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FUNCTIONAL PROPERTIES AND POTENTIAL UTILIZATION OF STARCH ISOLATED FROM CHAYOTE FRUIT

Moufida Abdelhai[1], Nadia Boudries[1], Houria Taibi[1], Christophe Blecker[2], Georges Sinnaeve[3] and Marianne Sindic[4]*

Address(es):

[1] Ecole Normale Supérieure Cheikh Mohamed El-Bachir El-Ibrahimi de Kouba, ENS-KOUBA, Laboratory of Bioactive Products and Valorization of the Biomass, Department of Chemistry, BO 92 Vieux-Kouba, Algiers 16050, Algeria.

[2] University of Liege, Gembloux Agro-Bo Tech, Laboratory of Food Science and Formulation, Passage des déportés, 2 B, Gembloux B-5030, Belgium. *TERRA* Teaching and Research *Centre*, Laboratory of Food Science and Formulation, Gembloux Agro-Bio Tech, University of Liege, Avenue de la Faculté 2 B BAT *B-5030, Gembloux, Belgium.*

[3] Walloon Agricultural Research Center CRA-W, Chaussée de Namur, 24 B-5030 Gembloux, Belgium.

[4] *Liege*University, *Gembloux*Agro-Bio Tech, Laboratory of Agro-food Quality and Safety, Analysis, Quality and Risk Unit, Passage des Déportés, B-5030, Gembloux, Belgium.

*Corresponding author: nadia.boudries@g.ens-kouba.dz

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ABSTRACT

Chayote, *Sechium edule* (Jacq.) Sw., is anoverlooked food plant despite its various potentialities. This study investigated the characteristics of starch isolated from chayote fruit cultivated in Algiers which is known for its Mediterranean climate. The granules morphology, functional properties and amylose content of Algerian chayote fruit starch were examined. Spherical, oval and polygonal shapes and smooth surface were observed using three imaging techniques: a normal and polarized light microscopy with Lucia software and Scanning Electron Microscopy (SEM). Starch granules size was in the rang 3.56-37.24 µm, and for the chayote tubers, it was 7-50 µm. The amylose content (20.36%) is different from that of the chayote tubers (12.81%), but close to those of conventional sources. The Algerian chayote fruit starch showed higher swelling strength and lower melting index. The Rapid Visco Analyzer pasting profile revealed a lower peak viscosity (2158.3 cP) than that from chayote tubers (14746 cP). However, Differential Scanning Calorimetry analysis showed higher gelatinisation temperatures (66.89 °C) and transition enthalpy (15.79 J/g). X-ray diffraction profile showed B-type. The digestibility (D∞), hydrolysis index, HI, and average glycemic index, ,(GI) were estimated at 50.66 %, 52.16% and 70.16 % respectively. The results showed that starch has an acceptable nutritional value with significant *in vitro* digestion properties and it is suitable for human health and nutrition. The starch of Algerian chayote fruit showed interesting functional characteristics which makes it suitable for relevant applications in both the field food and cosmetics industries. It can, also, be a raw material for starch processing.

Keywords: *Sechium edule*, starch, morphology, pasting properties, thermal properties, digestibility

INTRODUCTION

Starch is an unmissable edible component used as a thickener, colloidal, stabilizer, gelling agent, bulking agent, water retention agent, and adhesive in food, foodstuff, cosmetics, textile and papermaking industry **(Singh *et al*., 2003)**. The interest in starch is linked to its availability and its functional properties, which differ according to the botanical source **(Pascoal, *et al*., 2013)**. The functionality of starch depends on the granule size, structure and physical arrangement of amylose, amylopectin, and residual components as proteins and lipids, which affects the adhesion, ductility, viscoelasticity, and rheological properties **(Bahnassey & Breene, 1994)**. These properties define the potential applications of starch as well as its modification **(Kobayashi *et al*., 1986)**.

Corn, wheat, potato and cassava are the main feedstock for starch production. Corn starch represents about 80% of the plant source in the global market and it is widely used in the production of foodstuffs **(Zhu *et al*., 2013)**. Because of the increasing demand for native and modified starch along with the search by manufacturers for specific functional properties, scientists set out to isolate new starches from unconventional botanical sources such as fruit, roots and tubers. It is in this perspective that this investigation was conducted with the aim of isolating starch from chayote cultivated in a Mediterranean ecosystem and determining its morphological, thermal and rheological properties. Theses functionalities allow the selection of the most appropriate starch for specific applications.

The chayote, of its scientific name *Sechium edule* (Jacq.) Swartz, is also called mirliton in USA, Chritophine in France and Ezzenbaa in Algeria. It is an edible plant belonging to *Cucurbitaceae* family and recognized for its nutritional and biofunctional properties **(Lombardo-Earl *et al*., 2014)**. It was discovered in Central America and introduced to Europe during the Columbian exchange and probably brought to Algeria via the Mediterranean Sea in the 19th century.

Vieira *et al.* (2018) have reported that only a few researches have been conducted on the isolation and identification of individual chemical constituents and their biological efficacy. It should be noted that some researchers like **Lira Saade (1996)** have even published relevant results which were interested in promoting the conservation and use of neglected plants as chayote with the collaboration of International Plant Genetic Resources Institute (IPGRI). Starch content in chayote fruit of Guadeloupe was 27% while, it was 72% db in tuber **(Monnerville *et al*., 2001)**. A close content (72.8%) was, also, found in Mexican chayote tubers, (MCHT), which is characterized by higher viscosity than potato and maize starches and similar thermal properties to potato starch **(Jiménez-Hernández *et al*., 2007)**. It is, so, clear that the tubers are more profitable in the isolation of starch, but the Algerian variety of chayote does not tuberize as is the case with the chayote of the Antilles. This may be due to the thermoperiodicityconditions encountered all year long in the region **(Zinsou *et al*., 1988; Monnerville *et al*., 2001)**.

