RESEARCH ARTICLE

Isolation and characterization of Mexican chayote tuber (*Sechium edule* Sw.) starch

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Starch was isolated from Mexican chayote tubers (*Sechium edule* Sw.) and the yield, physicochemical, rheological, and molecular characteristics were investigated. Commercial potato was used for comparison. Starch yield was 49% with a purity of 89.1%. AM content of chayote tuber starch was 26.3%. Birefringence was shown in chayote tuber starch and granules had diverse shapes such as spherical, oval, and polygonal, with size between 10 and 25 μm. B-type XRD pattern was shown by chayote tuber starch. Chayote tuber starch had higher peak viscosity (PV) than potato starch and the maximum PV for chayote tuber starch was obtained at lower temperature. Flow properties of chayote tuber starch showed higher hysteresis than potato at the same concentration. In chayote tuber and potato starches, $G' > G''$ at both temperatures. Chayote tuber starch presented slightly lower gelatinization temperatures, but slightly higher enthalpy of gelatinization than potato starch, and similar retrogradation rate (due to the enthalpy value of the phase transition) were found in both tuber starches. Chayote tuber starch presented higher Mw and Rz values than potato starch. Chayote tuber could be an alternative for starch isolation with specific physicochemical and molecular characteristics.

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1 Introduction

The chayote is an herbaceous perennial, monoecious, vigorous creeper, or climbing plant. From the record it is known that, at least in Mexico, chayote has been cultivated since pre-Colombian times. The first description of chayote was probably that of Francisco Hernández, who was in Mexico from 1550 to 1560 [1]. Chayote is basically used for human consumption, not just in the Americas but is many other countries. In addition to the fruit, stems, tender leaves (usually know as quelites), the roots (in Mexico called "chayotextle," "cueza," "camochayote," and "chinchayote") are also edible. They are also appreciated as a vegetable and dessert [2–4]. There are information that mention the use of chayote in baby foods (giving consistency) and medicinal use [5, 6]. Its cultivation in Mexico has been focused on fruit production, harvesting approximately 100 452 tons/year, in 2007 [7]. However, there is no official information about of the harvesting of chayotextle in Mexico. The farmers of chayote in Tulancingo, Hidalgo, Mexico, reported tuber productions ("chayotextle") *per* plant up to four tubers, weighing an average of 6.5 kg [8], with 125 plants *per* hectare. In this sense, "chayotextle" can be an attractive agronomic alternative.

The importance of the chayote tuber is that it contains starch as its main component and can be used as an

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Abbreviations: BD, breakdown; **HPV,** hot paste viscosity; **Mw,** weight-average molar mass; **PV,** peak viscosity; **RVA,** Rapid Visco Analyzer, **Rz,** gyration radius.

alternative starch source for its isolation. However, limited information of chayote tuber starch [8] is available. Starch yield, purity of isolated starch and molecular information can be important to suggest novel industrial applications. However, to produce more information is necessary to compare with a commercial starch isolated from similar botanical source (tuber) such as potato starch.

Alternative starch sources such as Andean tubers, roots, fruits, and seeds have been studied and the potential for diverse applications reported [9, 10].

The objective of this study was to isolate starch from chayote tuber (“chayotextle”) and evaluate the morphological, physicochemical, and molecular structure.

2 Materials and methods

2.1 Chayote tubers flour

Chayote flour was prepared using 10 kg of the tubers. Tubers were washed with tap water then cut into 1 cm slices. The slices were dried at 50°C in a convection oven, ground using a commercial grinder (Mapisa Internacional, Mexico, D.F.) to pass a US 30 sieve and stored at 25°C in sealed plastic containers until further analyses.

2.2 Starch isolation

Chayote (*Sechium edule* Sw.) tubers were collected in Tulancingo, Hidalgo, Mexico. The starch was isolated following the procedure of ref. [11]. The tubers were cut into 5–6 cm cubes and immediately rinsed with a citric acid solution (0.3% w/v) and then macerated at low speed in a blender (500 g root: 500 g tap water) for 2 min. The homogenate was consecutively sieved and washed through screens number 50, 100, and 300 US mesh, until the washing water was clean. The starchy solution was steeping for 4 h and dried in a spray dryer (Niro Atomizer, Model P-6.3. Copenhagen, Denmark), with a feeding temperature of 130–150°C, a solid concentration in the feeding line of 30–40%, and an outlet temperature of 70–80°C. The powder was grinded to pass a US 50 sieve and stored at room temperature (25°C) in a glass container. Starchy powder of chayote tuber and residue were obtained. Starch powder and residue were quantified and starch recovery was determined. Commercial unmodified potato starch from Penford Food Ingredients Company (Centennial, Colorado) was used for comparison.

