Figure 4), which are consistent given their similarity to that reported for starches from the botanical source HAS, according to the research of [8, 29], and with the values of potato starch reported by [44, 46].

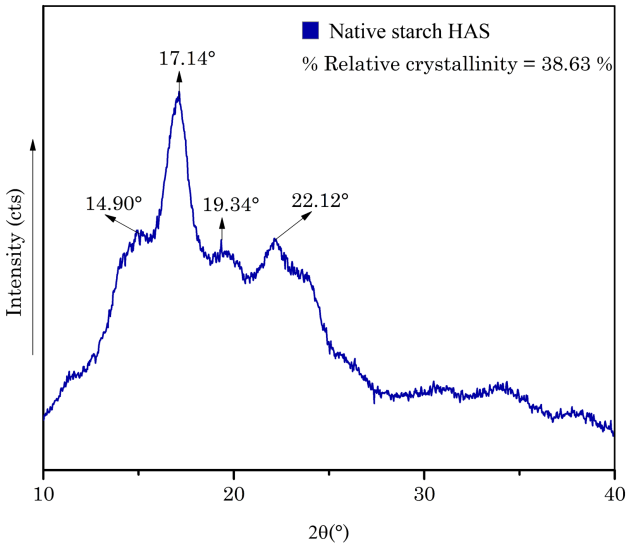

Figure 4: XRD diffractogram of native HAS starch.

Regarding the HAS type B starches, it is important to say that their structure is configured within a hexagonal polymorphic unit cell, in which different water molecules are distributed in an irregular and asymmetrical manner. These molecules, 36 in total, are located in the central channel of the double helices, but not inside them, which suggests a higher level of hydration compared to type A starches. It should be noted that the close relationship between amylose and amylopectin is based on a hexagonal distribution [48].

It is worth adding that, although there are reports on other crops, such as starch from Mill-type avocado seeds, information about the XRD patterns and the crystalline or amorphous nature of Hass avocado seed starch is still limited. This makes this study one of the few works focused on the structural characterization of this type of starch.

The relative crystallinity of 38.63% for this type of starch is higher than that reported by [29] with 17.23%, by [49] with 13.03%, and by [27] with 25.70%. It is also higher than that of lotus seed starches at 1.49% [33], yam at 6.63% [50], corn at 0.40% [51], cassava at 13.83% [52], and rice at 12.93% [53]. However, it is lower than the 56.09%, the highest

crystallinity so far found by [8] and [54]. It is understood that the crystalline phase in the range of 38% is attached to the amylopectin molecule, thus typifying highly amorphous starches [55].

It is relevant to highlight that the method at low solvent concentrations is considered suitable for obtaining a starch with a higher degree of crystallinity. This improves the amylose/amylopectin ratio, the success of which results in the formation of crystallites that make the starch more resistant. This results in a greater molecular association, an increase in the melting and gelatinization temperature, as well as greater thermal and granular compaction and stabilization [30, 45].

3.5 Chemical configuration of HAS Starch

The FTIR spectrum of HAS starch, shown in Figure 5, displays the characteristic absorption bands that reveal the presence of typical functional groups of starch, allowing the identification of its molecular structure and confirming its purity and chemical composition. The stretching band observed between 3000 to 3750 cm^{-1} the hydroxyl bonds OH, which are attributed to water molecules. In the range of 2750 to 3000 cm^{-1}, specifically at the sharp wave number of 2927.94 cm^{-1}, the presence of C-H bonds is observed. In the transmittance width vibration at 1639.49 cm^{-1}, the absorption of the hygroscopic water molecule OH in the amorphous regions of the starch is detected [21, 56]. The results mentioned coincide, as expected, with those reported for Hass avocado seed starches [7, 13, 19, 29], and corroborate the quality of the starch by being analogous to the polysaccharide extracted from the botanical source in question.