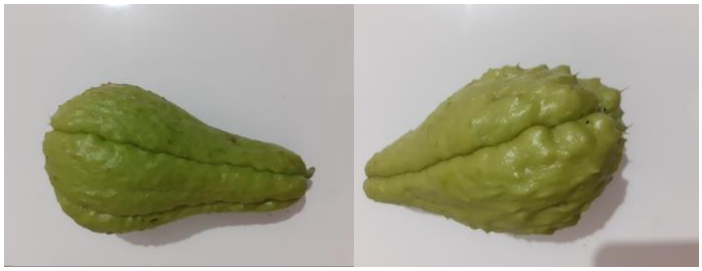
Few studies have been carried out on chayote starch despite its very interesting morphological, thermal, rheological, crystallinity and digestibility properties and which can provide it a place in the world market of starches or its derivatives.

With its low calorie intake, chayote is very appreciated in Algeria. It is cooked in savory dishes as couscous, gratin, in sweet receipt as jam and cake and in medicinal uses as tea from leaves. Currently, its use became rare due to the changing food culture and lack of cultivation. However, some farmers still continue to maintain this crop in different regions along the Mediterranean coasts. To the best of our knowledge, there are no reports regarding the starch in chayote cultivars growing in the Mediterranean ecosystem. This study aims to physically and functionally characterize the starches isolated from a variety of chayote grown in the coastal region to highlight its distinctive characteristics comparatively to starches from Mexican chayote, cereals grains (corn, wheat, sorghum) and tubers (potato, cassava). The market currently needs starch with specific properties for some particular applications, despite its low content, as in the case of sources with high water content. On the other hand, resistant and non resistant starches with their correlation to GI are of great interest in food.

MATERIAL AND METHODS

Chayote pretreatment

The Chayote fruits used for starch isolation were collected in mid-November from a farm situated in the east of Algiers (Algeria). They were cultivated under Mediterranean climate, characterized by dry summers and mild wet winters. The fruits purchased were fresh without mechanical damage. The fruits used were light green and elongated with deep ridges lengthwise. They appear in two forms; smooth and spiny (Figure 1). They had a light green color and a pear shape with 5 furrows.



A **B**

Figure 1 Fruits of Mediterranean chayote (**a**: smooth fruit and **b**: spiny fruit**)**

Chayote fruit dimensions were in the range of 6.70-17.82 cm and 3.31-9.83 cm for length and width with mean values of 12.75±0.23 cm and 7.41±0.16 cm respectively. Fruit of the Algerian chayote is not different in size from that reported by **Lira Saade (1996)** which were 4.3-26.5 cm and 3-11 cm for length and width, respectively.
Fruit weight varied over a wide interval 93.59-794.78 g with mean mass of 353.12 ± 0.43 g. The smooth and spiny fruits, in sample ratio of 5/9, were washed with water, dried and then peeled with a commercial potato peeler.

Starch isolation and purification

Starch was isolated from Chayote fruits according to the **Ganga and Cork (1999)** method with some modifications. Fresh fruits were cut into small cubes and grounded in a Condor MX-D1552 blender with 2 volumes of distilled water at high speed until the mixture was smooth and then left to stand for 20 min. The total ground was filtered through a 250 µm sieve and centrifuged at 3000 xg for 10 min. The obtained starch was washed several times until the wash water became transparent. The residual solid retained in the sieve underwent the same treatment. All starch recovered was dried in an air oven at 40 °C for 24 h.
The residual proteins of isolated starch were estimated according to the AACC 46-13 Crude Protein-Micro-Kjeldahl method.

Starch color

The HunterLab *MiniScan* spectrophotometer (Virginia, USA) was used to determine the color of the isolated starch powder. The device was calibrated using white and black panels. This measurement is quantified by Hunter lab system (1958) given by L, a, and b parameters.

Glucose quantification

Starch content was determined according to the method of **Goñi *et al*. (1996)**. Granules were solubilized in KOH solution 2 mol/L, followed by enzymatic hydrolysis using fungal α-amylase from *Aspergillus orysae* (26 U/mg, Fluka 10065), afterwards amyloglucosidase from *Rhizopus mold* (23000 U/g, Sigma A7255) at pH 4.6 and 55°C for 45 min. Glucose quantification was performed by DNS method **(Southgate, 1976)**. Starch content was determined by multiplying the glucose concentration by 0.9, a factor conversion from glucose to starch.

Amylose content determination

The amylose was estimated by the color iodine method approved by **Juliano *et al.* (1981)** and widely described by **Beta *et al*. (2001)**. A mass of 100 mg of extracted starch were added to 1 mL of ethanol (95%) and 9 mL of NaOH (1 mol/L). The mixture was heated in a water bath at boiling point for 10 min, cooled and completed with distilled water to 100 mL. A volume of5 mL of standard solutions and obtained homogeneous solution was taken for calibration curve and starch sample, respectively. Then, 1 mL of aqueous solution of acetic acid 1N, and 2 mL of iodine solution (0.2% I_2 dissolved in 2.0% KI) were added. The mixture was left in dark for 20 min at room temperature. The absorbance of the blue complex formed was measured with a Jasco V-630 UV-Visible spectrophotometer at 620 nm.

Microscopy granules starch analysis

Shape and size of chayote starch granules were examined using three imaging techniques: an optical microscopy (Nikon, Japan) under daylight and polarized light at 40x magnification were processed by a DS-Fi2 camera and Lucia software and Scanning Electron Microscopy (SEM) with FEI Quanta 650 equipped with EDX system and XFlash 6|10 Detector (Bruker Nano GmbH Berlin, Germany). Window type is Slew AP3.3. Magnification of 6000x and 24000x were used.

Granule size determination

The size distribution of the granules was determined by laser diffraction using Malvern Mastersizer 2000 (Worcestershire, England). A few drops of starch suspension (about 10%) were poured into the measurement cell. Ultrasounds were applied at ambient temperature for 30 s to disperse any blocks of granules. The particles were deviated from the parallel beam of the monochromatic light. The results express the granules size as a function of the occupied volume ratio. The measurements were repeated 3 times and before each analysis, 2-3 wash cycles were performed.

