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Characterization of Starch from two Ecotypes of Andean Achira Roots (*Canna edulis*)

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Starches from two ecotypes of achira roots (*Canna edulis* Ker-Gawler) were characterized and compared to commercial potato and corn starches. This included scanning electron microscopy (SEM) of starch granules and amylose content determination of starch. Starch solutions or gels were tested by rotational viscometry, Rapid Visco Analyzer (RVA), and texture analysis. Some starch samples were subjected to various treatments: pH reduction, autoclaving at high temperature, and high shear before testing by rotational viscometry. Achira starch showed some unusual properties, such as very large oblong granules (~45−52 μm major axis and ~33−34 μm minor axis) and relatively high amylose content (~33−39%). The San Gabán achira ecotype formed high-consistency gels upon cooling, both in RVA study (5% starch) and in texture analysis (8% starch), compared to other starch gels and also exhibited higher thermal resistance to viscosity breakdown.

KEYWORDS: Starch; rheology; microscopy; viscomylography; texture analysis; achira; *Canna edulis*

INTRODUCTION

Starch is one the most used food ingredients worldwide due to its diverse functionalities, year-round availability, and low cost. Some of the main uses of starch in foods are as a viscosity enhancer and as a gelling agent (*1*).

The main sources of starch are cereal grains (corn, wheat, and rice), roots (tapioca and sweet potato), and tubers (potato). This diversity of sources of starch is reflected in their properties and functionalities. Furthermore, some of the properties of starch can be modified by physical and/or chemical means to suit an intended commercial application (*2*).

There is a growing demand by consumers for natural ingredients for use in foods, as opposed to those chemically derived. Therefore, there is interest in finding new sources of starch with novel and unique properties that could potentially replace chemically modified starches in some applications.

The Andean region is home to a host of crops little known outside the region (*3*). One of these crops is achira (*Canna edulis*), also known as edible canna and as Queensland arrowroot. In Peru, achira root consumption is very low, mainly in isolated and very poor areas by rural farmers. The method of consumption is by simple boiling of the rhizomes without further processing performed. In Southeast Asia, especially in Vietnam, achira is used as a source of starch for noodle production (*4*). It is considered to be the cheap substitute for the more expensive mungbean starch for this purpose.

Previous studies have reported the physicochemical properties of achira starch, especially Asian and non-Peruvian varieties. These studies included differential scanning calorimetry and pasting properties (*5*−*8*). However, to our knowledge, research on achira varieties or ecotypes from Peru has not been reported previously. Considering that the eastern slopes of the South American Andes are believed to be the center of origin of the achira, it is likely that achiras with a greater diversity in properties are to be found in this area. Therefore, we considered it worthy of investigating the properties of achira starches from this region. The objective of this research was to characterize the starch from two Peruvian ecotypes of achira (San Gabán and Sandia) with emphasis on rheological behavior. This information would be of value for applied research by starch technologists.

MATERIALS AND METHODS

Raw Material and Proximate Analysis. Achira (*C. edulis*) roots (70 kg of each ecotype) were obtained in the year 2001 from the San Gabán and Sandia regions of Puno in southeastern Peru (**Figure 1**). These are mountainous jungle areas on the eastern slopes of the Andes. Both locations have a warm and humid climate with rain year round, and cultivation is based on traditional practices without the use of synthetic fertilizers and pesticides.

The achira roots were analyzed (*9*) for moisture content (AOAC method 950.46), crude fat (AOAC method 948.16), crude protein (AOAC method 984.13), ash (AOAC method 942.05), and crude fiber (AOAC method 962.09), and the carbohydrate content was calculated by difference.

Starch Extraction. Starch was extracted within a few days after arrival of the achira roots at the laboratory. Washed achira roots were disintegrated in a blender with enough water to form a slurry, which was filtered with a nylon filter medium (200 μm). The starch-containing filtrate was transferred to a vessel for starch sedimentation. When starch sedimented, the supernatant was decanted and fresh tap water was added. This step was repeated at least twice. Finally, the starch was dried in an oven at 40 °C to an approximately 11% (wb) moisture content. The starch samples were stored in a closed container until characterization studies were carried out within the next few months.

