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Extraction and characterization of the starch present in the avocado seed (*Persea americana mill*) for future applications



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ARTICLE INFO ABSTRACT Keywords: Agro-industrial waste causes environmental impacts, and the use of new technologies and materials emerge as Residue minimization alternatives. This work aimed to extract and characterize starch from avocado seeds for future Lump applications. The grains were removed, the starch extracted with a yield of (19.54%), moisture (41.35%), ash Avocado (0.33%), titratable acidity (4.64%), lipids (1.68%), proteins (1.60%) and carbohydrates (55.07%). The water Starch activity was 0.986 g/g, the water absorption index 0.333 g/g, the oil absorption capacity 0.691 g/g, and the water solubility 0.888%. The transmittance was 14.30%. The most significant water release in the third freeze cycle. The extracted starch has a high amylose content (39.56%). Thermogravimetric analysis showed stability up to 366 °C, and the granule had an oval shape and smooth surface. With regard to pasting properties of the starches, RVA shows an initial temperature of 88.5 °C and a viscosity of 2880.5 cP at setback. The results suggest that the starch extracted from the avocado seeds provides opportunities for further application of this material to prepare edible and/or biodegradable films.

1. Introduction

Fruit farming is one of the most prominent sectors in Brazilian agribusiness because Brazil is the third-largest producer of fruit in the world, behind only China and India. There is a wide variety of crops produced throughout the country in different climates therefore fruit growing achieves expressive results and generates opportunities for small Brazilian businesses. The fruit growing sector in Brazil is expanding, about 53% of the production is sold in fresh form, and 47% is destined for the agro-industrial sector for the production of juices, teas, frozen pulps, jellies, and others [1]. Per capta fruit consumption in Brazil and in the world should continue growing, according to the Food and Agriculture Organization of the United Nations [2]. Fruit farming in the country covers around 3 million hectares, generating at least 6 million direct jobs, and 3% of the 37 million tons produced is exported (Abra-frutas, 2019). Despite the global crisis caused by COVID 19 [3], Brazil's Gross Domestic Product (GDP) grew between 2019 and 2020.

This high consumption of products from the agroindustry is responsible for a greater demand for natural resources, and therefore, a large amount of waste and emissions can be generated, increasing environmental impacts [4]. Brazil is also one of the largest contributors to the world production of agro-industrial residues, mainly fruit processing by the pulp industries [5]. A large part of the waste generated in fruit processing is discarded in the environment, used for composting purposes, or used as a food source for animals due to its low cost [6]. According to Costa et al. [7]; during the last 30 years, the environmental impact of agro-industrial activities has become a constant concern of environmentalists, legislators, customers, public authorities, and society in general. The world production of agro-industrial residues is estimated to reach 1.3 billion tons per year, considering that 1/3 of the food potentially destined for human consumption is wasted, either as waste after processing or as loss in the production chain.

The constant search for alternatives that aim to reduce the impacts caused by waste has been highlighted in recent research. The search for new technologies, new materials, and new ways of use requires investigating these residues' composition and nutritional value. Their efficient, economic and safe use must further be investigated when developing new products [8]. Among the alternatives for the reuse of

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agro-industrial residues, Jacob et al. [9] studied the production and characterization of starch biofilms for packaging to replace materials such as polyethylene, provided that these packagings offer better degradation time when compared to those from conventional polymers. Several materials have been studied to replace conventional polymers. Historically, the main natural materials studied for preparing edible films or coatings are starch, proteins, cellulose derivatives, alginates, pectins, and other polysaccharides [10,11].

Several actions that encourage research for packaging development from alternative and renewable sources are becoming a reality [12]. Biodegradable films are an excellent alternative because they are environmentally friendly in addition to being biodegradable and renewable [13]. These films can be used as an alternative for transporting and releasing food additives, such as antioxidants and antimicrobials [14], for reducing the weight and waste of packaging, for seeds encapsulation, as well as for covering fruits in plantations [15].

Starch is the primary source of carbohydrates among the existing polysaccharides, widely applied in industry. Starch extraction has a consolidated market and an estimated yield of 77 billion dollars for the year 2018 [16,17]. Besides being used as an ingredient in numerous industrial processes in the food sector, starch is also applied in textile, cosmetics, pharmaceutical, packaging, synthetic polymer industries, and other technological processes [18]. Starch has some attractive characteristics, is available in nature, has relatively low prices and good performance [19]. Its renewable source is another highly relevant issue, making its production economically viable [20]. The use of starch is a great opportunity, and some studies can be highlighted: the production, and mechanical and barrier characterization of biodegradable sweet potato starch films [21]; the development and characterization of macaçar bean starch-based films (Vigna unguiculata (L.) Wap) [7]; the use of starch from peach palm fruit (Bactris gasepaes Kunth.), its isolation, granule characterization and application in biodegradable thermoplastic as an opportunity to reuse industrial waste [22].