2.3 Moisture content

Moisture content was determined by gravimetric heating (130 ± 2°C for 2 h) using a 2–3 g sample. Ash, protein, and fat were analyzed according to AACC methods 08-01, 46-13, and 30-25, respectively [12].

2.4 Starch content

Total starch content was determined by the method of ref. [13]. In brief, a 50 mg sample was dispersed in 2 mol/L KOH to hydrolyze all the starch (30 min) and subsequently incubated (60°C, 45 min, pH 4.75) with amyloglucosidase (Roché no. 102 857, Roche Diagnostics, Indianapolis, IN, USA); glucose content was then determined using the glucose oxidase/peroxidase (GOD/PAD) assay (SERA-PAK® Plus, Bayer de Mexico, SA de CV). Total starch content was calculated as glucose (mg) × 0.9; potato starch was used as a reference.

2.5 AM content

The AAM content was determined by the test of ref. [14]. Starch (20 mg, db) was dissolved in 90% DMSO (8 mL) in 10 mL screw-cap reaction vials. The contents were vigorously mixed for 20 min and then heated in a water bath (with intermittent shaking) at 85°C for 15 min. The vials were then cooled to ambient temperature, and the contents diluted with water to 25 mL in a volumetric flask. The diluted solution (1.0 mL) was mixed with water (40 mL) and 5 mL of I_2/KI solution (0.0025 M I_2 and 0.0065 M KI) and then adjusted to a final volume of 50 mL. The contents were allowed to stand for 15 min at ambient temperature, before A measurements at 600 nm.

2.6 Light microscopy

A polarized light microscope (Eclipse 80i, Nikon, Japan) was employed with 40× objective lenses equipped with a digital camera (Digital Imaging Head, DC330 camera MTI, Japan). Dry native starch was sprinkled on a slide and a cover was added.

2.7 SEM

For SEM study, the samples were fixed to a conductive tape of copper of double glue, which was covered with a layer of coal of 20 nm thickness. The layer was deposited in vacuum with a JEOL evaporator (JEOL, Tokyo, Japan). Subsequently, the tape was covered with a layer of gold of 50 nm in thickness in a JEOL metal ionizer. The procedure was observed in the microscope and registered photographically.

2.8 XRD

The XRD was obtained from a D/max 2500 X-ray difractometer (Tokyo, Japan), a conventional X-ray tube set to

40 kV and 200 mA. The X-ray source was Cu Kα radiation. Data were collected from 2θ of 5 to 35° (θ being the angle of diffraction) with a step width of 0.02° and step time of 0.4 s, scanning speed of 8°/min, divergence slit width of 0.2 mm, scatter slit width of 0.6 mm, receiving slit width of 0.2 mm, at room temperature. Starch samples were dried at 50°C to a constant moisture (10%) in a vacuum oven, then 50 mg samples were added into the slide for packing prior to X-ray scanning. Starches were equilibrated at 50°C to constant moisture (10%) prior to analysis.

The crystallinity percentage (%C) was determined from the diffractogram by calculation of the area corresponding to the crystalline peaks (A_p; from the difference between the area under the curve and the area of the amorphous halo), the total area under the curve (A_t), and the instrumental noise (N) according to the following equation [15].

$$\%C = \frac{A_p}{(A_t - N)} \qquad (1)$$

The amorphous halo was determined with the amorphous component of starch obtained with an extraction procedure reported elsewhere [16].

2.9 Pasting profile

Pasting properties of starches were evaluated with a Rapid Visco Analyzer (RVA; RVA-4, Newport Scientific, Sydney, Australia) [12]. Starch (2.24 g, 8% moisture db) was weighed directly in the aluminum RVA sample canister, and distilled water was added to a total constant sample weight of 28 g. The slurry was then manually homogenized using the plastic paddle to avoid lump formation before the RVA run. A programmed heating and cooling cycle was set for 23 min where the samples were held at 30°C for 1 min, heated to 95°C in 7.5 min, further held at 95°C for 5 min before cooling to 50°C within 7.5 min, and holding at 50°C for 2 min. Parameters recorded were pasting temperature (P_{temp}); peak viscosity (PV); hot paste viscosity (HPV; minimum viscosity at 95°C; cool paste viscosity (CPV; final viscosity at 50°C); breakdown (BD = PV − HPV); and set back (SB = CPV − HPV). All measurements were replicated three times.

