

PHYSICOCHEMICAL PROPERTIES OF STARCH FROM AVOCADO SEED (PERSEA AMERICANA MILL)

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The extraction and characterization of avocado starch isolated from seeds (*Persea americana* Mill) were studied. The starch was extracted by steeping, wet grinding and sedimentation process and calculated its yield. Isolated starch was then characterized for chemical composition, morphology and granules size distribution, X-ray diffraction and mid-infrared spectra, swelling power and solubility, pasting and thermal properties and clarity and syneresis behavior. The starch yield was 42.2%. The results showed starch granules were predominantly ellipsoidal with an average granule size of 17.83 μm . Exhibited B-type X-ray diffraction pattern with 25.7% of crystallinity, and apparent amylose content of 21.5% with similar mid-infrared spectra to other starches. Onset and peak gelatinization temperatures were 67.6 and 76.0 °C, respectively, and gelatinization enthalpy was 14.9 J/g. Starch suspensions showed peak viscosity at 4421 cP and high retrogradation tendency, which was evidenced by opaque gels and syneresis.

KEYWORDS: RAPID VISCO ANALYZER; DIFFERENTIAL SCANNING CALORIMETRY; X-RAYS; SWELLING POWER AND SOLUBILITY INDEX; CLARITY AND SYNERESIS.

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1 INTRODUCTION

Starch is the main carbohydrate storage in plants. It is abundant, renewable and nontoxic relatively easy to extract with high purity. It can be converted into various compounds by chemical and biochemical process (BELLO-PÉREZ *et al.*, 2010).

It consists essentially of two main polysaccharides, amylose and amylopectin, and some minor components such as lipids and proteins (ROCHA, DEMIATE and FRANCO, 2008). Amylose consists of glucose units connected by α -glycosidic linkages $\alpha(1,4)$, essentially yielding a straight chain. Amylopectin consists of glucose units joined in $\alpha(1,4)$ and $\alpha(1,6)$ links, forming a branched structure (CHUNG, LIU and WEI, 2011). The proportions in which these structures appear differ according to their botanical origin and to their varieties within same species and even within the same botanical specie according to the degree of plant maturity (TESTER, KARKALAS and QI, 2004).

The physico-chemical and functionality of starches are influenced by the botanical source where it originated and by granular and molecular structure that determine their applications in industry (SINGH *et al.*, 2003).

The most important properties for defining starch uses in the preparation of food and other industrial applications, besides the size and shape of the granules transparency and opacity, include the physicochemical properties such as gelatinization and retrogradation, water absorption and solubility, swelling, syneresis and the rheological behavior of their pastes (HERNÁNDEZ-MEDINA *et al.*, 2008).

The main commercial sources of starches are corn, wheat and cassava, however the need of continually searching for other non-convention sources that can be economically viable is very important (HERNANDEZ-URIBE *et al.*, 2011). There are many botanical sources of starch poorly explored such as avocado (Tango *et al.*, 2004).

The fruit of avocado is formed by pericarp (hull), mesocarp (pulp), and endocarp (seed), wherein the pulp is only used in oil production or consumed *in natura*, generating a seed as a residue (Mooz *et al.*, 2012). Tango, Carvalho and Soares (2004), studying several varieties of avocado found that the seed corresponds to approximately 17% of the fruit and presents around 20% of starch.

According to Food and Agricultural Organization of the United Nations (FAO), estimated who in 2010 global production of avocado was about 4 million tons, covering an area 423,000 hectares, where Mexico, Indonesia, United States, Colombia, Chile and Brazil are the largest producers (FAO, 2003). A large number of avocado varieties are found in various regions of Brazil and São Paulo is the largest producer in the country (TANGO, CARVALHO and SOARES, 2004). Despite being produced in large quantities and its seeds are rich in starch, the seeds of the avocado is considered a waste hence not appropriately used. Moreover, there is currently little information on the physico-chemical and functional application of starch extracted from avocado seed.

Given the importance of avocado crop and the possibility of using the seed as alternative source of starch, the present work aimed to study the physical and chemical properties of starch extracted from the seed of the Margarida avocado variety.

2 MATERIAL AND METHODS

Avocados of Daisy variety were collected from healthy plants from the vicinity of the city of Anápolis (GO/Brazil), located at 1000 m altitude in the coordinates: 16°19'43" south latitude and 48°57'12" west longitude.

The experiment was conducted in a completely randomized design. Avocado fruits (70 fruits) were collected near their maturity stage when they were still firm. They were stored at room temperature until they reached their point of maturation, when they become soft under manual compression. The fruits were weighed and separated into its three parts: skin, pulp and seed. The percentage of each of them was determined.

2.1 EXTRACTION OF STARCH

To obtain the starch, it was followed the methodology described by Ascheri *et al.* (2010), with modifications. The fine dark peel over the seeds was manually removed. The peeled seeds were chopped and placed in buckets containing sodium metabisulfite (5 g/L), in proportion of 1 kg seed per 2 L metabisulfite solution (1:2 w/v). The milling was performed in a slicer machine MA-580 (Marconi, Piracicaba, Brazil), fitted with an aperture size of 1 mm, that produced a dough that was manually compressed with a mesh screening cloth made of polyester with opening size of 100 µm and washed successively with water in order to separate the starch from larger particles. The starch was filtered under vacuum, dried at 40 °C for 12 h.