The starch comprises of two types of glucose polymers, amylose and amylopectin, with different structures and functionality. Amylose is a linear polymer composed of D-glucose units linked by α - $(1 \rightarrow 4)$ bonds, with polymerization degrees from 200 to 3000, depending on the starch source. Due to their linearity, amylose molecules, when in solution, tend to orient themselves parallel to each other, getting close enough to form hydrogen bonds between hydroxyls of adjacent polymers. Consequently, the polymer affinity for water is reduced, favoring the formation of opaque pastes and resistant films [23]. Amylopectin is a highly branched polymer, with D-glucose units linked through α - $(1 \rightarrow 4)$ bonds and branches in α - $(1 \rightarrow 6)$ [24]. The linear part of the amylopectin molecules forms double helical structures, stabilized by hydrogen bonds between hydroxyl groups, giving rise to the crystalline regions of the granules. The amorphous region is composed of amylose chains and amylopectin branches [25].

Starch can be obtained from industrial residues as seeds of fruits, mostly underused as ingredients for feed and fertilizers. The avocado seeds are an alternative source for extracting starch since it is considered industrial waste. The seed composition of the avocado fruit, when in natura, presents a large amount of oxygen (48.4%), carbon (44,6%), hydrogen (6,3%), and nitrogen (0,7%), where 97,2% of its weight is related to organic compounds, and the remaining 2,8% corresponds to silicon and potassium oxides [26]. Avocado (Persea americana Mill.) is a fruit originating in Central America, and cultivated in several countries. Brazil is the eighth largest world producer [2], having reached a production of more 180 thousand tons in 2015 (IBGE/PAM, 2016).

Builders et al. [27] and Brito [28] studied the composition and contents of amylose and amylopectin present in the starch of avocado seeds, whereas Oliveira et al. [29] studied protein levels in avocado varieties. Despite of that, few studies reported in the literature address avocado seeds. This paper aims to extract and characterize the starch extracted from the avocado seed for application in the development of biodegradable and active films. This study will support potential applications of the starch from avocado seeds, adding value to a residue from agroindustry and offering new sources of renewable feedstock for biopolymer production.

2. Material and methods

2.1. Material

Avocado fruits (120 unripe fruits) were purchased from the supply center in Feira de Santana-Ba in Brazil, produced in Lavrinhas, s/n – Venda Nova do Imigrante – ES and sold by Peterfrut, the only supplier with traceability code 13125. These fruits were manipulated in the Laboratory of Dietary Techniques at Faculdade Pitágoras Feira de Santana, and the residues were made available for this study.

2.1.1. Preparation of material

To ensure the monitoring of the ripening process, the unripe fruits were packed in paper bags. The fruits were later pulped and their seeds reserved for later removal of the integument present. Randomly, the skins were removed from the pits, which were washed and kept under freezing until the time of testing. These were ground in a Willye Micro Mill Type TE-648 to homogenize the sample as each batch was carried out for starch extraction.

2.2. Starch extraction and yield

Starch extraction was performed based on the method by Loos et al. [30] adapted by Silva et al. [31]. The avocado pits, already washed and cut into small pieces, were infused with distilled water containing sodium metabisulfite (0.2% v/v) for 24 h under refrigeration. Starch was extracted by crushing the raw material with 0.2% (v/v) sodium metabisulfite solution in an industrial mixer for 5 min. After homogenization, the mixture was sieved through 200 mesh (0.074 mm). Decantation was performed for 24 h with resuspension in 0.2% sodium metabisulfite solution (v/v) and centrifuged at 1105 g for 12 min, and the supernatant was discarded. The mucilage formed on the surface of the residue was removed. The starch residue obtained was dried in an oven at 40 °C for 12 h and stored in a clean container under refrigeration. The yield (RSA) of avocado seed starch was calculated according to:

RSA
$$\left(g.\frac{100}{g}\right) = \frac{me(g)}{mc} \cdot 100$$
 (1)

where me is the mass of dry starch after extraction and mc is the mass of the seed without husk.