2.10 Starch gelatinization

Gelatinization parameters were measured using a DSC(TA Instruments, Newcastle, DE), equipped with a thermal analysis data station (TA Instruments) and following the procedure of Paredes-López *et al.* [17]. Starch (2 mg db) was weighed directly in an aluminum DSC pan (TA series 900796.901) of 20 μL capacity. Deionized water was added with the help of a Hamilton microsyringe to achieve a starch–water suspension containing 70% water. Pans were hermetically sealed, and allowed to stand for 1 h at room temperature for even distribution of water before DSC measurements. Indium and empty aluminum pan were used as reference to calibrate the DSC. Sample pans were heated at a rate of 10°C/min from 20 to 120°C. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and heat of gelatinization (ΔH_{gel}) were calculated automatically. ΔH_{gel} were calculated on starch dry basis.

2.11 Starch retrogradation

After gelatinization test the samples was stored at 4°C for 7 and 14 days before rescanning. Temperature range and heating rate were 25–120°C and 10°/min, respectively. An empty pan was used as reference for all measurements.

2.12 Molar mass

The solubilization procedure was carried out using the procedure reported previously [18]. The supernatant solution was filtered through a 5 μm filter paper (Millipore, Bedford, MA, USA) and dilution series were made at room temperature yielding five or six concentrations. The samples were injected into the DAWN detector (MALLS, Dawn8^+), Wyatt Technology Corporation, Santa Barbara, CA, USA) using the microbatch mode. The carbohydrate concentration of the supernatant solution after filtration was measured by the sulphuric acid–orcinol colorimetric method [19]. The weight average MW (M_w; g/mol) and z-average gyration radius (R_G; nm) were determined with the second-order Berry plot using the ASTRA software for Windows, version 4.90.07 (Wyatt 1993). Optical alignment was checked over the angular range described using filtered (0.1 μm) toluene [19, 20]. The solubilization was calculated using the total carbohydrate calculated in the supernatant after filtration divided by the amount of initial sample (0.5 mg/mL) multiplied by 100 [19].

2.13 Statistical analysis

The means (at least four replicates) and SDs were determined for all determinations. A commercial software program (Sigma Stat ver. 2.03, Jandel Corporation, San Rafael, CA) was used to evaluate by one-way analysis of variance (ANOVA) to determine differences in mean values based on data collected from replications of each measurement. Statistically significant differences ($p < 0.05$) were evaluated using the Tukey multiple comparison procedure.

3 Results and discussion

3.1 Starch yield and AM content

Moisture content of chayote tuber was 74.2% with 25.8% solids. The solids contain 59.4% starch and consequently

Table 1. Moisture content, starch content, and AM of chayote tuber and potato starches (%)$^{a)}$

Starch	Moisture	Protein	Fat	Ash	Starch	AM
Chayote	4.7 ± 0.08^{a}	0.33 ± 0.01^{a}	0.34 ± 0.03^{a}	0.39 ± 0.01^{a}	89.1 ± 0.96^{a}	26.3 ± 0.38^{a}
Potato	11.2 ± 0.08^{b}	0.22 ± 0.02^{b}	0.24 ± 0.01^{b}	0.35 ± 0.0^{b}	93.4 ± 2.2^{b}	27.6 ± 0.5^{b}

a) Average of three replicates ± SE. Means in column not sharing the same letter are significantly different ($p < 0.05$).

in 10 kg of chayote tuber there is 1.5 kg of starch potentially extractible. The amount of starch recuperated was 0.82 kg (dry basis) with moisture content of 4.7% (Table 1). The starch yield was 0.55 kg/kg of chayote tuber with a purity (assessed as total starch content, Table 1) of 89.1%. Commercial potato starch has 11.2% of moisture content and purity of 93.1% (Table 1). Other components, protein, fat, and ash are present in starch as minor constituents (Table 1), but likely oligosaccharide and non-starch polysaccharides could be present in starch of both tubers, because soluble sugars were not detected in tuber starches [8]. Differences in moisture content can be related with the granule size (superficial area) and molecular starch structure (hydroxyl groups), but the isolation process used for chayote tuber produced starch with high purity. However, the starch yield of chayote tuber was lower than those reported for other tuber (taro) of different varieties used for starch isolation, ranged between 51.4 and 57.9% [21].

AM content of chayote tuber starch (26.3%) is slightly less than commercial potato starch (27.6%) used as reference. Another cultivar of chayote tuber (*S. edule*) was used for starch isolation, reporting lower AM content (12.9%), but similar for commercial potato starch (25.9%) [8]. The cultivar and the method for AM determination can be responsible of this difference in AM content.

AM content for tuber and root starches ranged between 15 and 38% [14], for different cassava varieties between 13 and 27%, and different potato varieties between 8 and 38% [22].