Swelling power (SP) and water solubility index (WSI) determination

SP and WSI were determined according to methods of **Radosta *et al.* (1991)** and **Tang *et al*. (2004)**. The palatability hydration properties of starch granules were estimated for suspensions of 100 mg of starch in 10 mL of distilled water. They were constantly stirred and heated in a thermostatic water bath for 30 minutes at temperatures ranging from 50 to 95 °C, and then cooled to room temperature. Finally, they were centrifuged at 2000 rpm for 10 minutes. The supernatant was collected, and evaporated in an oven at 130 °C. The remaining gel was weighted. SP is expressed in g of gel/g starch db and WSI correspond to the ratio between the mass of the evaporation residue of the supernatant and the mass of starch (db). WSI and SP were calculated respectively according equations 1 and 2 below:

$$WSI(\%) = \frac{W1}{0.1}\,100 \qquad \text{(eq. 1)}$$

$$SP(g/g) = \frac{Ws}{0.1\,(100\% - WSI)} \qquad \text{(eq. 2)}$$

Where W_1: mass of the solid precipitated in the supernatant solutionand W_s: mass of the precipitate (g).

Rapid Visco Analyzer (RVA) measurements

Pasting properties of ACHFS were performed according to the ICC-Draft- method N° 126 (1995) using Rapid Viscosimeter Analyser RVA-4 Newport Scientific (Warriewood, Australia). 3g of starch (4.5% db) were suspended in 25 g of distilled water in an aluminum RVA sample canister which was introduced in the RVA apparatus. The following profile of heating and cooling of 13 minutes was applied: holding at 50°C for 1 min, heating at 95 °C for 3.7 min, holding at 95 °C for 2.5 min, cooling to 50 °C for 3.8 min and finally holding for 2 min. The mixture was stirred at 960 rpm for 10s for strong homogenization and then at 160 rpm for the remainder of the test. Peak viscosity (PV ,cP), trough (TV, cP), breakdown (BD, cP), final viscosity (FV, cP), setback (SB, cP), peak time (PT ,min) and pasting temperature (GT, °C) were obtained from the pasting curves.

Differential Scanning Calorimetry (DSC)

To study the thermal properties of ACHFS, Differential Scanning Analyzer DSC 2920 (TA Tools, New Castle, USA) was used and both eicosane (T_0 = 36.8 °C and ΔH = 247.4 J/g) and indium (T_0 = 156.6 °C and ΔH = 28.71 J/g) were applied to calibrate the DSC. Calorimetric measurements were carried out on samples of 5 mg (db) loaded into 10 µL of distilled water. The samples were hermetically sealed in an aluminum capsule and allowed to stand for 1h at room temperature. The suspensions were then heated in DSC at a rate of 5 °C/min from 10 to 120 °C. An empty aluminum pan was used as reference. Temperatures of onset (T_0), and peak (T_p), of gelatinization and endothermic enthalpy (ΔH) were deduced from DSC .curves

X-ray diffractometry (XRD)

X-ray diffraction techniques were used for the identification of crystalline phases of starch which affect significantly the properties and functions of starch. XRD analysis was performed using a Philips X Pert PRO MPD, Analytical diffractometer configured for Bragg-Brentano, BB, configuration) with Cu Kα radiation (154 nm) at 45 KV and 40 mA. The range of 10-90 was recorded with 0.1 resolutions.
The relative crystallinity index was evaluated according to the Hayakawa method (**Chakraborty *et al*., 2004**) by determining the ratio of the area occupied by the main diffraction peaks and the total area bounded by the curve. The areas were

determined by weighing the two sections and reported to the unit area by measuring the mass of a known surface (50.00 mg/cm^2).

Starch digestion procedure

The *in-vitro* starch digestibility was studied according to the modified method of **Goñi *et al.* (1996)** using α-amylase type VI.B from porcine pancreas (A3172, Sigma-Aldrich). Starch (300 mg) was dispersed in 25 mL of phosphate buffer solution (pH = 6.9) to which 5 ml of enzyme solution (0.02 % m/V) were added. The mixture was left for 3 hours at 37 °C with continuous shaking. Samples of 0.2 mL were taken every 30 min over 3 hours. The starch digestion was stopped by placing reactor in a boiling water bath for 5 minutes. After that, 0.833 µL of amyloglucosidase from *Aspergillus niger* (300 U/mL, Sigma, A-7095) in sodium acetate buffer pH = 4.75 was introduced and incubated at 60 °C for 45 min. The reaction volume was adjusted to 20 mL with distilled water. The glucose concentration C_G was measured using glucose-oxidase and peroxidase method. Percentage of digested starch was determined as follows (eq. 3):

$$D_t = [(0.9 \times C_G \times (1/1000) \times V]/ [\ Ws \times (TS\ (\%)/100)] \qquad \text{(eq. 3)}$$

Where the factor of 0.9 represents stoichiometric constant of glucose content conversion into starch; V, volume of digesta (mL); Ws, sample weight (mg); TS (%) corresponds to the total starch expressed as percentage in dry matter.
The first-order exponential model in the kinetic study of enzymatic hydrolysis allows to estimate the starch digestibility, or what is known as the glycemic index, GI, as given by **Goñi *et al.* (1996)** by integrating the area under the kinetic curve, $D_t = f(t)$, limited by $t_0 = 0$ min and $t_{final} = 180$ min. This area is designated by AUC_{exp} and determined according to equation (4):

$$AUC_{exp} = D_\infty\ t_f - \frac{(D\infty)}{(K\,(1-\exp\,(-k\ tf))} \qquad \text{(eq. 4)}$$

Where D∞, digested starch at infinite time (g/100 g dry starch); K, rate constant (min^{-1}) and t_f, final time (min).
The hydrolysis index, HI, is expressed by the ratio between the value of the two areas under the kinetic curve in the case of the reaction of the sample and the reaction of white bread, estimated at ~15500 min g/100 g dry starch. The glycemic index, GI_{HI}, was calculated using the following formula (eq. 5) (**Goñi *et al.*, 1996)**.

$$GI_{HI} = 39.51 + 0.570\ HI \qquad \text{(eq. 5)}$$

The glycemic index value at 90 minutes (GI_{H90}) was calculated by equation 6

$$GI_{H90} = 39.21 + 0.803\ D_{90}) \qquad \text{(eq. 6)}$$

Then the mean value of the glycemic index (GI_{avg}) was measured with equation 7

$$GI_{avg} = (GI_{HI} + GI_{H90})/2 \qquad \text{(eq. 7)}$$

Statistical analysis

All the assays related to characteristics determination were performed in *quadruplet*. The mean and standard deviation of measurements were calculated using Excel.