2.3. Starch characterization

2.3.1. Moisture content

To determine the moisture content, standards were used in accordance with the Analytical Standards of Instituto Adolfo Lutz (2008). The Moisture Content was calculated according to:

$$TU\% = \frac{(MRAU(g) - MRAS(g))}{MAU(g)} \cdot 100$$
 (2)

where MRAU is the vessel/wet sample mass, MRAS is the vessel/dry sample mass and MAU is the wet sample mass.

2.3.2. Proteins

For the quantification of proteins, the method of determination of total nitrogen, adapted from Kjeldahl [32] was performed. The total nitrogen was determined as:

$$N\% = VHCl \cdot 0.1 \cdot f \cdot 14 \cdot \frac{100}{P}$$
(3)

where N% is the total nitrogen in the sample, in percentage; VHCl is the

volume of the hydrochloric acid solution used in the titration of the sample, in milliliters; f is the correction factor for 0.01 mol/L hydrochloric acid and P is the sample mass, in grams. After the calculation of total nitrogen, crude protein was determined, multiplying the value of total nitrogen by the factor 6.25 (the nitrogen converter).

2.3.3. Lipids

The analysis of total lipids was performed by exhaustive extraction with hexane in the soxhlet apparatus, according to Williams et al. [33].

$$TL\% = \frac{(Balloon and oil weight (g) - balloon weight(g)) \cdot 100}{Aliquot (g)}$$
(4)

2.3.4. Ashes

To determine the Total Ash Content, standards were used according to the Analytical Standards of Instituto Adolfo Lutz (2008), according to:

$$TC\% = \frac{(MRAC (g) - MR(g))}{MA (g)} \cdot 100$$
(5)

where MRAC is the calcined vessel/sample mass, MR is the vessel mass and MA is the sample mass.

2.3.5. Carbohydrates

The determination of the probable content of carbohydrates in the sample was carried out by the difference between 100 (total percentage) and the sum of the percentages found for moisture, ash, protein fraction and fat, according to:

$$TCarb\% = 10 - (\%moisture + \%ashes + \%protein + \%lipids)$$
(6)

2.3.6. Determination of water absorption index (IAA), oil absorption capacity (CAO) and water solubility (SA)

To determine the water absorption index (IAA), the methodology of Okezie and Bello [34] was applied. To determine the oil absorption capacity (OAC), the same methodology was used using soybean oil instead of water. For Solubility in Water (SA), the supernatant liquid obtained from the IAA was used, drying in an oven at 105 °C until constant weight. These indexes were computed according to:

$$IAA = \frac{\text{water absorbed by sample }(g)}{\text{sample weight }(g)}$$
(7)

$$CAO = \frac{\text{oil absorbed by the sample } (g)}{\text{sample weight } (g)}$$
(8)

$$SA = \frac{\text{evaporation residue } (g)}{\text{sample weight } (g)} \cdot 100$$
(9)

2.3.7. Total titratable acidity

The total titratable acidity was determined by titration with 0.1 N NaOH, using a 1% phenolphthalein solution as indicator and expressed in mL of 0.1 N NaOH per 100 g of sample [35,36].

2.3.8. Clarity of starch paste

To determine the clarity of the paste, the methodology described by Lawal [37] was used. In the process, the retrogradation trend was monitored, the samples were stored for 24 h at 4 $^{\circ}$ C for nucleation. They were stored afterward at 30 $^{\circ}$ C for 1–9 days to determine the absorbance on days 1, 2, 3, 5, 7, 8 and 9.

2.3.9. Starch freeze and thaw cycles

The three repetitions of the sample were suspended at the proportion of 8% (p.p–1) in deionized water, gelatinized and kept in boiling water, and stirred for 10 min. The gel was divided into three 50 g portions and frozen (–18 °C) in airtight plastic containers. The samples were subjected to three freezing cycles, each 72 h. All samples from the first,

second and third cycle were frozen at $-18\ ^\circ C$ and thawed at 45 $^\circ C$ for 3 h [38].

2.3.10. Determination of water activity (Aw)

Starch water activity (Aw) data were obtained by the dynamic method, with the Aw tester (Aqcua Lab, CX-2, Washington, USA), at a constant temperature of 24 \pm 1 °C.

2.3.11. Amylose content

The enzymatic analysis and verification of the amylose content were performed according to the guidelines described in the Megazyme Assay Kit for Amylose/Amylopectin Assay Kit manufacturer's manual. The calculation of the amylose content was performed as follows:

Amylose content (%) =
$$\frac{\text{Abs. do Com A (g)}}{\text{Abs. total starch(g)}}$$
.66.8 (10)

2.3.12. Thermogravimetric analysis (TG)

The starch extracted from the avocado kernel was analyzed by TG, using a Shimadzu TG 50 type thermal analyzer. The samples were heated from room temperature to 600 °C with a heating rate of 10 °C/ min in an air atmosphere.