3.2 Microscopy studies

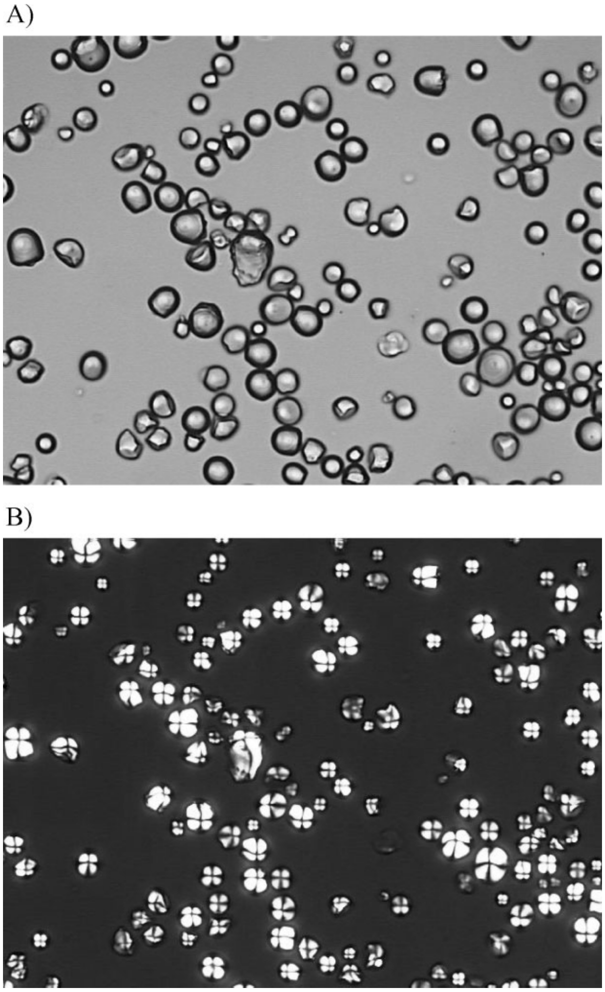


Figure 1. Micrographs of chayote tuber starch. (A) Light microscopy; (B) Polarized-light microscopy.

Light microscopy of chayote tuber starch (Fig. 1) showed spherical, oval, and polygonal shapes; while potato shown (Fig. 2) rounded and oval granule shapes, with smaller granule size for chayote tuber starch. Both starches presented a structure exhibiting a well-defined birefringence pattern with a dark cross. The birefringence in both starches showed that the isolation process did not modify the molecular orientation of starch components (AM and AP) within the granule. The SEM (Fig. 3) of chayote tuber starch clearly shows a mixture of shapes such as spherical or dome-shaped and split, oval and polygonal, with granule size between 10 and 25 μm. Shapes for potato starch were rounded and oval, the surface of the granules was smooth, the granule size was larger than chayote, with size between 10 and 50 μm. Sizes and shapes of starch granules influence some physicochemical and functional characteristics, because larger granules develop a high paste viscosity. Jiménez-Hernández *et al.* [8] studied chayote tuber starch from another cultivar, presenting a granule size between 7 and 50 μm and commercial potato starch between 10 and 85 μm; both starches showed oval,

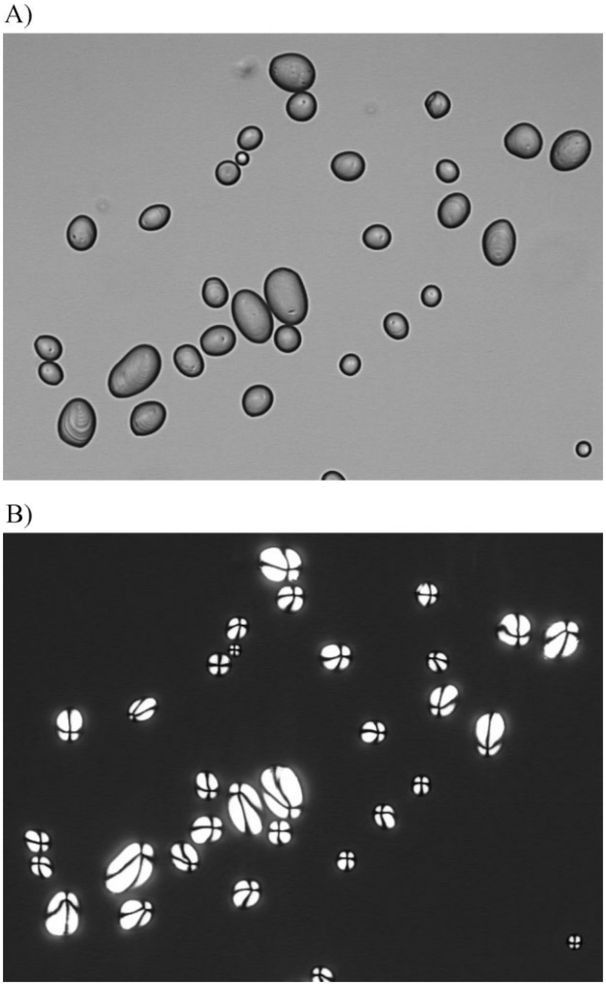


Figure 2. Micrographs of potato starch. (A) Light microscopy; (B) Polarized-light microscopy.