RESULTS AND DISCUSSION

The starches have been reported to differ in granules size, shape and amylose/amylopectin ratio according to botanical source and environment conditions during cultivation. These characteristics affect rheological, thermal and other functional properties and distinguish one starch from another. These chayote starch characteristics are given below and compared to starch from tuber chayote and other conventional sources.

Isolated and purification of chayote starch

Moisture content of chayote fruit was 90.14%. This high water content may have advantages in industrial starch extraction processes, as in the case of potatoes **(Bergthaller *et al.*, 1999)**. Starch content was estimated at 0.35 ± 0.01%. So, in 100 g of fresh chayote fruit there is 0.35 g of starch potentially extractible. Starch content, evaluated at 0.33% (db), was slightly higher than the Mexican chayote fruit (0.20 %) reported by **Garzón (2006)**. It was included in the range of 0.20-1.56% (db) given by **Vieira *et al*. (2018)**. Starch content in fruit is much lower than in tubers. Indeed, **Jiménez-Hernández *et al*. (2007)**, **Hernandez-Uribe *et al*. (2010)** and **Aila-Suarez *et al*. (2013)** have estimated it, respectively, at 60.00; 72.80 and 89.1%. Relatively to potato (70.01%) and cassava (84%), starch in chayote landrace was lower. Also, as expected, in comparison to cereal, starches content of ACHFS had lower values than those given by **FAO (1999)** for wheat (69.7%), corn (63.6%), barley (55.8%) and sorghum (66.8%). Furthermore, as mentioned by **Jiménez-Hernández *et al*. (2007)**, chayote could constitute a non-conventional starch source of specific starch competing with conventional sources, especially since it presents interesting functional properties.
Using the method of **Ganga and Cork (1999)**, starch was isolated from chayote fruit with a purity of 65.30%. The isolation yield (0.35%), as weight of starch isolated from 100g of chayote fresh fruit, is higher than that given by **Monnerville *et a*l. (2000)** which is between 0.20-1.56%. It is, obviously, smaller than those obtained for chayote tubers by **Jiménez-Hernández *et al*. (2007)** and **Hernandez-Uribe *et al*. (2010)** which were 72.80 ± 0.60% and 89.10 ± 0.96% respectively. The starch was isolated at a rate of 3.35% in term of db. This value is within the range of 1.8-31.2% given by **Monnerville *et al*. (2001)**.
Starch production from chayote fruit has reached 0.16% (starch db per fresh fruit). So, 45.71% of starch was extracted from total starch contained in the fruit with 65.30% purity. This is a small value compared to the yield obtained from MCHTS estimates at 55% by **Hernandez-Uribe *et al*. (2010)** with a purity of 89.1%. Significant loss observed during the purification step had the effect of reducing the extraction yield. The isolated starch holds in 5.50 ±0.13% of moisture and 0.23% of residual proteins. According to **Bergthaller *et al*. (1999)**, moisture content down to 20 % is permitted for commercialization. So, edible ACHFS can find a place in the local and global market as a raw material for many food and non-food applications.

Amylose/amylopectin content

The amount of amylose present in the granule starch affects, significantly, its physicochemical and functional properties. It varies depending on the botanical source and is affected by climatic conditions and soil **(Singh *et al.*, 2006)**. In ACHFS, amylose content was 20.36% and so, 79.64% were amylopectin. This content is different in MCHTS as reported by **Jiménez-Hernández, *et al*. (2007)** and **Hernandez-Uribe *et al*.(2010)** who gave the range of 12.90 ± 0.64 and 26.3 ± 0.38% respectively. However, it is close compared to that in potato (table 1) **(sing *et al.*, 2003)** and Cassava **(Yuan *et al.*, 2007)** and it is closer than the amylose content found in cereal starches such as corn starch **(López *et al.*, 2010)**, wheat starch **(Sing *et al*., 2003)**, barley starch **(Ellis *et al*., 1998)** and sorghum landrace starch **(Boudries *et al*., 2009)**.
In general, starches with lower amylose content are more susceptible to chemical and/or physical modifications than those with higher content, because amylose is linear and has crystalline structure, whereas amylopectin is amorphous. The cross-linked starch with low amylose content showed a higher phosphorus content than the other modified starches. In addition, the starch sample with lower amylose content had higher water absorption due to the greater stiffness of the hydrogel structure that resisted swelling. Indeed, amylose plays a role in the initial resistance of granules to swelling and solubility. Lower amylose content indicates that starch needs less energy for its gelatinization. The formed paste has a higher viscosity with fewer tendencies to retrogradation. Differences in granule swelling (onset of viscosity), PT, PV, shear-thinning during gluing, and gel stability are mostly attributed to the difference in amylopectin structure **(Ring, 1985)**, while the differences in relapse and FV during gluing is due to the amylose structure **(Leloup *et al*., 1991)**. As industrial application, amylose can form strong films.

Table 1 Amylose and amylopectin contents of starches from chayote and other conventional sources.

Source	ACHFS	MCTS	Barley	Wheat	Cassava	Corn	Potato	Sorghum
Amylose (%)	20.36	12.90	19-22.1	18-30	23.7	23.86	20.1-31.0	24.8-27.1
Amylopectin (%)	79.64	87.1	77.9-88	70 -82	76.3	76.14	69-79.9	72.9-75.2
References	**This study**	**Jiménez-Hernández *et al*., 2007**	**Ellis *et al*., 1998**	**Sing *et al*., 2003**	**Yuan *et al*., 2007**	**López *et al*., 2010**	**Sing *et al*., 2003**	**Boudries *et al*., 2009**

Starch color

The isolated starch has a high degree of whiteness. The L value was 93.57 ± 0.40 with low **a** (-0.30 ± 0.01) and **b** (3.92 ± 0.19). The obtained whiteness, redness and yellowness given by **L, a** and **b** confirm the high purity of starch. **Wang *et al*. (1993**) have estimate that **L** higher than 90, gives a satisfactory whiteness for the isolated starch. Starches from corn and sorghum had similar color **(Boudries *et al*., 2009)**.