2.3.13. Paste viscosity (RVA)

Viscosity was performed on a Rapid Visco Analyzer 4 RVA (Newport Scientific Pty Ltd., Warriewood, Australia) (Santos et al., [39]. starch suspension (3 g starch in 25 mL of water) adjusted to 14% moisture (wet basis). The time-temperature profile included mixing with the spoons, rotating at 960 rpm for the first 10s and 160 rpm until the end. The samples were heated between 50 and 90 °C at a constant rate of 6 °C/min, and then cooled to 50 °C. The bonding curve readings were the maximum peak viscosity, paste temperature, rupture viscosity, final viscosity and regress or retrograde viscosity.

2.3.14. Differential scanning calorimetry (DSC)

The starch samples were analyzed by power compensation DSC, using the Perkin Elmer DSC 8000 model equipment, with heating at 10 $^{\circ}$ C per minute, between the temperatures of 40 and 240 $^{\circ}$ C.

2.3.15. Scanning electron microscopy (SEM)

For SEM analysis, samples were diluted in 100% ethyl alcohol (1/10 p.v -1) and placed two drops in the stubs. After this procedure, the samples were covered with 10 mm of metalizing gold (MED-010 from Balzers) and analyzed in a Scanning Electron Microscope (SEM 515 Philips), under a voltage of 15 kV or 20 kV.

3. Results and discussion

3.1. Starch extraction yield

The yield observed in the starch extraction process from avocado seeds was 19.54%, similar to the 20.1% found by Kowalski et al. [40] and higher than the 11.36% found by Silva et al. [31]; when extracting starch from the avocado kernel. Different values are observed when comparing different types of raw material for starch extraction because of its species and extraction method. Vegetable raw material, extraction method, and soil composition are factors that directly influence the yield of the starch extractive process [41]. Another relevant factor is the avocado variety, which can directly affect the extraction results.

3.2. Centesimal composition

The average moisture found in the avocado seeds was 63.37%. Moisture values for avocado seeds converge with those found in other studies reported in the literature: Melo et al. [42] found the moisture values of avocado seeds at 62.78%; Tango et al. [43] characterized pits

Table 1

Chemical composition of starch extracted from avocado seeds.

Analysis/Product	Moisture content (%)	Ash content (%)	Titratable Acidity (%)	Lipids (%)	Proteins (%)	Carbohydrate Content (%)
Starch extracted	41.35	0.33	4.64	1.685	1.60	55.074

Source: the author

of different varieties of avocado, finding values between 53.6% and 73.9%; Silva et al. [44] obtained mean values of 61.9%.

The proximate composition of the starch extracted from the avocado seed is shown in Table 1. The moisture analysis of starch extracted from avocado seeds presented values much greater than those observed in the literature. [28]; when analyzing starch extracted by different methods from avocado seeds, found an average moisture value of 10.59%. Rengsutthi and Charoenrein [45] found values of 9.59% moisture in jackfruit starch and 11.74% in corn starch. Falade & Ayetigbo [46] analyzed the moisture in tubers and reported mean values of 14.87%. The moisture in the seed depends on the variety and drying conditions to which the seeds were submitted. Knowledge of the moisture content of the samples is essential, as this indicates the water content present in the sample, also demonstrating important parameters regarding its degradability such as stability, quality, and composition, parameters that define the degree from sensitivity to deterioration. It is noteworthy that the moisture in the kernel depends on the variety and drying conditions to which the kernels were submitted.

The average value of ash found in the avocado kernel was 1.07%, which is lower than those found in the literature. The ash content found in avocado species studied by Melo et al. (2014) was $2.21 \pm 0.02\%$, whereas the value found by Tango et al. [43] was 2.07%. This comparison indicates the smaller amount of inorganic residue, which is mainly made up of minerals, in the investigated samples. Ash determinations in plant samples indicate the minerals present in the sample or, in some cases, residues of products used during sample preparation. The analysis of ash content can help, for example, in predicting contamination with sand or soil from which the sample was extracted [47–49].