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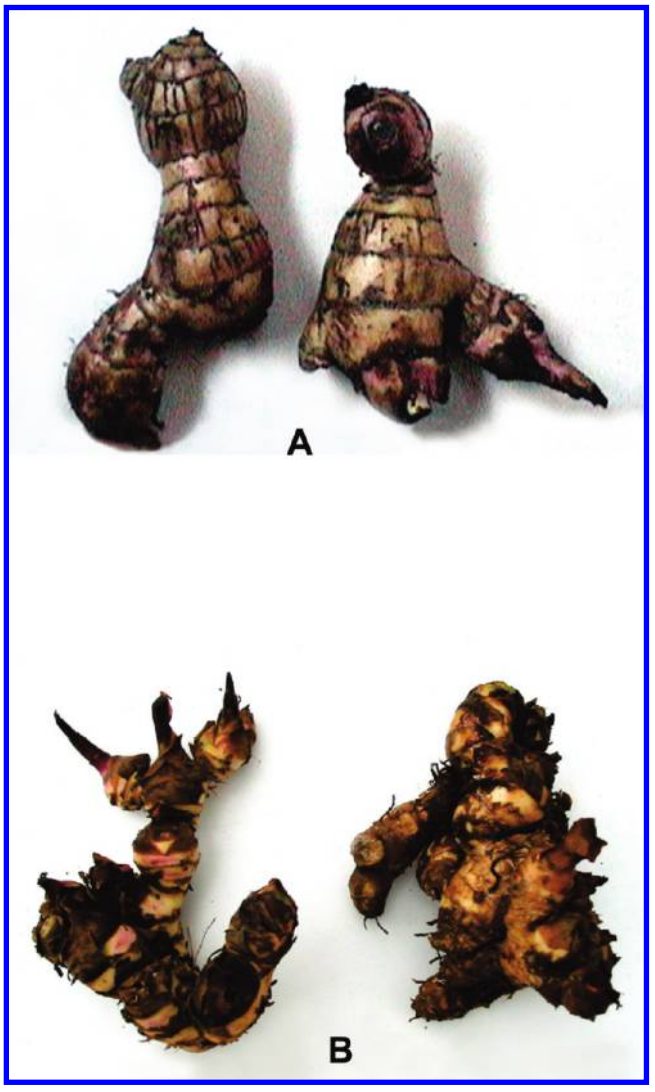

Figure 1. Achira rhizomes: (**A**) San Gabán and (**B**) Sandia ecotypes.

Achira starch samples as well as commercial starch samples from potato and corn (bought from a local supermarket) were subjected to several analyses for characterization as described below.

Amylose Content. AACC method 61-03 (*10*) was used for apparent amylose content. This method is based on blue color formation upon reaction of amylose with iodine (starch−iodine complex). Color was measured at 620 nm with a spectrophotometer (Spectronic 20 Genesys, Spectronics Instruments).

Scanning Electron Microscopy (SEM). SEM was used to visualize starch granule morphology and size. An ESEM E-3 Electroscan was used under the following conditions: working distance, 7.5 mm; incident angle, none; acceleration voltage, 25 kV; condenser lens position, 60.

The magnifications used for microphotographs were ×400 and ×1000 (only ×400 is shown). For granule size determination, 16 granules were selected at random from microphotographs. For spherical granules, the diameter was measured, whereas for oval granules, the major and minor axes were measured.