Morphological properties

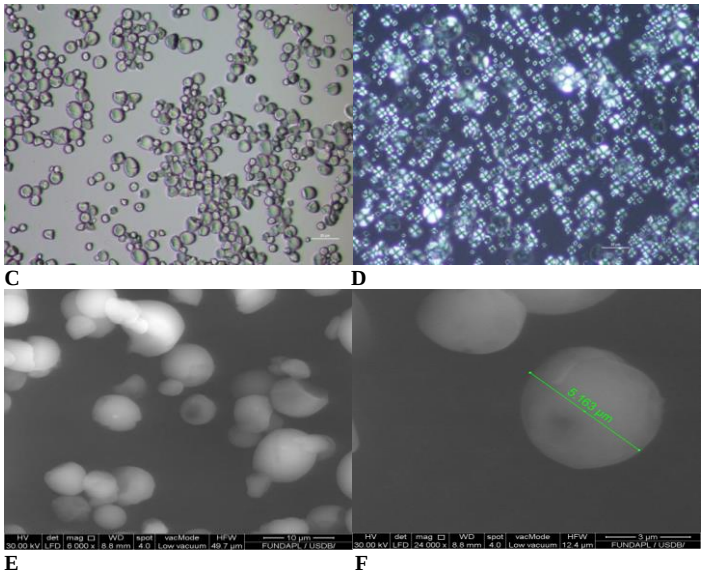
Starch granules vary in shape and size. They are related to the botanical species and affect starch functionality. Sometimes, they allow orienting the applications.

The optical microscopy 3D image of granules of ACHFS (Figure 2, (c)) shows heterogeneity in morphology. Spherical, oval and polygonal forms can be observed. The surface of the granule was smooth without pinholes. Similar observations were made by various authors for MCHTS **(Jiménez-Hernádez *et al*., 2007)**.

Exposed to polarized light (Figure 2, (d)), the granules shine, and a cross appears on the surface due to the birefringence. This phenomenon indicates that granules are native and intact. **Bergthaller *et al*. (1999)** reported that the extraction process controls the quality of granule in the ratio of the presence of broken and cut granules. So, the absence of damaged granules is the result of the precautionary follow-up in the mechanical steps (milling) which reduces broken and truncated granules. Damaged and broken granules affect the characteristics of starch. However, according to **Bergthaller *et al*. (1999**), it is not considered in the quality standard of starches.

The SEM micrographs of ACHFS granules in figure 2 (e) and (f) respectively for magnification of 6000x and 24000x show, more precisely, the round and oval shapes and the surface appears smooth with absence of pinholes.

Granular structures of starch from conventional sources show significant differences in size and shape when viewed by SEM. Typically, rice and maize starch have angular (polyhedral) granules; potato starch has oval-shaped granules. Wheat starch consists of spherical and flat circular (lens)-shaped granules. Sizes also vary widely. Rice starch granules are very small (Ø=6 µm), while potato starch granules can exceed 100 µm. They differ from those of tubers and roots which are oval but look like those of sorghum as described by **Boudries *et al*. (2014**). Wheat starch has a bimodal distribution of granular sizes-small (B) granules average 4 µm, while the large (A) granules average 14 µm. Compared to available conventional starches, shape of granules of chayote are considered as small and oval with diameters lower than 60 µm. So, they are desirable in cosmetic industries.



C **D**

E **F**

Figure2 Micrographs of ACHFS granules, (**c**: normal-light microscopy (x40); **d**: polarized-light microscopy (40x); **e**: SEM (x6000) and **f**: SEM (24000x)

The granule size of ACHFS ranged between 3.56-37.24 µm with a mean size of 10.98 µm in unimodal distribution as shown in figure 3. Granules are smaller than those in MCHFS (61 µm) **(Garzón, 2006**) and MCHTS (7-50 µm) **(Jiménez-Hernández *et al*., 2007**). However, they are close to those of barley starch (19-30 µm) with oval, irregular or cubic surfaces (**Morrison *et al*., 1986**). The size of the irregular cassava starch granules (11.9-12.2 µm) was close to the mean value given by **Mishra and Rai (2006**). Spherical and oval-shaped forms were observed in potato granules starches in the range 14.3-53.6 µm (**Jane, 2009**). The granule size indicates some possible applications. Indeed, starches with diameters lower than 60 µm, as in the case of with chayote starch, are desirable in cosmetic industries (**Paredez-López *et al*., 1989**).

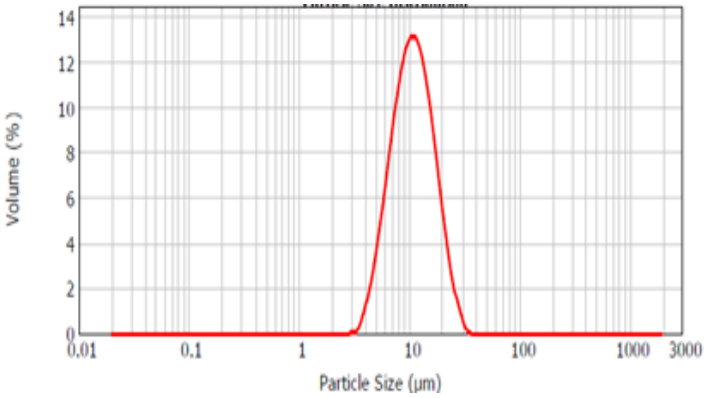


Figure 3 Granule sizes distribution of ACHFS

Swelling Power (SP) and Water Solubility Index (WSI)

The difference in starch swelling is essentially due to the granule size and the complex amylase-lipids. It is also due to the chain structure because of a ramification on the chain of amylopectin This leads to the further penetration of water **(Bertolini, 2010)**. When heated, granules in water absorb water and swell. This behavior has been described as a loss of radial regulation of amylopectin and amylose chains **(Muñoz et *al*., 2015)**. Starch of chayote increased in swelling with temperature to 22.12 g/g at 75 °C. After that, a decrease of SP reaching 19.97 g/g at 95 °C was observed (Figure 4). This value is higher than that of starches isolated from corn (14 g/g) **(Morrison *et al*., 1986)** and sorghum (12-15 g/g at 95°C) **(Boudries *et al*., 2009)**.