The Lipid Content found in the extracted starch sample is close to the values reported by Cladera-Olivera et al. [50]; $1.24 \pm 0.09\%$ for pine nuts. According to Buléon et al. [51]; there are three types of minor components associated with starch granules, among which there are the particulate materials, the internal components and the surface components. In a study that sought to characterize starch isolated from different sorghum cultivars, the lipid contents did not vary according to the type of sorghum [52]. Ehtiati et al. [53]; in a study with white sorghum, reported a lipid content of 0.08%. Rivera-Corona [54]; on the other hand, found 0.88% at sweet sorghum.

The Protein Content may reveal the easiness to extract and purify the starch. When analyzing the protein content present in several avocado varieties, Oliveira et al. [29]; observed results ranging from 0.74 to 1.9% of proteins. Henriquez et al. [55] evaluating native pine seed starch found values for protein 0.94%.

The mean Aw value in the avocado seed starch sample was 0.986. Neto [22] analyzed the water activity of starch extracted from peach palm (Bactris gasepaes Kunth.), obtaining an average of 0.550, a value below that found in the starch extracted in this study. According to Neto [22]; there are no legal parameters for measuring water activity. Lima et al. [56] characterized the starch extracted from the organic arrowroot and observed a value of 0.32. According to Gutkoski et al. [57]; the value found by Lima et al. [56] may guarantee the stability of the product, hindering the development and growth of groups of microorganisms with potential for damaging the starch, limiting its application. The literature reports that AW < 0.60 for microbial growth in food products [58,59]. In addition, if 0.4 < Aw < 0.8, chemical and enzymatic reactions are trigged [60].

The water absorption index (IAA) had a mean value of 0.334 ± 0.014 (g/g). This value indicates the amount of water absorbed by the starch

Table 2				
Effect of storage	time on	starch	paste	clarity.

Repetitions	% of Transmittance (650 nm)							
	Day 01	Day 02	Day 03	Day 05	Day 07	Day 08	Day 09	
R1	14.6	13.7	12.5	11.2	10.1	9,3	8.4	
R2	13.9	12.8	11.9	10.7	9.9	8,9	8.1	
R3	14.3	13.2	12.4	11.6	10.8	9,3	8.5	
Average	14.3	13.2	12.3	11.2	10.3	9.2	8.3	

Source: the author

granules in a given sample submitted to a heat treatment; therefore, it is considered a hydration property [61]. Ferreira et al. [62] observed in organic arrowroot starch a water absorption index ranging from 0.79 to 0.83 g/g. High water absorption in starch is desirable for products with a high amount of water to increase the product's viscosity [63].

The oil absorption capacity (OAC), which is a variable that is mainly related to the binding of protein parts of the sample to oil molecules, presented a value of 0.691 ± 0.032 (g/g). Ferreira et al. [62] observed a variation between 1.05 and 1.14 g/g when analyzing the starch from the organic arrowroot kernel. This is related to the starch's ability to hold oil in its structures in addition to the presence of hydrophobic groups [64].

Solubility in water (SA) presented a value of 0.888 \pm 0.016%. It reflects the degradation suffered by the fiber constituents, that is, the sum of the effects of gelatinization, dextrinization and, consequently, solubilization (Gutkoski, 2003).

According to Lawal (2004a), starches present a hydrophilic tendency; however, this decreases as the starch is submitted to the acidification process. Grossmann (1986) highlights that the IAA and the SA have variations correlated to the degree of dextrinization suffered by the starch during the extrusion process. The IAA increases with increasing gelatinization because, when gelatinizing, there is an increase in free hydroxyls to form hydrogen bonds with water. As for SA, there is a growth with increasing dextinization, that is, the greater the degradation of starch molecules, the greater the solubility.

Machado et al. [65] analyzed fermented and sun-dried cassava starch, and the result of the water absorption index at a temperature of 90 °C was 4.07 g/g. Bezzea et al. [66] analyzed the starch extracted from the breadfruit, presenting a water absorption index (IAA) of 1.06 ± 0.07 (g/g) and an oil absorption capacity (CAO) of 1.44 ± 0.08 (g/g).

According to Carvalho et al. [67]; the solubility in water is related to the number of soluble solids in a dry sample, allowing to verify the degree of severity in the treatment as a function of degradation, gelatinization, dextrin, and consequent starch solubilization. For Hoover [68]; when starch is heated with excess water and subjected to temperatures above those expected in the gelatinization process, the crystal structure is broken due to the relaxation of hydrogen bonds. In these cases, water molecules interact with the hydroxyl groups of amylose and amylopectin, causing an increase in granule size due to swelling, leaving it partially solubilized.