The yield of avocado seed starch was calculated according to Equation (1), from pelled seeds.

$$\text{Starch yield (\%)} = \frac{\text{Mass } \mathbf{\delta} \text{ dry after starch extraction (g)}}{\text{Mass } \mathbf{\delta} \text{ shelled shelled roots (g)}} \times 100 \quad (1)$$

2.2 CHEMICAL COMPOSITION

The methods of the Association of Official Analytical Chemists (1990) were used for analyzes moisture (925.09), protein (979.09), lipids (923.05), fiber (962.09), and ash (923.03) content. The apparent amylose was determined using the method of Rincón and Padilla (2004).

2.3 OPTICAL AND SCANNING ELECTRON MICROSCOPY

Starch samples were suspended in water distilled and drop visualized directly on optical Leica DM E microscope (Wetzlar, Alemanha) and analyzed by Leica Application Suite LAS EZ v. 2.0.0 software and to provide a sharper image of the granules and analyzed by Leica Application Suite LAS EZ v. 2.0.0 The granule size distribution was determined by image analysis (Leica LAS EZ) recorded directly from a Starch samples were suspended in water distilled and to provide a sharper image of the granules softwar. Granule size distribution was determined by image analysis (Leica LAS EZ) recorded directly from an optical Leica DM E microscope (Wetzlar, Alemanha).

The starch granule of avocado was also observed on a scanning electron microscope TM 3000 (Hitachi, Tokyo, Japan) at 15 kV acceleration and magnification of 500 and 4000x. The sample was left in a desiccator with silica gel for 24 h for drying, set on double-sided adhesive carbon tape attached to an aluminum support and taken directly to SEM vacuum chamber.

2.4 SPECTROSCOPY IN FOURIER TRANSFORM INFRARED (FTIR)

FTIR spectra were recorded using a Perkin-Elmer Spectrum Frontier FT-IR/NIR (Perkin-Elmer Corp. Norwalk, CT) equipped with a deuterated tryglycine sulfate (DTGS). The sample holder was used for the background spectra without KBr and scans were taken for each sample from 4000 to 400 cm⁻¹ 1 cm⁻¹ at a resolution of 4 cm⁻¹.

2.5 X-RAY DIFFRACTION

The starch was manually compacted in specific media and subjected to an X-ray diffraction Rigaku RU 200 (Rigaku, Osaka, Japan) for evaluation of crystallinity of the granules. The sample was scanned using a radiation Cu Ka (k = 1,54056 Å) at 40 kV and 150 mA. The spectral angle (2q) was measured between 5° a 40° at a speed of 2°/min. The interval of the reading was 0.02 at time of 0.2 s. The degree of crystallinity was quantified according to the method of Nara and Komiya (1983).

2.6 DETERMINATION OF PASTING PROPERTIES

For the analysis of the viscosity was carried out on a RVA (Rapid Visco Analyzer 4, Newport Scientific Pty Ltd, Warriewood, Australia). Starch suspension (3 g of starch in 25 mL water) adjusted to 14% moisture (wet basis). The time-temperature profile included mixing with the paddles rotating at 160 rpm at 50 °C, for 1 min, heating to 95 °C at a constant rate of 14 °C/min, holding at 95 °C for 3 min, and then cooling to 50 °C in 5 min at the same rate. The readings from the paste curve generated were paste temperature, maximum peak viscosity, breakdown viscosity (difference between the maximum and the paste, maintained at 95 °C for 5 min), final viscosity and set back viscosity or retrogradation (difference between the final viscosity at 95 °C for 5 min).

2.7 DIFFERENTIAL SCANNING CALORIMETRY

The starch gelatinization analysis was performed on a differential scanning calorimeter (DSC) model Q200 (TA Instruments, New Castle, USA) calibrated using the indium as standard. Approximately 3 mg of sample of known moisture content was weighed directly in a hermetic aluminum pan with distil water (at least twice the amount of starch). The sample pan and lid was hermetically sealed and allowed to stand overnight. The sample was heated at 10 °C/min from 5 °C to 110 °C under flow of nitrogen at 50 mL/min. From the curve, the onset temperature, peak temperature, end temperature and the enthalpy of the starch gelatinization were calculated using the enthalpy of gelatinization was calculated with the help of Universal Analysis software, version 4.3 A (TA Instruments New Castle, USA).

2.8 SWELLING POWER AND SOLUBILITY

The swelling power and solubility index was determined according to the methodology described by Leach *et al.* (1959).