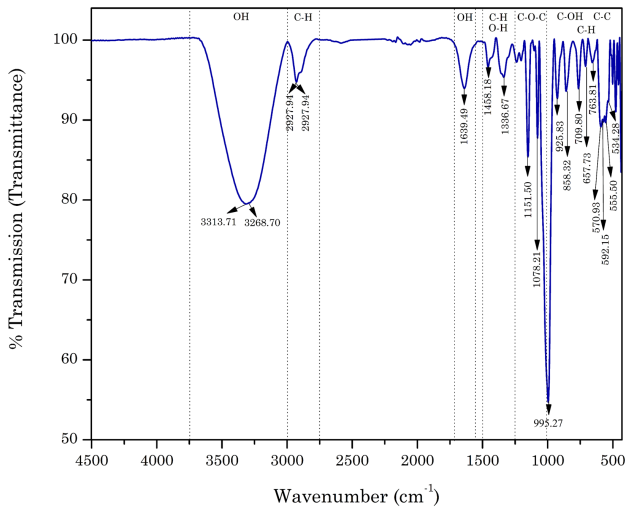

Figure 5: FTIR infrared spectrum for starch obtained from HAS.

In the angular deformation region, between 1250 to 1500 cm^{-1}, the peaks observed at 1458.18 and 1336.67 cm^{-1} correspond to C-H and O-H bonds. The peaks with wavenumbers at 1151.50 and 1078.21 cm^{-1} are associated with the vibration of the C-O-C ether bonds in the bending zone, related to amylopectin [10]. The vibration at 995.27 cm^{-1} can also be highlighted, which indicates the presence of 1,4-glycosidic C-O-C bonds [19]. In the fingerprint region, the functional groups C-C, C-OH and C-H stand out, corresponding to the D-glucose rings [27], whose molecular association belongs to amylose, where the polymer and its hydration are configured [21].

3.6 Thermal analysis of native HAS starch

Figure 6 shows the thermal analyses performed on the starch extracted from Hass avocado seeds. In panel A, the TGA/DTGA analysis identifies the mass loss stages associated with the thermal decomposition of the starch. In panel B, the DSC analysis reveals the characteristic thermal transitions, such as the gelatinization temperature, highlighting the thermal stability and calorimetric behavior of the material. The TGA and DTGA results for the native starch from HAS are presented in Figure 6A. As expected, mass losses are observed at three moments, which is characteristic of this type of starch.

These weight losses remain stable between period one and the subsequent stage [14]. During the first stage, which spans approximately 25 to 120°C, "water evaporation and release of volatile compounds" [55] occur, in the order of 13.84%, a value higher than that shown by [3] of 5 to 9% before 200°C, being attributable to a greater amount of the mentioned substances, especially bound water.

In a second instance, the first degradation of the organic matter content occurs at 220°C, which could be defined around 63.16% with a maximum peak at 380°C. Simultaneously, depolymerization occurs or, in other words, thermal stability is achieved at 305°C, as evidenced by the DTGA curve. This result is 78°C higher than that reported by [57] for potato starch and 55°C in wheat starch [58]. This difference is due to "a higher crystalline portion associated with the amylopectin molecule, which leads to a shift of the decomposition to higher temperatures" [10]. In addition, it can be explained by the "release of some glucosidic derivatives and compound gases" [55], or by the "dehydration of the hydroxyl groups of the glucose ring and the release of low molecular weight substances" [59].

At time three, the temperature rose from 380.13 to 590.37°C. According to [14], in this phase "oxidation of organic matter" occurs, which causes a weight loss of 21.73% and the formation of ash as a residue of the thermal study process.