The maximum swelling is observed near the clotting temperature, but all starches continue to absorb water above the coagulation temperature. The swelling continues until balance is reached and does not stop at the coagulation temperature, which is a critical reference point for starch granules **(Li & Yeh, 2001)**. It was reported that the swelling power of small granules at 95°C was higher **(Sasaki & Matsuk, 1998)** as confirmed by ACHFS.

Increased heating reduces friction and adhesion between particles, resulting in increased release of pellet components towards the medium. For starch isolated from chayote (Figure4), WSI was 6.81% at 65 °C, it increased until 85 °C, where it remained nearly constant, at 12.31%. This value is lower than those given by **Radosta *et al.* (1991)** for potato starch (25%) and barley (18.40%). On the other hand, it was similar to starches of corn (11.21%), wheat (12.72%) **(Morrison *et al*., 1986)** and sorghum (10%) **(Boudries *et al*., 2009)** . High WSI suggests the presence of high proportion of short amylose chains which increases viscosity. **Garzón (2006)** gave values of 112 g/g and 0.13% for water holding capacity and soluble substances for MCHFS.

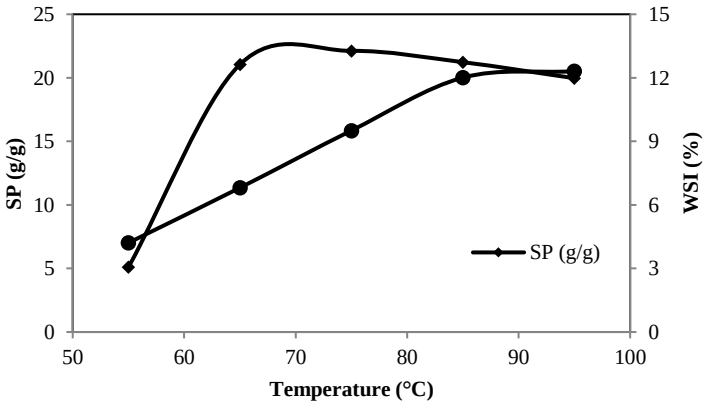


Figure 4 Swelling power, SP, and water solubility index, WSI, profiles of ACHFS

Pasting properties

Pasting properties are intimately linked to granule swelling, amylose leaching, crystallinity, lipid content and degree of polymerization (DP) of starch chains. PV and BD were negatively correlated with starch granule size and the ratio of long chain, and positively correlated with starch content. In addition, PV and BD values either increased or decreased, depending on protein type and concentration (**Zhang & Xu, 2019**).

The pasting profile of chayote starch and parameters recorded by Rapid Visco Analyzer (RVA4), are presented, respectively, in figure 4 and Table 2. The curve shows a distinct profile where the viscosity peak and breakdown are not

accentuated, and the viscosity continue rising even during the cooling step. The results of the rheological properties (RVA) of ACFS dispersion were as follows: PV=2158.25 cP, BD=40.75 cP, FV=3328.75 cP, TV=2117.5 cP, respectively, for peak viscosity, breakdown, final viscosity, and trough. ach with high PV and FV can be used as thickening agent in food dispersants, where high viscosity is required.

The RVA profile is different from those obtained for conventional starches isolated from potato, corn, rice and tapioca given by **Horstmann *et al*. (2016)** and sorghum starches given by **Boudries *et al.* (2009)** .However, the thermal behavior of chayote starch looks similar to wheat starch below 95 °C, but different above that temperature.

When comparing the viscosity of starch from chayote fruit to tuber, it appears that the profiles are very different. That of chayote tuber starch seems similar to potato starch **(Jiménez-Hernández *et al.*, 2007)** with higher PV and PT relatively, and in an inverse pattern to those reported by **Hernandez-Uribe *et al.* (2010)**. High PV reflects the ability of starch granules to swell freely before their physical breakdown **(Singh *et al*., 2003 & Hernandez-Uribe *et al*., 2010)**. The noticed differences on viscosities can be attributed to amylose/amylopectin ratio as well as to the chain length of the two polymers.

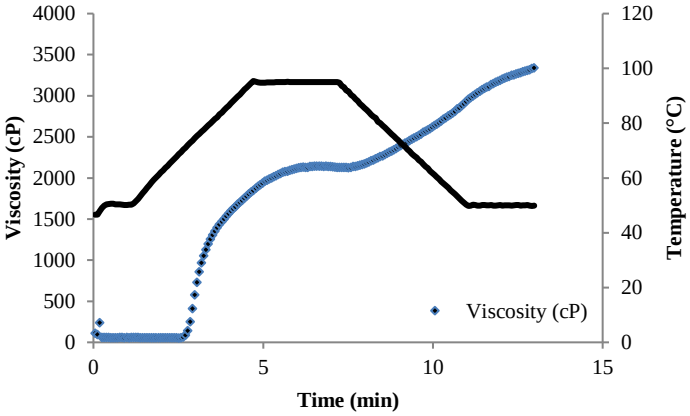


Figure 5 Pasting profile of ACHFS

The dispersion properties of starch are affected by the chain length distribution of amylopectin more than by the molar mass (**Srichuwong *et al*., 2005)**. Also, the viscosity during gelatinization is significantly affected by amylose and amylopectin (**Ai & Jane, 2015)**. Compared to chayote starch, potato starch showed a slight increase in viscosity as noted by (**Mali *et al*., 2003)**. The increased viscosity results from the difference of amylose and amylopectin network while retaining water in gel formation.

Table 2 Viscosity parameters of ACHFS suspension

Starch source	PV (cP)	TV (cP)	BD (cP)	FV (cP)	SB (cP)	PT (min)	Tg (°C)
ACHFS	2158.25 ±21.31	2117.5 ±13.52	40.75 ±18.22	3328.75 ±12.01	1211.25 ±10.05	6.62 ±0.29	71.08 ±0.05
Chayote tuber	14746 ±787	2329 ±166	12417 ±853	4939 ±118	2610 ±87	3.59 ±0.26	67.75 ±1.36
Potato	9412 ±61	1987 ±210	7424 ±148	4253 ±136	2455 ±213	2.97 ±0.15	67.75 ±1.36
Corn	4959 ±101	3231 ±307	1724 ±75	4237 ±168	1037 ±101	6.08 ±0.79	78.55 ±3.79

Thermal properties

DSC pattern of transition, as assessed by DSC, is shown in figure 6 and characteristics T_0 (64.29°C), T_P (66.89°C) and ΔH(15.79 J/g) are tabulatedin table 2. These characteristics qualitatively reflect the importance of crystallinity through the transition enthalpy. The pattern shows only one peak. Many authors reported that, at intermediate water levels of moisture, a second peak associated to melting, can appear due to the disorganization of starch crystallites. The endotherm of starch gelatinization represent essentially the difference between the endothermic energy, associated with granule swelling, melting of crystallites and the exothermic energy associated with hydration of starch and formation of amylose-lipid complexes **(Lindeboom *et al*., 2004)**.