2.9 CLARITY FOLDER AND SYNERESIS

The method of Singh *et al.* (2003) was used to determine the clarity of starch pastes, with modifications. A dispersion of 8% starch (dry basis) was boiled for 30 min under constant stirring, then transferred to plastic tubes and stored at 4 °C for 4 days. Absorbance was measured every day with a UV-Vis spectrophotometer at 620 nm. The mass difference before and after gel filtration was measured. The water loss and syneresis from the starch gels was calculated based on the percentage of water loss compared to the initial mass of water contained in the gel.

2.10 STATISTICAL ANALYSIS

The experimental data were described by mean and standard deviation. Statistica for Windows, version 8.0 (StatSoft, Inc., Tulsa, USA) was used for data treatment and statistical analysis.

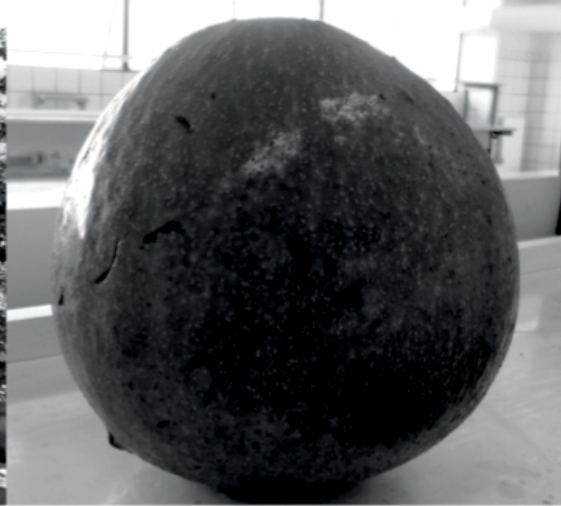
3 RESULTS AND DISCUSSION

3.1 STARCH YIELD AND CHEMICAL COMPOSITION

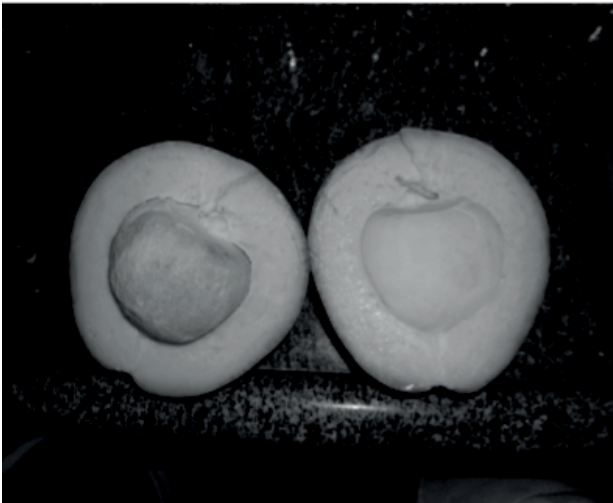
The plant variety of avocado used in this work can grow rapidly reaching 9 m high (Figure 1A). Figure 1B and 1C show the fruit and its interior. The kernel has an oval shape presenting a darker peel (Figure 1D).



(A)



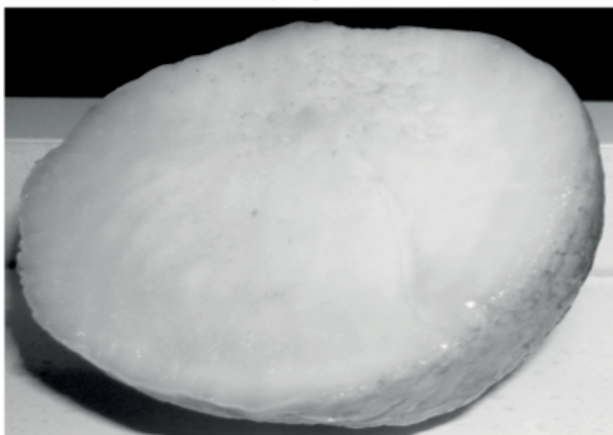
(B)



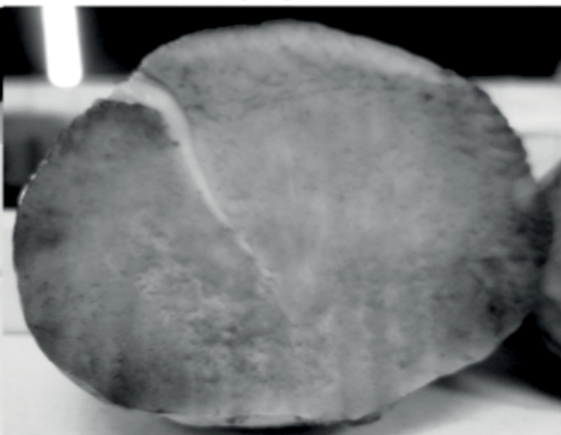
(C)



(D)



(E)



(F)

FIGURE 1 - (A) AVOCADO TREE; (B) AVOCADO FRUIT; (C) LONGITUDINAL CUTTING OF THE FRUIT SHOWING THE INTERNAL PARTS: PULP AND SEED; (D) SEED; (E) FRESH CUT LONGITUDINAL SECTION OF THE SEED; (F) APPEARANCE OF THE SEED AFTER 45 MIN CUT, IT TURNS ORANGE.