The DSC transitions results for the onset (Ti), maximum (Tm) and final (Tf) temperatures of HAS starch were 58.70°C, 110.2°C and 182.32°C, respectively (see Table 2). These values indicate that the maximum temperatures of native HAS starch in this study are higher and do not coincide with those reported in previous investigations [14], where the maximum peaks were less than 100 °C. Likewise, they are notably higher compared to the values reported by [56, 60] in native or acetylated potato starches, and HAS starches [27, 30] whose maximum (Tm), final (Tf) temperatures and melting enthalpies (ΔH) do not exceed 67 °C, 85°C and 15 J/g, respectively. This reaffirms the high crystallinity evidenced in the XRD patterns. It is crucial to emphasize that a higher crystalline phase influences the increase in initial temperature (Ti), suggesting "the formation of a more stable structure

Table 2: Thermal properties DSC of HAS starch. Ti = Initial temperature, T_m = Maximum temperature, T_f = Final temperature, ΔH = Enthalpy of gelatinization, T_r = Total temperature range of gelatinization.

Specimen	Peak	T_i (°C)	T_m (°C)	T_f (°C)	Enthalpy ΔH (J/g)	$T_r(T_f - T_i)$ (°C)
HAS starch	P1→	58.70	110.23	182.32	541.76	123.62

with stronger chemical bonds, a higher melting point and a better interaction between ionic and atomic bonds" [30]. In other words, the resulting structure is distinct from the botanical sources of HAS currently investigated.

The enthalpy and the gelatinization temperature range (Tr) were 541.76 J/g and 123.62°C consecutively, which is consistent with a "higher energy requirement to produce gelatinization" [30] (see Figure 6B). These "maximum temperatures are typical of the double helix structures of starch" [55]. Thus, the enthalpy of 541.76 J/g in the HAS starch allows to affirm the crystalline stability of the material achieved thanks to the extraction process, that is to say, the shorter chains are not restructured nor the amorphous areas increase.

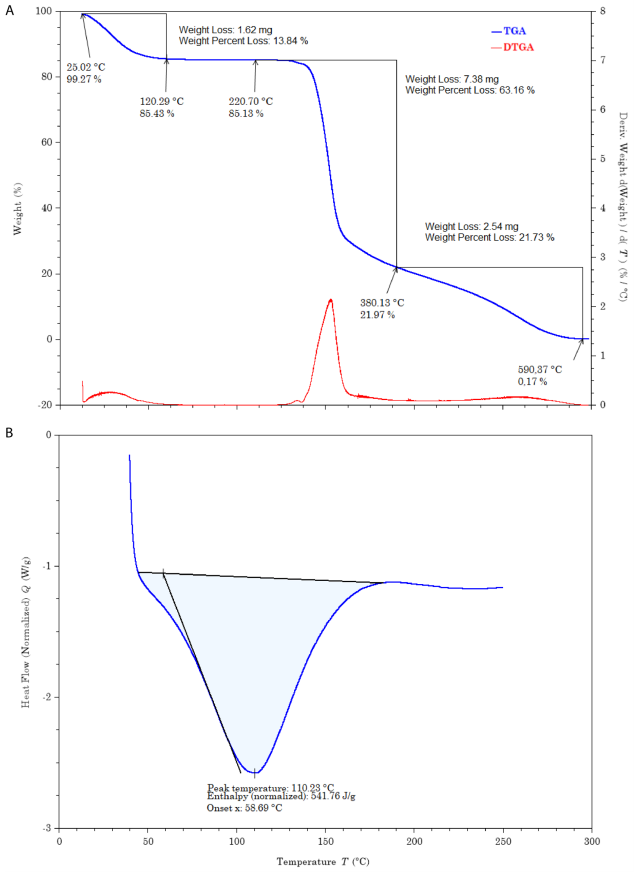

Figure 6: A) TGA and DTGA analysis of HAS starch B) DSC analysis of HAS starch.