The titratable acidity presented a value of 4.64 \pm 0.358%. Ferreira et al. [62] analyzed the starch from the organic arrowroot kernel and found for the titratable acidity the value of 6.68%. After performing a clarity analysis of the paste for the starch extracted from the avocado seeds, after 24 h, an average transmittance of 14.30% was observed, reaching a value of 8.3% on the ninth day of observation. The values of the three repetitions, obtained along the storage time, are shown in

Table 3

Starch freezing and thawing cycles.

Repetitions	Cycle 01 (%)	Cycle 02 (%)	Cycle 03 (%)
R1	32.8	55.9	68.2
R2	33.1	56.6	68.7
R3	31.9	56.3	68.5
Average	32.60	56.27	68.47

Source: the author

Table 4

Val	lues of	amyl	lose	and	amy	lopectin	present	in	avocad	lo seed	l starch	n.
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	Amylose (%)	Amylopectin (%)	Reference	
Avocado starcl	h 39.56	60.44	The author	
Avocado starch	32.80	67.20	[28]	
Avocado starch	32.50	6750	[27]	
Zingiber officina	le 22.20	77.80	[77]	
C. edulis	28.60 a	71.40 a 67.00	[78]	
	33.00			
Castanea sativa, Mill	21.50	78.50	Demiate, oetterer e wosiacki, 2001	
Cicer arietinum I	L 28.60 a 34.30	71.40 a 65.70	[79]	
Potato	8.50 a 38.00	91.50 a 62.00	[80]	
Manioc	13.60 a	86.40 a 73.00	[68]	
	27.00			
Maize starch	16.80 a	83.20 a 78.70	[81]	
	21.30			
Buckwheat,	19.60	81.40	[82]	
Wheat	25.60	74.40	[83]	
Barley	22.10	77.90	[83]	

Source: the author

Table 2. In all repetitions, a reduction in the transmittance percentage with the advance of the storage days can be observed. Singh, McCarthy & Singh [69] also considered this analysis. Chezine et al. [70]; when analyzing the lightness of the starch extracted from beans, observed transmittance values ranging from 3.25% to 4.68%. Wang et al. [71] stated that starches with high amylose content have lower transmittance values.

Among the analyzes carried out with the extracted starch, the verification of resistance to freezing and thawing cycles is extremely important, given the need to characterize the type of starch according to its applicability, especially with regard to water release rates. The values of the repetitions referring to the results obtained in the starch freezing and thawing cycles are shown in Table 3. The percentage of water released from the pastes increased along with the cycles, being the 3rd cycle the one that promoted the greatest water release.

According to Takizawa et al. [72]; when studying the native starches of cassava and waxy corn, they observed a resistance to the freezing and thawing cycles when compared to parsley, potato, sweet potato and regular corn starches. Furthermore, the study suggests that, in cases of application of chemical treatments, it is possible to observe the fragmentation of the constituent chains of starch, which may be associated with the high release of water due to intensive molecular reassociation. Silva et al. [73,74] analyzed the starch gel extracted from pigmented rice and observed that, under the experimental conditions, the red rice starch gel lost 66.46% of water, a value higher than the others. The syneresis of these starches was associated with the amylose content of each sample as well as the retrogradation tendency observed by the viscoamylographic properties. In addition, an increase in water loss was also observed with the increase of the freezing and thawing cycles, which behavior was also observed in this whole, showing similar values in the third freezing and thawing cycle.

Takeiti et al. [75] analyzed starch gels from three different sources and observed that starch extracted from Peruvian carrots (cassava) had lower syneresis than waxy corn (7.81%) and sweet potato starches (16.74%) in the 5th freeze-thaw cycle. Weber et al. [76] studied the behavior of waxy corn starch gels that presented lower syneresis (69.55%) to normal corn starch gels (74.45%) observed in the third cycle. When studying the freezing and thawing cycle of starch extracted from rice, Silva et al. [73,74] observed an increase in water loss with the increase in the freezing and thawing cycles. After five freeze-thaw cycles, the red rice starch gel showed 66.46% syneresis, while the black and white rice starch gels showed 51.39% and 31.18%. These values are also close to the results found in this study. This behavior possibly occurred due to the retrogradation of amylopectin chains, which have a branched structure, is smaller and slower, and due to the difficulty that molecules have to rearrange themselves.

3.3. Enzymatic analysis of starch amylose from avocado seed

To guarantee the purity of the starch, the sample was previously



Fig. 1. Viscoamylographic properties (RVA).