The average fruit weight was 757 g, with average proportions of peel, pulp and seed around 8.7%, 67.1% and 24.2%, respectively. The seed had a diameter of 6.5 cm (Figure 1D) of creamy endocarp (Figure 1E), which turned orange in contact with the environment by the action of the intrinsic enzyme system after 45 min cut (Figure 1F).

The moisture content of the avocado seed was 66.3% and the starch yield was 14.2% (wet basis). The starch fraction presented a darker color (pinkish) than the known white characteristic color. On a dry basis, the starch sample presented 0.09% fat, 0.38% ash, 0.07% protein and 0.14% crude fiber. These results indicate that the extraction of starch from the kernel was successful, particularly because of the low content of protein and fat.

Starch avocado presented 21.5% of amylose, which was higher than those recorded by Peroni, Rocha and Franco (2006) for starch from cassava (19.8%), but lower than those reported by Sandhu and Singh (2007) for normal corn (24.5%) and Ashogbon and Akintayo (2012) for rice (26.4%).

3.2 SCANNING ELECTRON MICROSCOPY AND PARTICLE SIZE DISTRIBUTION

The morphology, size and location of the hilum of starch granules are related to the botanical origin. The starch granules showed different shapes, however, it was observed that the ellipsoidal shape was predominant (Figure 2A), with smooth surface and the hilum located at one end, characterized by a hole of 2.5 μm diameter (Figure 2B and 2C). The granule size distribution varied from 1.9 to 57.6 μm and the average size of 17.8 μm (Figure 3D). With the use of optical microscopy, the minimum and maximum diameters varied from 9.37 μm to 27.7 μm .

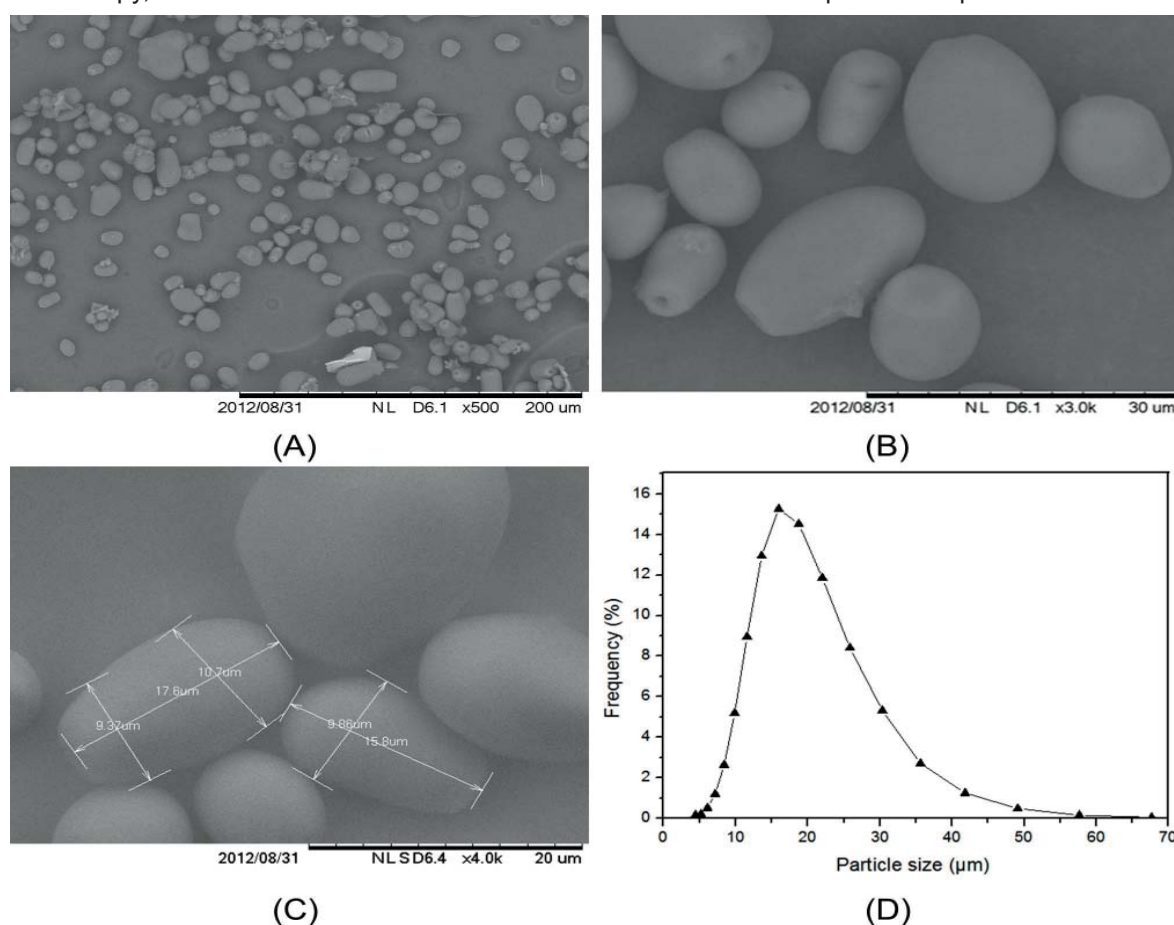


FIGURE 2 - MICROGRAPHS OF STARCH GRANULES EXTRACTED FROM AVOCADO SEEDS: (A) 500X, (B) 3000X, (C) 4000X; AND (D) DISTRIBUTION OF PARTICLE SIZE USING AN OPTICAL MICROSCOPE.