3.7 Technological applications of HAS starch

The starch extracted by the wet alkali method has a B-type structure with high crystallinity, a lower amount of soluble amylose, better stability in the amylose/amylopectin ratio, and a higher proportion of double helix structures. It also stands out for its high-water absorption capacity, swelling power, low water solubility, and higher viscosity. Therefore, starch processed by this method is suitable for various industrial applications, both food and non-food, such as:

In food systems, in view of the physicochemical, structural, thermal and granular attributes, they can be applied in the production of thickeners, stabilizers, emulsions, gelling agents [8, 14], frozen foods, resistant starches [59].

While in the non-food industry, its applications are favorable for the plasticization of biodegradable materials [4, 8, 41] the development of films for single-use products [33], the advancement of agrobiotechnological technologies, and packaging coatings [29, 31, 37].

4 Conclusions

The starch extraction yield achieved in this study, using low solvent concentrations with $Na_2S_2O_5$ and NaOH, was 14.93%, a value that is within the range reported in the literature for Hass avocado seed. Also, an improvement in color appearance was observed, since enzymatic darkening was moderated, and savings in water use during starch washing were achieved. In summary, the extraction method, seed variability and precipitation time are key factors in the wet alkali process, since they allow for optimization of extraction and affect the results obtained.

The starch extracted from Hass avocado seed showed a higher water absorption index and viscosity, suggesting a higher water retention capacity, improved structure and higher intergranular strength. Moreover, its behavior in terms of swelling and gelatinization temperature reveals a more stable and resistant composition. Overall, Hass avocado seed starch presents suitable physicochemical properties that could expand its use in various applications in both food and non-food systems.

The Hass avocado seed powders, extracted and free of impurities, have been confirmed as starch polysaccharides by SEM and EDS analysis. The particle size distribution denotes an average size of tenths of microns, larger than that reported in previous studies. The absence of conglomerates and a greater surface fluidity, observed in the SEM analysis, reinforce the physicochemical properties of starch, contributing to an increase in viscosity and swelling power.

The X-ray diffraction (XRD) of HAS starch shows a type B structure with peaks at 17.14° and 22.12°, similar to potato starch. The degree of crystallinity is 38.63%, which is above the values of other polysaccharide sources, although lower than the maximum crystallinity recorded in HAS starches. Its hexagonal structure shows a high level of hydration, highlighting the importance of this research in the structural characterization of starches from HAS.

The FTIR spectrum of HAS starch exhibits characteristic bands that confirm its quality and composition. The detected absorptions show the presence of functional groups such as hydroxyl (OH), C-H, O-H, C-O-C, C-OH, and C-C bonds, typical of amylose and amylopectin molecules and their hydration. Likewise, 1,4-glucosidic bonds and D-glucose rings are identified, which are representative of HAS starches.

The thermal properties of HAS starch indicate a high thermal stability in the range of 305°C, with three stages of mass losses, mainly related to the elimination of water, volatile compounds and organic matter. The transition temperatures measured by DSC are remarkably higher than those reported in the literature for HAS starch, specifically regarding the maximum or peak temperature, suggesting an improved and stable crystalline structure. Furthermore, the gelatinization enthalpy of 541.76 J/g reinforces the structural stability of the starch.

The use of HAS promotes intelligent utilization and can contribute to food security by reducing dependence on food inputs such as potatoes, cassava, corn, bananas or similar, which contributes to the balance in global food consumption. By incorporating native starch from HAS into the bioeconomy model, its valorization advantages are taken advantage of, since it can serve as raw material in specific industries with alternative uses.

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Authors Conflicts of Interest

The authors declare that they have no financial, professional, or personal conflicts of interest in the research manuscript reported in this document.

Authors contributions

Edgar Farid Carreño Flórez: Conceptualization, Methodology, Formal Analysis, Investigation Development, Drafting and Original Writing, Project Administration. Carlos Alberto Meza Barbosa: Conceptualization, Methodology, Formal Analysis, Investigation Development, Drafting and Original Writing, Project Administration. Gabriel Peña Rodríguez: Conceptualization, Methodology, Formal Analysis, Review and Editing, Acquisition of Funding, Project Supervision.

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