Fig. 2. TG and DTG diagram.

degreased, a procedure that is mandatory before the quantification of amylose. The analysis of amylose and amyloptin contents found in this study compared with studies from the literature is presented in Table 4. The values referring to the content of amylose present in the starch vary according to the species, cultivation places, harvesting times, etc. [68, 80,81]. The differences can also be attributed to the analytical method, variations in genes, or climate. For Simkova et al. [84]; the amount of amylose can also vary depending on the stage of maturation, and these factors should be considered when comparing the results [85,86]. The amylose content of starch is important to elucidate its properties, including crystallinity, gelatinization temperatures, paste behavior, and nutritional properties.

According to Gao et al. [85]; amylose contents above 39.0% can produce functional foods with retrograde starch and, therefore, low glycemic index. According to Lacourse and Altieri [87]; starches with higher amylose content present better characteristics for film formation, as their structures have a greater capacity for reorganization during the drying period. As for the amylopectin content, studies bring values of long-chain fractions for rice (7.0–11%), wheat (3.4%), corn (5.6%), and sweet potatoes (5.4%) [88]. Izidorczyk et al. [89]; when comparing cereal starches, found a relatively large amount of longer linear amylopectin chains in wheat starch, which may explain its gelling potential, high gelatinization temperature and low digestibility.

The starch obtained in this work has an amylose content of 39.56%, much higher than the averages of those present in starch from other sources and other species of avocado fruit, as shown in Table 4. This result is relevant because the higher the amylose content, the better the conditions for film formation and the better the potential for coatings. Most starches have amylose content between 18 and 30% and amylopectin content between 70 and 82%, in addition to other constituents (lipids, proteins, and minerals). The smaller the amount of these other constituents and the greater the amount of amylose, the better the characteristic in the formation of films and coatings as amylose is directly linked to the chemical and physical characteristics of the film [90–92]. One of the most used starches due to the amylose content for producing biodegradable films is the starch extracted from cassava, which presents contents between 13 and 27% [68].

3.4. Paste viscosity (RVA)

The starch gelatinization process was carried out by heating under excess water. During this process, the breakdown of crystalline regions was observed, which was characterized by an increase in viscosity between 3 and 5 min of heating [93]. The starch studied showed a gelatinization process as can be seen in Fig. 1.

In this work, a slight variation in viscosity can be observed at low temperature, which may indicate the presence of some impurity in the sample, a consequence of the extraction of starch from the avocado seed. The initial paste temperature is 88.5 °C (Fig. 1), a value higher than that found by Ramos et al. [94] for white (80.6 °C) and red rice starch (79.1 °C), and similar as that found for black rice starch (88.8 °C).

For Franco et al. [95]; starches with a high amylopectin content have high percentages of branched chains and, in these cases, need a higher gelatinization temperature. Fig. 1 showed that after 5 min, at a temperature of 95 °C, the process of elaboration of the viscosity in the RVA showed a considerable increase, reaching about 1400 cP. A possible molecular arrangement of the amylose and amylopectin chains is also observed, much narrower than the common starches, which leads to the intuition of the presence of a structure with a higher percentage of the crystalline structure than the amorphous spaces.

The studied starch has a maximum viscosity of 5385,5 cp, maximum viscosity on cooling of 3085,5 cp, and 2880,5 cP of setback. This is an indication of the retrogradation of the starch, which is favorable for some applications, such as the elaboration of films.

With regard to the tendency to retrograde, the starch studied can be classified as an intermediate, not being considered to have a high tendency to retrograde, nor as a waxy starch. For Kahn [96] in the Rva, the viscosity curves of avocado seed starch do not have a pronounced peak collar. Lacerda et al. [97] also studied the thermal, structural and rheological properties of avocado seed starch (Persea americana, Mill) and observed that in RVA the starch viscosity curves do not have a pronounced peak collar.

According to Ordónez [98]; the firmness of the gel will depend on the forces with which the crystalline zones are joined. Thus, when these zones are numerous, large and strongly joined, firm and stable gels are obtained, but when they are few and small or when the forces that unite the molecules are insufficient, weak and unstable gels are obtained. The determination of this parameter is essential for the starch application industry, as in the development of biodegradable packaging.

3.5. Analysis of TG, DTG and DSC curves

Thermogravimetric analysis is very useful for obtaining information about phase transitions and determining the thermal properties of polymeric materials, allowing the analysis of thermal stability and degradation temperature for different materials. Through the curves

Table 5

Thermogravimetric data of avocado kernel starch.