3.3 INFRARED SPECTRA AND X-RAY DIFFRACTION

Starch is constituted mainly of carbohydrates: amylose and amylopectin and water, which are shown in the spectral bands of Figure 3A. Generally, the hydroxyl groups present on the D-glucose unit of starches from different botanical sources appear as a broad band rounded in a wavelength region between $3900\text{--}3300\text{ cm}^{-1}$ (WANG *et al.*, 2009). Avocado starch showed a broad spectral band (width at half height of 758 cm^{-1}) with value of maximum absorption wave number in 3435 cm^{-1} . The peaks characterizing the links C-H of the ring and connecting stretch C-O gave values of wave number maximum absorption in 2928.24 and 1645.98 cm^{-1} , respectively, close to those values found by Nakason *et al.*, (2010) and Wang *et al.*, (2009), which were for C-H between 2923 and 2926 cm^{-1} and for C-O of 1650 cm^{-1} , respectively.

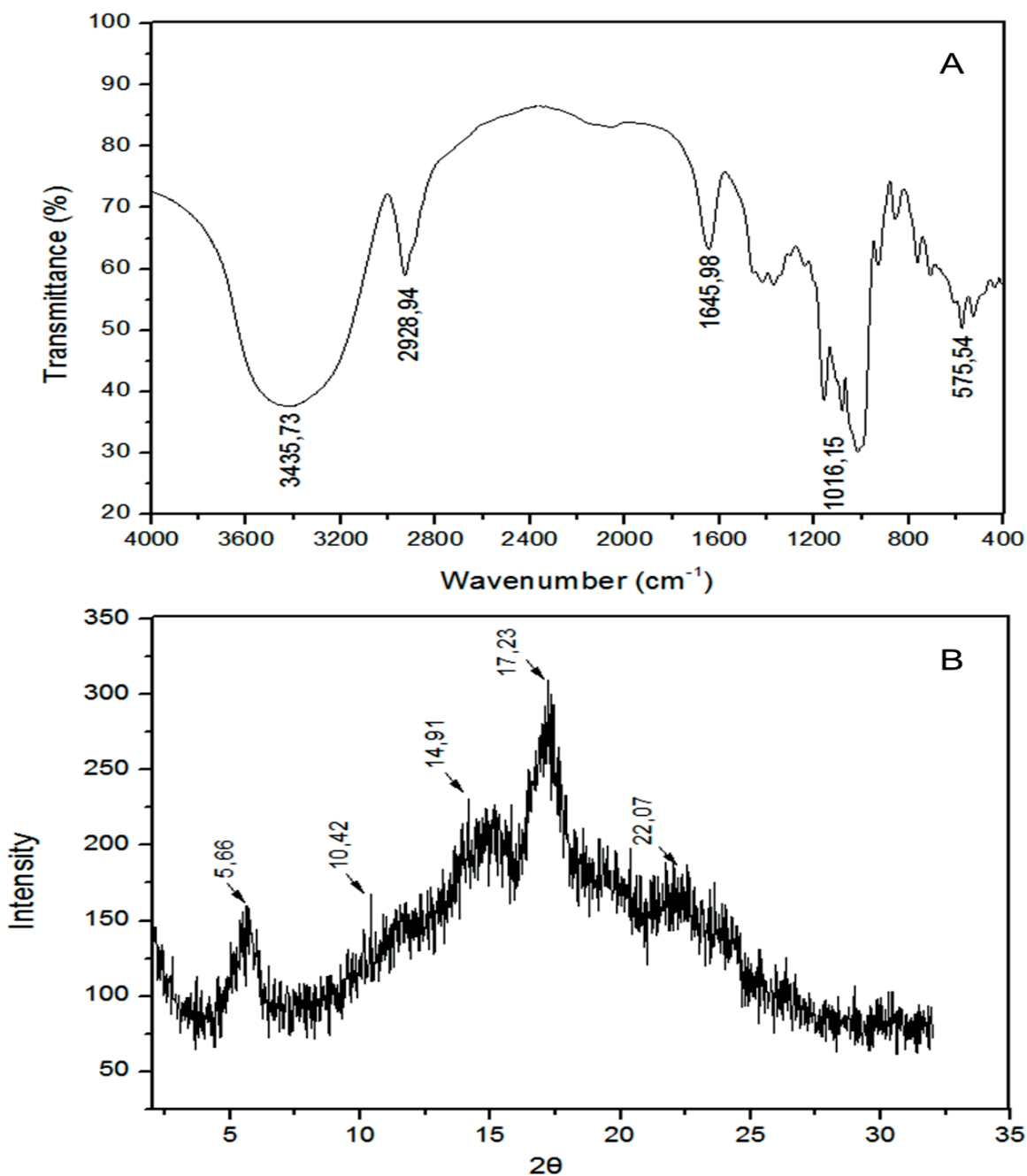


FIGURE 3 - (A) INFRARED SPECTRUM (FTIR) OBTAINED BY THE KBR PELLETT TRANSMISSION AND (B) X-RAY DIFFRACTOGRAM OF AVOCADO STARCH GRANULE EXTRACTED FROM THE SEEDS.