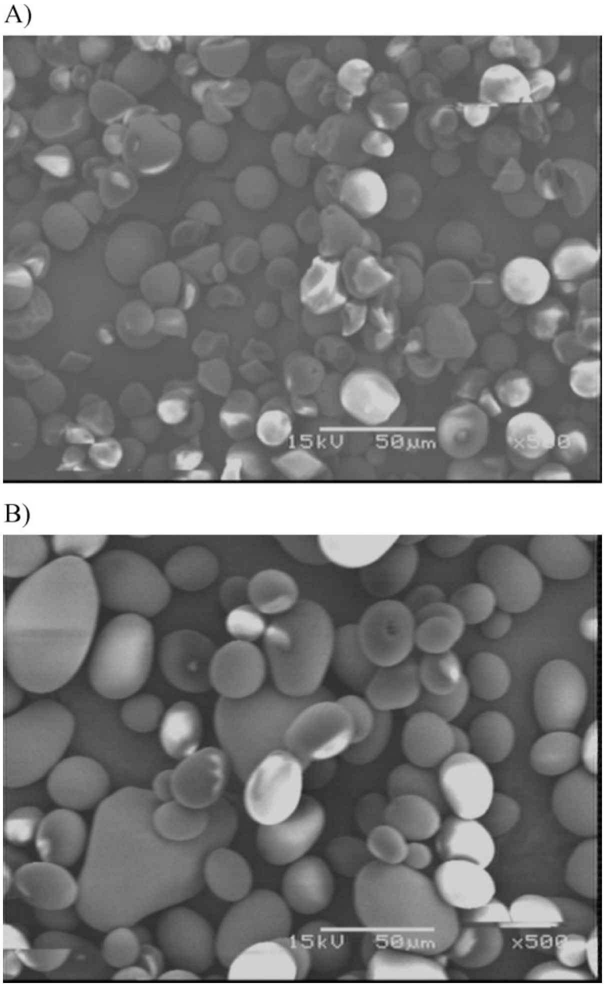


Figure 3. SEM of starches: (A) chayote tuber; (B) Potato.

rounded, and truncated forms, and some granules were broken.

3.3 XRD

Figure 4 shows the XRD pattern of chayote tuber and potato starches. Both starches presented B-type XRD pattern with two principal peaks at $2\theta = 17$ and 23°; with potato starch having a broader second peak. Various root and tuber starches presented XRD patterns of A, B, and C. For example, cassava and sweet potato starches had A and C types depending on the variety. Differences in crystallinity level were found in chayote tuber and potato starches, with potato starch having higher crystallinity than chayote tuber starch. Oca starch isolated from Mexican variety presented B-type XRD pattern, similar to that determined in chayote tuber starch [13]. The XRD pattern and the crystallinity level (long range order) give information of the arrangement of AP chains in double helices. This is important in properties such as starch digestibility [23] and retrogradation [24]. This is the first report of the XRD pattern and crystallinity level of chayote tuber starch.

3.4 Pasting profile

RVA-curves of chayote tuber and potato starches are presented in Fig. 5. When a starch suspension is heated at a constant rate, the viscosity increases gradually until a maximum value is reached. Chayote tuber starch presented lower PV than potato starch, and the PV of the former was obtained to higher temperature. The maximum of the PV reflects the ability of starch granules to swell freely before their physical BD [25]. It was reported that the starch dispersion properties were more affected by the chain-length distribution of AP than by the molar mass [26]; however, viscosities during gelatinization/pasting are also very much affected by the AM/AP ratio [27]. During heating the viscosity decreased and this fall in the viscosity