1st Stage			Stability		2nd Stage			3rd Stage		
Δm(%)	$\Delta r(\circ C)$		$\Delta r(\circ C)$		Δm(%)	$\Delta r(\circ C)$		Δm(%)	$\Delta r(\circ C)$	
6,27	22	113	133	260	4,4	260	366	1,56	366	550

Source: the author



Fig. 3. DSC diagram.

obtained in the DTG, it is possible to observe the peaks related to mass variations. The thermogravimetric curve of the starch sample is shown in Fig. 2, and the values obtained in the thermogravimetric analysis are shown in Table 5. The analyzed sample portrayed the mass loss in the three stages event and demonstrated only a period of stability between the first and second stages.

According to the data obtained, the decay in the first stage is inherent to the loss of water from the material. According to Lacerda et al. [99]; the greater the loss in the first stage, the more humid the sample. The thermal degradation process can be seen in the second stage and represents the decomposition of the organic matter of the analyzed starch, also observing the depolymerization of the material. According to Santana et al. [100]; starch depolymerization occurs in the range of 300 °C. In this phase, some glycosidic derivatives (such as Maillard reaction) and complex gases are released. The third step corresponds to the oxidation of organic matter, leaving only the ash mass.

DSC is a thermal analysis technique where the heat difference required to raise the temperature of a sample and a reference sample is measured as a function of temperature. This analysis provides quantitative measurements of the heat flux needed for gelatinization to occur, which provides endothermic peaks represented by curves in a characteristic range for each botanical source [101]. The curve obtained from the DSC analysis of starch is shown in Fig. 3. The peak temperature was found at 65.65 °C, close to the peak at 64.7 °C found by Biscudo (2008) when evaluating the native starch of cassava. Freitas et al. [102] also assessed cassava starch and observed a peak at 63.5 °C. These values demonstrate a critical event to be considered in the food industry, such as gelatinization. The starch gelatinization curve can be observed from the analysis of temperature variation between the end and beginning of the event. This analysis makes it possible to assess the degree of purity of the extracted material. Factors such as the formation of lipid complexes, molecular organization of amylopectin, and degree of crystallinity, can influence starch gelatinization temperature [103,104].

According to the value observed in this study, it can perceive lower stability in terms of endothermic transition, presenting a lower temperature when compared to other studies. Silva et al. [73,74] found peak temperature values at 117.8 °C, 119.6 °C and 111.6 °C for white, red, and black rice starch, respectively. Among the pigmented varieties, black rice starch showed the endothermic transition at the lowest temperature regarding its higher water content.



Fig. 4. Morphological image of starch granules extracted from avocado seeds.

According to Brito [28]; the choice of extraction methodology can directly influence the increase of this peak, thus observing a change in the event's temperature. In these cases, a slight chemical modification is suggested at the time of extraction due to the presence of the alkalizing reagent. For Molavi et al. [104]; certain chemical modifications make the starch more resistant to water entry and retention, requiring more energy to form the paste.

3.6. Scanning electron microscopy (SEM)

Fig. 4 illustrates the starch granules. These are free of impurities, attesting to the good extraction of starch from the avocado seeds. The starch granule extracted in this study has an oval shape and a smooth surface, which may be a characteristic arising from its plant origin [31]. The minimum diameter of the starch granules was measured at 18.454 μ m, while the maximum diameter was 32.885 μ m and the average diameter was 24.598 μ m. Very similar values were observed by Brito [28] and by Silva et al. [31] when analyzing morphologically the starch extracted from avocado seeds. The existence of a possible morphological variation was likely influenced by the type of extraction used to obtain the starch [28]. The images do not show damaged starch granules, indicating that the starch extraction process was adequate.

4. Conclusion

This study extracted and characterized the starch from the avocado seeds with a reasonable yield (19.0%). The extraction process was efficient since scanning electron microscopy showed the starch granules without impurities, which was also proven by the low protein and lipid content found in the starch. Starch had a high moisture content and 32% resistance to the first freezing cycle. Due to the high amylose content, the viscosity values found, the low clarity of the paste and the good stability, its use in processing up to approximately 366 °C can be indicated, the extracted starch can be used to prepare edible films and/or biodegradable. As starch is extracted from the avocado seed, which is an agro-industrial residue and consequently pollutes the environment, its use reduces the environmental impact caused by its disposal.

In this context, this study allowed us to know the characteristics of the starch extracted from the avocado seeds, which offers new challenges in the study and application of this material. The need to develop materials with biodegradable characteristics is growing in the research environment. The findings obtained in this work indicated a good direction for using this raw material with the attribution of one more mode of use and exploitation, thus contributing for the reduction of agroindustrial waste.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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