The region 1565-400 cm^{-1} can be analyzed in two sub-regions. One between 1565 and 1285 cm^{-1} , corresponding to the normal modes of angular deformation of C-H, and another between 1285 and 400 cm^{-1} , corresponding to the normal modes of C-O and C-C stretch belonging to a ring of D-glucose. They are vibrations network in which all atoms of the chain of the macromolecule vibrate in phase and normal modes resulting from the coupling of stretches C-C and B-C. The long polysaccharide chains of the starch granules are associated with each other through hydrogen bonds, resulting in crystallinity that is assigned to amylopectin Zobel (1988).

Concerning crystallinity, starch can be classified into patterns A, B and C. According Nwokocha and Williams (2011) the diffraction pattern of type A is commonly found in cereal starches and shows characteristic peaks at approximately 15, 17, 18 and 23°, while the diffraction pattern of type B is found in starches of tubers, varieties with high amylose starches, showing characteristic peaks around 5.8°, 15° and a single peak at 17° (rather than a peak in another 17 and 18 as in type A) and two small peaks at about 23 and 24°. The diffraction pattern of type C corresponds to a polymorphic form and may be regarded as a mixture of types A and B (Zobel, 1988). The avocado shows a pattern of crystallinity of type B with major peaks appearing at 5.66, 10.42, 14.91, 17.23, 22.07° in 2θ (Figure 3B). The degree of crystallinity found for this starch was about 25.7%.

3.4 PASTING PROPERTIES AND DIFFERENTIAL SCANNING CALORIMETRY

The apparent viscosity of avocado starch is presented in Figure 4A as a function of time and temperature. During the first 3 min, the starch granules showed no change in viscosity. The temperature of initial paste viscosity, 76.0 °C, was detected after 3.1 min of run and it was very close to that reported by Sandhu and Singh (2007) for maize starches (76.5 °C).

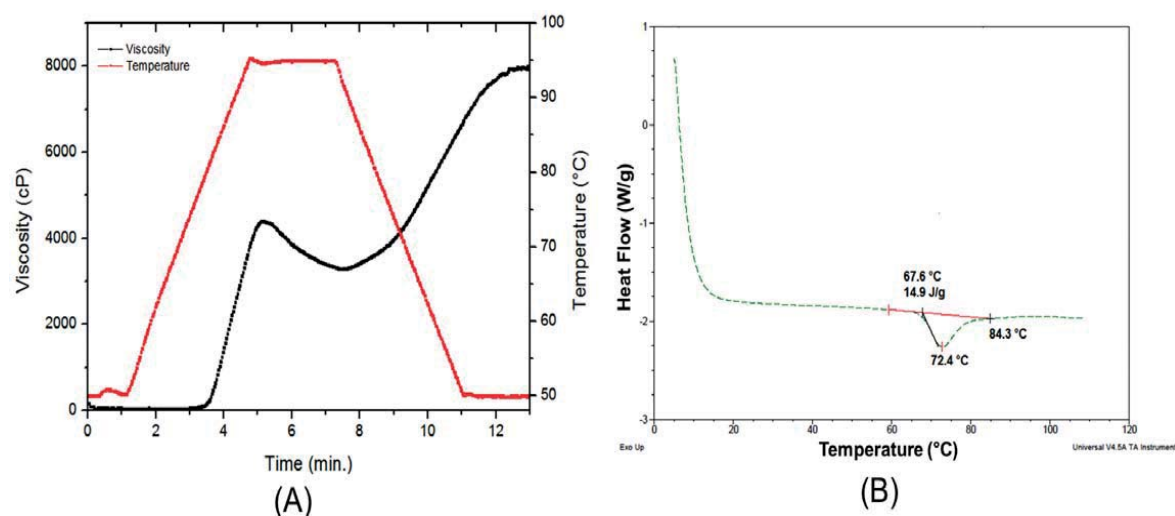


FIGURE 4 - (A) PASTE VISCOSITY OF AVOCADO STARCH SEED AND (B) THERMOGRAM OF AVOCADO STARCH.

After reaching the initial paste viscosity, the starch granules started to swell, increasing the viscosity from 50 to 4421 cP (peak viscosity) in 5.1 min at 95 °C. During the heating cycle, the paste viscosity profile showed a round shape with a peak viscosity reading. It was an indication of non-homogeneity of the structure of the granules, which is characterized by the simultaneous phenomenon of swelling and collapsing of the starch granules under continuous stirring, hence causing a drop in viscosity (1151 cP) at 7.5 min, when collapse prevails leading to the minimum paste viscosity value after the heating cycle. During the cooling period, the increase in viscosity was 4703 cP (tendency to retrogradation), reaching a final viscosity of 7973 cP showing a substantial gel recovering viscosity, also known as retrogradation.