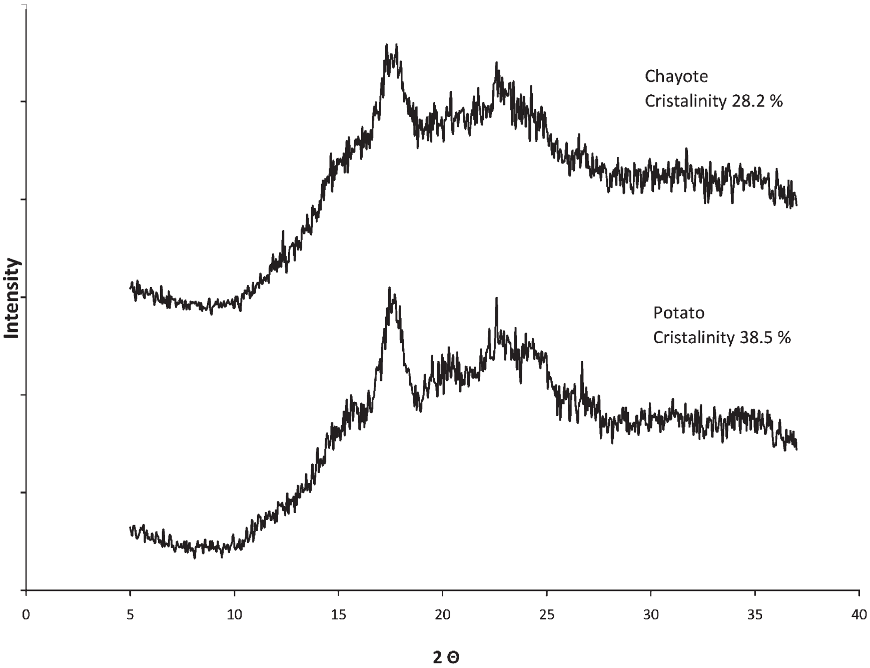


Figure 4. XRD and crystallinity (%) of potato and chayote tuber starches.

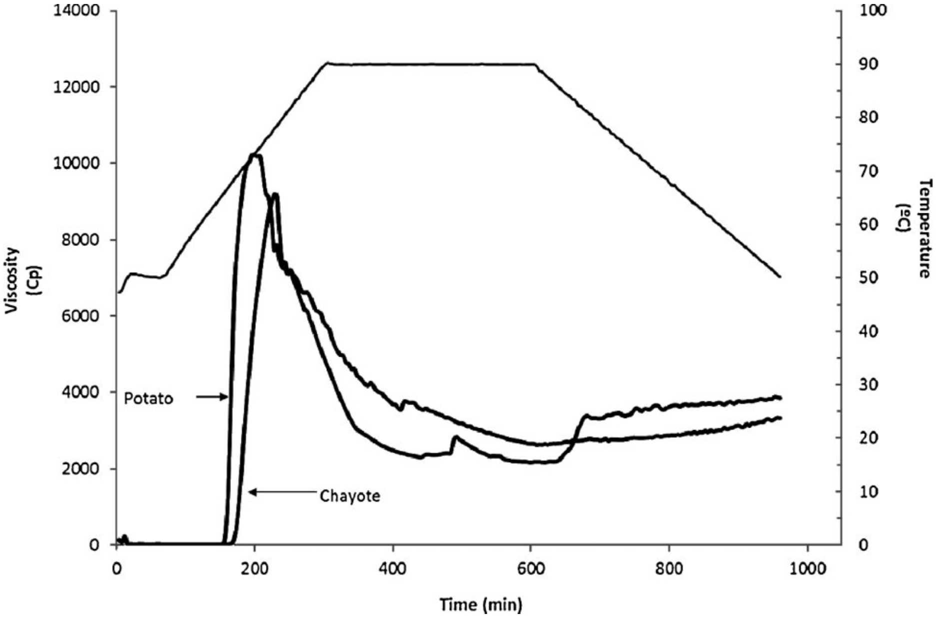


Figure 5. RVA pasting curves of potato and chayote tuber starches.

continued during holding at 95°C under shear, resulting from the BD of some swollen starch granules. When the temperature dropped the viscosity of chayote tuber starch presented a slight increase that was higher for potato starch. This increase in the viscosity resulted from network formation between AM and AP while retaining a certain amount of water [28, 29] and resulted in a gel-like characteristic. Larger granule size of potato starch is responsible of this pattern. Higher PV and P_{temp} were reported for chayote tuber starch than potato starch, an inverse pattern to those determined in this study. However, the pasting profile during holding was similar, and potato starch presented a similar pattern to that determined here during the cooling step [8].