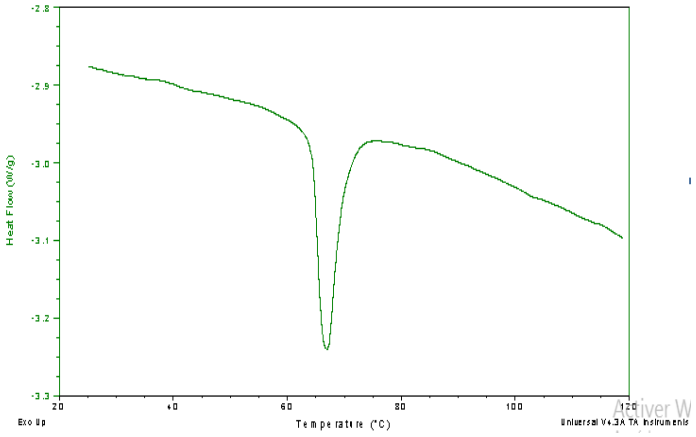


Figure 6 DSC Thermogram of ACHFS

Table 3 DSC transition parameters of ACHFS

T_0 (°C)	T_p (°C)	ΔH (J/g)
64.29±0.01	66.89±0.09	15.79±0.48

Onset temperature of gelatinization (T_0) of ACHFS was evaluated at 64.29 °C. It is close to that obtained for Spanish chayote starch **(Garzón, 2006)** and seems slightly lower to that of MCHTS given by**Jiménez-Hernández *et al*. (2007)** which was 65.18 °C. At the peak, the temperature $_pT$ of ACHFSwas 66.89 °C, while, the previous authors gave temperatures of 2 °C higher for both chayote tuber and fruit starch. Compared to other tuberous starches, **Morrison *et al*., (1986)** gave a similar T_p (64.4°C) for potato starch, whereas **Muñoz *et al*. (2015)** reported slightly higher value (68.69°C) for cassava starch. Cereal grains starches present higher gelatinization temperatures and lower gelatinization enthalpies. Nevertheless, the differences between the endothermic enthalpy of transition are very significant. Indeed, local ACHFS enthalpy was evaluated by DSC at 15.79 J/g. It seems similar to potato starch (16.8 J/g) as reported by **Li & Yeh (2001)** but less than that of SCHFS (26 J/g) **(Garzón, 2006)**. ΔH of chayote tuber starch of 1.13 J/g was very weak. This lower enthalpy value suggests a lower proportion of organized structures or a lower stability of the crystalline regions in granules. Gelatinization enthalpy corresponds to overall crystallinity of amylopectin and loss of double helical order is considered to be responsible for enthalpic transition. **Hoover (1996)** reported that higher amylose content leads to higher gelatinization temperatures indicating that these granules could be more resistance to gelatinization and swelling.

Crystallinity in chayote starch

Figure7 shows the XRD pattern of starch isolated from chayote fruit and this could be the first report of aXRD pattern and crystallization level of chayote fruit starch. Two main peaks appeared at 17.12° and 23.48° (2 θ). The same profile and same peaks were recorded in starch of chayote tubers as reported by **Hernandez-Uribe *et al*. (2010)** at figure 7. B-type XRD pattern was observed for both chayote fruit and tuber starch. Starches with *B*-type crystalline structure is typical of high-amylose starches of tubers, fruits, and stems. Potato starch had two distinct and wide peaks which seem more crystalline than the chayote tuber starch. The cassava and sweet potato starch show A and C type according to the variety. Differences in crystallization level was found in chayote fruit and tubers and potato starches. Indeed, potato starch crystallizes more than fruits and tubers chayote. Compared to wheat starch, sorghum starch had higher crystallinity (**Chakraborty *et al.* 2004)**. It appears that the more crystalline starch comes from the more regular alignment of its chains. So, increasing the degree of crystallinity increases hardness and density. It is one of several factors that determine the digestibility of starch in animals **(Benmoussa *et al*., 2006)** and correlates with the molecular structure of amylopectin (chain units length, branching range, molecular weight, and polydispersity) **(Bao & Bergman, 2004)**. However, there is no empirical evidence to exclude the presence of amylose in the crystalline regions. The X-ray diffraction is important in starch properties such as digestion **(Aila-Suárez *et al*., 2013)**.

From the crystalline structure refinement work type A and B, proposed from purified extracts of amylose, it appeared that in the hexagonal mesh that characterizes type B, each double helix has 3 neighbors and the helices are arranged around a central cavity which contains 36 water molecules per cell. However, in the type A structure, possessing a monoclinic mesh, the structure is dense because each double helix has six neighbors. The hydration by mesh is then 4 molecules of water.

The crystallinity index (CI) which measures the proportion of matter in the crystalline structure, varies from 15 to 45% in starches **(Zobel, 1988b)**. CI of chayote fruit starch, deduced from XRD pattern, was evaluated at 29.0%. This value is very close to that obtained for chayote tuber (28.2%) (**Hernandez-Uribe *et al*., 2010)** and red sorghum (28.9%) (**Boudries et al., 2009**). It is lower than that of potato (38.5%) (**Hernandez-Uribe *et al*., 2010**) and higher than that of cassava, which is generally of type A or C with CI ranging from 8 to 14% (**Moorthy, 2002**).