Avocado starch showed maximum viscosity, breakdown and setback viscosities values higher than potato starch (KAUR *et al.*, 2007) and maize starch (SANDHU and SINGH, 2007), which were 1239.0; 883.2 and 673.7; 1063.0; 377.0 and 1345.0 cP, respectively. By comparing those data, they indicate that avocado starch showed higher thermal resistance and its gel may not be indicated to be stored under refrigeration due to the substantial gelation that might contribute to syneresis.

Results of gelatinization by differential scanning calorimetry (Figure 4B) showed that the onset temperature, peak temperature, final temperature and enthalpy equal to 67.6, 72.4, 84.3 °C e 14,9 J/g, respectively. The onset temperature was lower than the value of temperature of paste viscosity observed by the RVA, which was 76.0 °C. According to Pérez, Breene and Bhanassey (1998), the paste viscosity temperature obtained by RVA are higher due to the lower sensitivity in detecting early increases in viscosity of the starch paste, unlike the initial gelatinization temperature, which is detected when the crystalline regions of the first granules begin to disrupt.

Only one endotherm was obtained for the starch from avocado starch by DSC. The value of this endotherm was 14.9 J/g, which was lower than the endotherm of cassava starch (17.4 J/g), observed by Sangseethonga, Termvejsayanona and Siroth (2010) and superior to that of rice starch endotherm (13.5 J/g) found in the work of Chung *et al.*, (2011), demonstrating that avocado starch requires less energy to disrupt the crystalline region of amylopectin molecules than the cassava starch and more energy than the rice starch.

3.5 SWELLING POWER AND SOLUBILITY

The heating starch granules in excess water promotes a disruption of the crystalline structure favoring the formation of hydrogen bonds between the water molecules and the molecules of amylose and amylopectin, which culminates in increased solubility and swelling of the granules (SINGH *et al.*, 2003). The avocado starch showed no large change in swelling power and solubility index at 60 °C (Figure 5A). This behavior is consistent with the results obtained by RVA and DSC, once the initial gelatinization temperature observed in these analyzes were higher than 60 °C. However, above this temperature it was observed a fast increase of swelling, indicating internal breakdown of the internal granular structure of the granules.

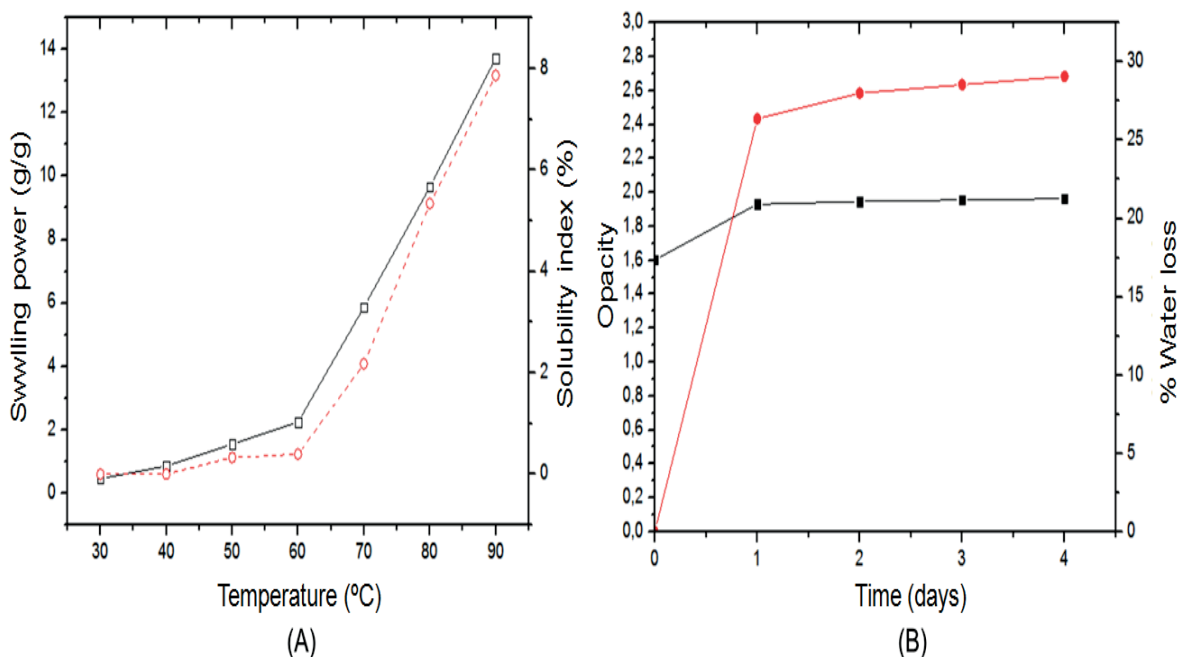


FIGURE 5 - (A) SWELLING POWER (-*-) AND SOLUBILITY INDEX (-j-), AND (B) OPACITY (-N-) AND % OF ACCUMULATED WATER LOSS (-L-) OF AVOCADO STARCH