Rotational Viscometry. Rotational viscometry measurements were made at ambient temperature (20−22 °C) in triplicate with a Brookfield viscometer LV model (Middleboro, MA) equipped with the small sample accessory (10 mL). The starch samples consisted of 2% (w/v) gelatinized starch solutions, which were prepared by suspending starch in distilled water and heating with agitation to a temperature of 95−98 °C for 20 min. This was achieved with magnetic stirring hot plates (Thermolyne Nuova). The starch solutions were allowed to rest at ambient conditions for 48 h before viscosity measurements were made to allow for viscosity stabilization.

Apparent viscosities were measured at different shear rates, and the linearized power law viscosity model was adjusted to the experimental viscosity data.

$$\tau = k\dot{\gamma}^{n} \quad \text{(power law model)} \tag{1}$$

$$\log(\tau) = \log(k) + n\log(\dot{\gamma}) \quad \text{(linearized power law model)} \tag{2}$$

τ is the shear stress, k is the consistency coefficient, n is the flow behavior index, and $\dot{\gamma}$ is the shear rate.

By applying linear regression using Excel computer software, the equation parameters, consistency coefficient (k) and flow behavior index (n), were determined for each sample.

Viscosity measurements were also made on starch solutions that had previously been subjected to different conditions often encountered during food processing: (a) Acid conditions: starch gelatinization (95−98 °C for 20 min) was carried out using water at pH 3 and 4, adjusted with citric acid. (b) Autoclave temperature: freshly prepared gelatinized starch solutions contained in 4 oz glass jars were autoclaved at 121 °C for 20 min (with a laboratory autoclave). (c) Shear treatment: gelatinized starch solutions were blended at minimum setting for 10 s using a kitchen blender (Braun).

Rapid Visco Analyzer (RVA) Studies. A RVA, model 3 D (Newport Scientific, Sidney, Australia), was used to monitor the gelatinization−retrogradation characteristics of aqueous starch solutions. AACC method 61-02 (*10*) was used with some modifications in starch concentration, temperature, and time to obtain better differentiation between curves from different samples.

In this test 5% (w/v) starch aqueous suspensions were subjected to a heating−cooling program with mixing. The initial rotation speed was set at 960 rpm for the first 10 s of the test; then it was lowered to 160 rpm for the rest of the test. The temperature program started with progressive heating from 40 to 95 °C in 9 min, followed by constant temperature at 95 °C for 4 min, then by cooling from 95 to 40 °C in a period of 5 min. Finally, the temperature was kept constant at 40 °C for 3 min, making a total test time of 21 min.

The following parameters were obtained from the viscosity curve generated: gelatinization temperature, temperature at which viscosity increases appreciably; peak viscosity, maximum viscosity during heating period; time to peak viscosity, time taken to reach peak viscosity; channel viscosity, minimum viscosity after peak viscosity; final viscosity, viscosity of the cooled paste at the end of the test; stability, difference between peak and channel viscosities; peak retrogradation, difference between final and peak viscosities; channel retrogradation, difference between final and channel viscosities.

Texture Analysis. Texture analysis was conducted to measure hardness (or gel strength) of starch gels (8% solids) at ambient temperature (20 °C). The instrument used was a TAXT-2 texture analyzer (Stable Micro Systems, Surrey, U.K.) fitted with a cylindrical plunger (25.38 mm diameter, 34.88 mm height) at a speed of 2.0 mm s^{-1} and a penetration distance of 15 mm.

The starch gels were made according to a procedure described by Takahashi et al. (*11*). The starch slurry was heated to 95 °C for 30 min, and then cooled to 50 °C. The hot paste was poured into a cylindrical container to form a disk gel with dimensions of 27 mm height and 55 mm diameter. The gels were stored at room temperature for 24 h before any measurements.

RESULTS AND DISCUSSION

Proximate Analysis of Raw Material. The proximate analysis results of achira roots are shown in **Table 1**. San Gabán achira roots showed a lower moisture content (77.5%) compared to Sandia roots (82.9%). Therefore, the total solids contents were 22.5 and 17.1% for San Gabán and Sandia, respectively, most of which is carbohydrate, 19.8 and 13.0%, respectively. On a dry matter basis this represents 88.0 and 76.0% of carbohydrate content for San Gabán and Sandia, respectively.