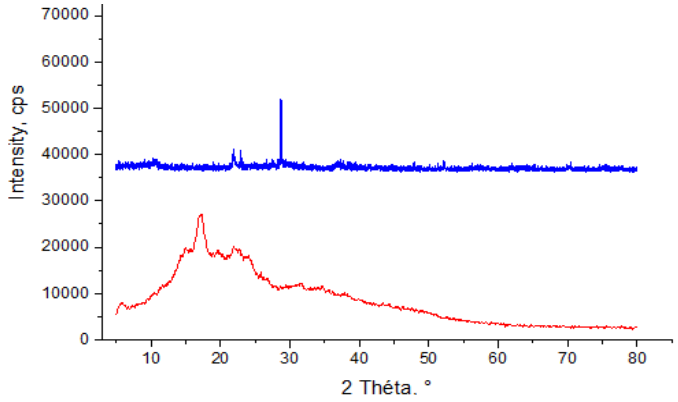


Figure 7 XRD pattern of ACHFS

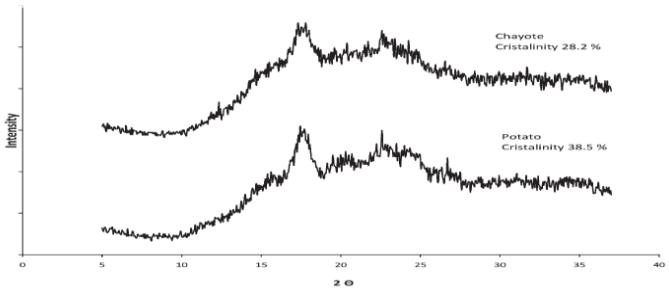


Figure 8 XRD of chayote tuber and potato starches **(Hernandez-Uribe *et al.* 2010)**.

***In vitro* digestibly of ACHFS**

The kinetic curve of enzymatic hydrolysis of ACHFS is shown in figure 9, representing the change in the mass of digested starch (D_t) over time. The hydrolysis reaction is subject to a first-order model and the parameters of the model as D_∞, K, hydrolysis index HI andaverage glycemic index (GI_{avg}), were estimated according to **Goñi *et al*. (1996)** and reported in Table 3. The digested starch in an infinite time D_∞ reached 50.66% for ACHFS. This percentage varies according to the structure of the starch and therefore of the botanical source. K value was 0.05 min^{-1}, which is close to what was recorded in sorghum starch 0.0131 min^{-1} by **Souilah *et al*. (2014)**. The hydrolysis index (HI) of chayote fruit starch was 52.16% and GI_{avg} was 70.16 according to the GI classification as suggested by **Hernandez-Uribe *et al*. (2010)**. In sorghum, maize (*Zea mays*) and brown rice, it was evaluated respectively at 76.86 and 85,94 % **(Hernandez-Uribe *et al*., 2010)**. The results indicate that chayote fruit starch has different GI from other sources starches. This provides several types of starch for feeding different segments of the population. Highly digestible starches for children, starch with low digestion or resistant starch for patients (diabetes, colon ...) and starch that releases energy slowly for athletes and hard workers. This *in-vitro* method estimate the metabolic glycemic response to a food. The percentage of starch hydrolysis at 90 min was the best correlated value with *in-vivo* glycemic responses.

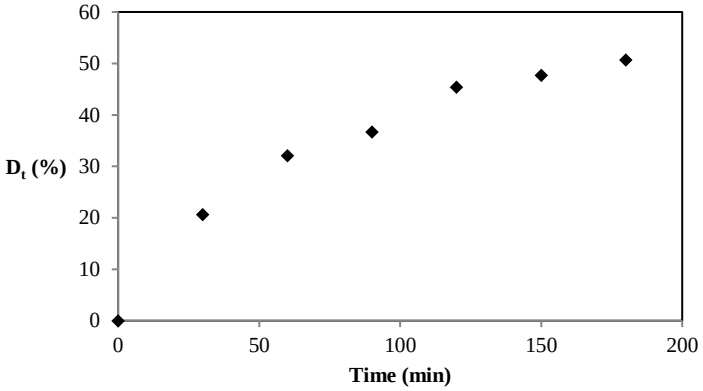


Figure 9 Change in the mass of digested starch (D_t) over time

Table 4 Digestibility and glycemic parameters of the first-order kinetic model

	K (min^{-1})	D_∞	AUC	HI (%)	D_{90exp}	GI_{D90}	GI_{HI}	GI_{avg}
ACHFS	0.05	50.66	8084.92	52.16	39.69	71.08	69.24	70.16

CONCLUSION

Native and modified starches isolated from different sources have potential applications in food and non-food industries. The potential applications of starch and starch-based compounds are related to their functional properties. Currently, word markets require starch with specific properties. In this study, starch was isolated from chayote fruit, cultivated in the Mediterranean ecosystem of Algeria, with appreciable purity despite extraction difficulties. These difficulties differ from those encountered with starch of conventional botanical source due to the differences in its functional properties, as well as in the presence of residual materials. The starch granule size ranged between 3.56 and 37.24 µm. The amylose was present at 20.36%, while amylopectin made up 79.64%.

Starch granules are smaller than those of other cultivars. Starch paste showed high viscosity peak, which reached 2158.25 cP. SP profile was singular, and a maximum of swelling strength was observed at 75°C with a decrease until reaching 19.97 g/g at 95°C. The XRD pattern of starch isolated from chayote fruit was of B-type and the crystallinity index was evaluated at 29.0%, which is close to that given for chayote tuber. The higher crystallization of starch indicates higher hardness and density. The digestibility of starch was of up to 90% and follows a first-order model. It can be affected by several factors including the genotype, environmental conditions, and structure of the starch and non-starchy components. The examination of functional properties of starch manufactured from novel materials allows considering its use in special products. Starches having small granules and tight distributions can be intended as a binder for fine printing paper and plastic sheets and as a component in cosmetic products. Small granule starches are also suitable for coating, paper, textile and photographic industries. Additionally, they are used as a cold-water laundry-stiffening agent. They offer superior penetration into the fabrics and their stiffness is less affected by humidity. This new starch has acceptable nutritional value with good *in-vitro* digestibility characteristics, which make it suitable for human nutrition. It is, also, appreciated as a functional ingredient in the starch-based food, as well as in biotechnological modifications. More investigations are required to shed light on the structure of amylose and amylopectin to assess the extent of the environmental and genetic influence on the properties of starch and to direct its use towards specific applications.

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