The values of power and swelling rate of 90 °C solubility of starch avocados were 13.72 g/g and 7.86%, respectively. These values were close to that of maize starch determined by Demiate, Oetterer and Wosiacki (2001), which were of 14.4 g/g and 16.7% at 90°C, respectively, however, much lower than the values found by Takizawa *et al.* (2004) for cassava starch were 34.9 g/g and 21.5%, respectively, at the same temperature studied. Comparison of these data indicates that the starch has avocado limited swelling, i.e., low water absorption and low solubility.

According to Jiang *et al.* (2012) the power of swelling is related to the amylose content, water retention capacity of the starch granules, hydrogen bonds and degree of crystallinity. The low swelling power observed may be attributed to the presence of a large number of crystalline regions formed through the association between long chains of amylopectin. Since the low solubility suggests that additional interactions may have occurred between amylose chains-amylose and amylopectin-amylopectin, although high retrogradation, as seen in the RVA (HUGHES *et al.*, 2009).

3. 6 PASTE CLARITY AND SYNERESIS

Initially, the absorbance of starch gels was 1.6, increasing its value by 11%, reaching 1.8 of absorbance after the first day of storage at 4 °C (Figure 5B) and remained stable until the end of the experiment. According to Silva *et al.* (2006) opaque gels have more organized granular structure, with greater association between the chains, which hinders the passage of light. According to these authors, starches with higher amylose content, high retrogradation tendency and opaque gels exhibit gels with firm structure. Craig *et al.* (1989) studying different gels at absorbance of 650 nm found that the following values: 0.036 for potato starch, 0.48 for cassava, 0.92 for wheat and 1.22 for corn starch. The value of absorbance of avocado starch was higher than those values indicating high opacity. It is noteworthy that in addition to high retrogradation viscosity tendency showed by avocado starch paste, the starch color might have contribute to decrease its clarity.

The stability of freezing and thawing cycles is very important to characterize the starch in terms of applicability in food process. In order to be refrigerated and/or frozen, the release of water generally occurs in detrimental to the quality of the final product (Silva *et al.*, 2008). According to Zhang, Tong and Ren (2012) the exudation of water is due to the reassociation of starch molecules, particularly amylose, that form ordered structure (retrogradation). As the avocado starch gels in the first day of storage, it lost 26.4% water, stabilizing on the fourth day at 4 °C and achieving a total water loss of 29.1%. This information indicates that avocado starch would not be suitable for using in chilled foods, contrary to what happens with tapioca starch and waxy maize starch, when the water loss can be as low as 0 and 9%, respectively (Takizawa *et al.*, 2004).

4 CONCLUSIONS

The starch extracted from avocado fruit seed (*Persea americana* Mill) has potential use as a raw material for the manufacture of starch, as the starch yield was 42.2%. The starch granules showed a B type crystalline structure with 25.7% of crystallinity. It has a predominant ellipsoidal shape with a characteristic hilum in one end with average starch granule size of 17.8 µm. The amylose content was 21.5%. The paste viscosity curve showed high value of peak viscosity and retrogradation. Gel was opaque and firm showing syneresis.

PROPRIEDADES FÍSICO-QUÍMICAS DO AMIDO DA SEMENTE DE ABACATE (*PERSEA AMERICANA* MILL)

RESUMO

No presente estudo se estudou o processo de extração e caracterização do amido da semente de abacate (*Persea americana* Mill). O amido foi extraído por meio de moagem úmida e decantação, calculando-se seu rendimento. O amido foi caracterizado quanto a sua composição química, morfológica e distribuição de tamanho dos grânulos, difração de raios-X, espectro no infravermelho médio, poder de inchamento e índice de solubilidade, propriedades reológicas e térmicas e claridade e sinérese de suas pastas. O rendimento de amido foi de 42,2 %. Os grânulos de amido foram predominantemente elipsoidal com tamanho médio de 17,83 µm. Exibiu padrão de difração de raios-X do tipo B, com 25,7% de cristalinidade e teor de amilose aparente de 21,5%, com espectros de infravermelho médio semelhante a outros amidos. Temperaturas inicial e de pico de gelatinização foi de 67,6 e 76,0° C, respectivamente, e entalpia de gelatinização foi de 14,9 J/g. Suspensões de amido mostraram viscosidade pico de 4.421 cP e alta tendência à retrogradação, evidenciado por apresentar géis opacos e sinérese.

PALAVRAS-CHAVE: ANALIZADOR RÁPIDO DE VISCOSIDADE; CALORIMETRIA DIFERENCIAL DE VARREDURA; RAIOS-X; PODER DE INCHAMENTO E ÍNDICE DE SOLUBILIDADE; CLARIDADE E SINÉRESE.

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