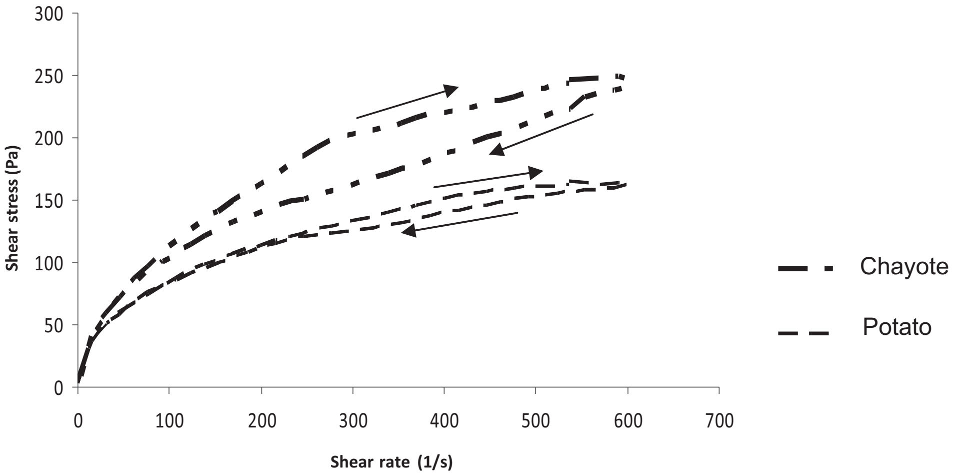


Figure 6. Flow behavior of potato and chayote starches at 7.5% and 60°C.

3.5 Flow properties

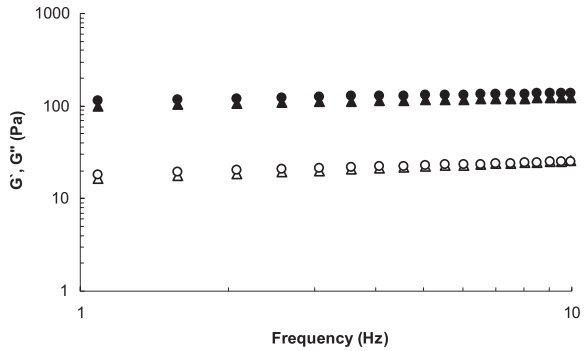
Figure 6 shows the flow behavior of chayote tuber and potato starches at 60°C at the same concentration (7.5% w/v). The shear stress *versus* shear rate curve shows the non-Newtonian shear thinning character of the pastes that is higher in chayote tuber starch than in potato starch. Figure 6 shows a higher hysteresis in chayote tuber starch than potato starch. The presence of hysteresis might be due to an increase of available water as a result of BD of starch granule structure. When a suspension of deformable particles, such as starch, shows no hysteresis, all solvent is absorbed by the particles, indicating that water is limited in this system [30], this pattern was exhibited in higher level in potato starch due to its higher granule size. Therefore the starch granules were partially swollen and consequently more resistant to stress [31].

3.6 Viscoelastic properties

Figure 7 shows the profiles of frequency sweep of starches at the second (90°C) and third stage (25°C) in heating–cooling process. The curves for chayote tuber and potato starches were similar ($G' > G''$) at both temperatures. According the rheological definition of gel [32]: both moduli, independent of frequency, G' greater than G'', and tan δ well below 1.0 (data not shown). This pattern is mainly due to the swollen granules, because the AM separate out from the granules during heating does not produce viscous solutions due to its disorder state (random coil) to this temperature.

When starch cools to ambient temperature the AM in the continuous phase forms a three-dimensional network resulting in a gel [33] where the minimum AM concentration is about 1 [34]. At 25°C (Fig. 7b), a characteristic of a gel: low dependence of G' with frequency ($G' \alpha\ \omega^{0.01}$), G' was significantly greater than G'', and tan δ was lower than 0.1, was observed (data not shown). At this temperature a three-dimensional AM network in the continuous phase of the starch pastes was expected; however, the disperse phase (AP swollen granules) have their contribution, so the rheological behavior is attributed to the AM gel and to the starch granules embedded randomly in the AM network [35]. In frequency sweep of chayote and potato starches, the modules were similar.

a) 90 ºC



b) 25 ºC

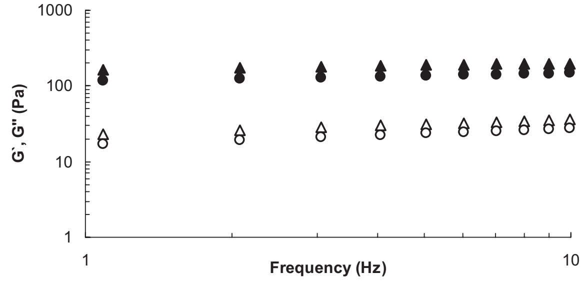


Figure 7. Frequency sweeps of (▲) potato and (●) chayote starches (7.5% w/w and 0.5% strain) at (a) 90°C and (b) 25°C. G' (filled symbols), G'' (empty symbols).

Table 2. Gelatinization and retrogradation properties of chayote tuber and potato starches[a)]

Starch		Gelatinization	Retrogradation 7 days	Retrogradation 14 days
Chayote	T_o (°C)	60.1 ± 0.07^a	46.9 ± 1.2^a	46.7 ± 2.14^a
	T_p (°C)	63.0 ± 0.13^c	59.0 ± 0.9^c	59.0 ± 1.10^b
	T_c (°C)	71.2 ± 0.16^e	71.8 ± 1.3^d	70.2 ± 0.73^c
	ΔH (J/g)	12.1 ± 0.20^g	4.5 ± 0.9^f	5.3 ± 0.49^d
Potato	T_o (°C)	61.0 ± 0.21^b	49.3 ± 1.4^b	47.0 ± 1.7^a
	T_p (°C)	65.8 ± 0.17^d	59.9 ± 1.0^c	58.7 ± 0.7^b
	T_c (°C)	73.0 ± 0.13^f	69.7 ± 0.9^e	69.1 ± 0.8^c
	ΔH (J/g)	10.9 ± 0.01^h	4.6 ± 0.7^f	4.5 ± 0.5^d

a) T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH, gelatinization or retrogradation enthalpy. Average of three replicates ± SE. Means in column not sharing the same letter are significantly different ($p < 0.05$).

3.7 Thermal properties

Thermal characteristics of gelatinization and retrogradation of chayote tuber and potato starches are shown in Table 2. Chayote tuber starch presented slightly lower gelatinization temperatures, but slightly higher enthalpy of gelatinization than potato starch. Gelatinization of starch is the loss of the crystalline structure of starch granule, which mainly results from crystalline regions of AP. The enthalpy value reflects the loss of the ordered double helices more than crystallinity loss. The gelatinization temperatures agreed with the crystallinity level that was higher for potato starch, but the enthalpy is more related with granule size, AM/AP ratio, and chain length distribution of AP [36, 37]. Higher gelatinization temperatures were determined in chayote tuber starch isolated from another cultivar, but enthalpy value was very low (1.13 J/g) [8].

Retrogradation temperatures of chayote tuber and potato starches were lower than gelatinization temperatures (Table 2), because during storage small and/or less perfect crystals were formed [17, 26]. Crystals formed during storage of gelatinized starch on both starches had similar thermal stability because they were dissociated with the same energy (enthalpy change), and this thermal parameter did not change at the longest storage time. Similar pattern in retrogradation and gelatinization temperatures was determined in potato starch stored for 7 days [38]; however, higher enthalpy value (7.5 J/g) was recorded. Unconventional starch such as mango [39] had similar enthalpy of retrogradation (5.4 J/g) to that of chayote and potato starches. There are no reports on retrogradation of chayote tuber starch.

3.8 Molar mass

Weight-average molar mass (Mw) and gyration radius (Rz) of chayote tuber and potato starches are shown in Table 3.

Table 3. Average molecular weight and gyration radius of chayote tuber and potato starches[a)]

Source	Mw × 10^7 (g/mol)[b),c)]	Rz [nm][b),d)]
Chayote	20.31 ± 0.65^a	315.4 ± 28.8^a
Potato	7.62 ± 0.80^b	240.5 ± 8.6^b

a) Means in column not sharing the same letter are significantly different ($p < 0.05$).
b) Averaged from three replicates.
c) Weight-average molar mass.
d) Z-average radius of gyration.

Chayote tuber starch presented higher Mw and Rz values than potato starch. The Zimm plot with a Berry second order treatment showed a good fit (Fig. 6) because the solubilization procedure produced a good dispersion of starch components in the solvent [19]. Mw and Rz values for chayote tuber starch were similar to that calculated for maize starch using the same solubilization procedure, method, and data processing [19]. These values can be important to explain the physicochemical and functional properties of both tuber starches, because it was reported that the molar mass has influence on the pasting viscosity of the starch [40]. The use of HPSEC with multi-angle light-scattering and RI detectors (HPSEC-MALLS-RI) in the structural studies of starches increased in the last years due to that both starch components (AM and AP) can be separated and Mw and Rz of both are determined. Mw and Rz of non-conventional starches such as mango and banana using HPSEC-MALLS-RI were 5.0 and 3.3×10^8 g/mol, respectively, and Rz of 298 and 267 nm, respectively, indicating that these structural differences are responsible of the physicochemical and functional characteristics of each starch [39]. Other studies of AP from diverse cultivars of Tef presented Mw between 1.0

and 1.5 × 108 g/mol and Rz between 156 and 250 nm [40].

4 Conclusions

The results obtained suggest that Mexican chayote tuber contain starch with morphological, physicochemical, and molecular characteristics that are comparable to a traditional starch source as potato. The higher maximum PV in the pasting profile of chayote tuber starch could be due to its higher molar mass. Gel was obtained for chayote tuber and potato starches with the elastic character (G') predominant over the viscous character (G''). Flow property of chayote tuber starch shows non-Newtonian character of the pastes with higher hysteresis than potato starch. Mexican chayote tuber could be an alternative for starch isolation with commercial potential.

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The authors have declared no conflict of interest.

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