Amylose Content. The amylose contents of San Gabán and Sandia achira starches were 39 and 33%, respectively. These values are relatively high when compared to traditional sources of

Table 1. Results of Proximate Analysis of Achira Roots (Wet Basis) Used as Raw Material for Starch

achira ecotype	moisture (%)	crude protein (%N × 6.25)	crude fat (%)	crude fiber (%)	ash (%)	total carbohydrate (%)
Sandia	82.9	2.2	0.3	1.1	1.6	13.0
San Gabán	77.5	1.1	0.4	0.9	1.2	19.8

starch such as potato (27%) and corn (24%) (**Table 2**). These results confirm the high-amylose values reported for achira starch in previous studies (*12*). The presence of high levels of amylose will influence starch properties and enhance functionality. For example, amylose content has been shown to correlate well with film-forming abilities of starch (*13*, *14*).

SEM. Starch granules of achira (San Gabán and Sandia) and those of potato and corn are shown in SEM micrographs with a magnification of ×400 (**Figure 2**). Achira starch granules have a characteristic large size and an oblong shape. San Gabán starch granules had the largest size, followed by that of Sandia, then by that of potato granules, and finally by corn granules (**Table 2**). Virtually all achira starch granules had an oblong shape as compared with those of potato, which had large oblong granules coexisting with small spherical granules, whereas corn starch showed basically spherical granules.

Rotational Viscometry. All starch aqueous solutions (2% w/v) showed a decrease in apparent viscosity with increasing levels of shear rate, indicating a pseudoplastic behavior. This decrease was much more pronounced in the lower shear rate range (**Figure 3**).

At lower shear rate values ($< 5\ s^{-1}$) achira starches developed intermediate viscosity values when compared to potato, which exhibited the highest viscosities, whereas corn starch recorded the lowest (**Figure 3**). No comparison could be made between the viscosities of achira and potato starch solutions at higher shear rates because no data could be obtained for potato starch due to instrument limitations. Achira starches showed a dramatic decrease in apparent viscosity up to a shear rate of $2\ s^{-1}$ and then leveled off at higher shear values (**Figure 3**).

By applying the power law model to the rheological data, the two parameters of the model (consistency coefficient, k, and flow behavior index, n) could be calculated. The consistency coefficient can be considered to be the apparent viscosity of the solution at a shear rate of $1\ s^{-1}$. The consistency coefficient values obtained were 9.15, 1.08, 0.66, and 0.17 Pa s^n, for potato, San Gabán achira, Sandia achira, and corn starch, respectively (**Table 3**). These values would suggest that achira starch does not develop the highly expanded granule structure of potato starch, which apparently is responsible for its unusually high viscosity in solutions under low-shear conditions (*15*). On the other hand, the higher viscosity of achira starch compared to that of corn would suggest that its granules swell more than the poorly swelling corn starch granule.

The flow behavior index values obtained in this study were < 1, indicating the pseudoplastic character of starch solutions (**Table 3**). The values of n for potato, San Gabán achira, Sandia achira, and corn starch were 0.32, 0.42, 0.47, and 0.47, respectively. These results suggest that achira starches have an intermediate pseudoplastic character compared to potato and corn starches, which showed the highest and lowest pseudoplastic character, respectively.

In starch rheology, the flow behavior index (n) can be used as an indicator of the integrity of the gelatinized structure, especially when for comparison of gelatinized starch under mild conditions with gelatinized starch that has been subjected to additional aggressive treatments. An undamaged well-developed (expanded) gelatinized starch granule structure will usually yield a rather low n value, whereas a greatly disrupted starch granule concomitant with an extensively fragmented starch molecule will give n values closer to 1, which is characteristic of Newtonian fluids.

Table 2. Amylose Content and Granule Size in Achira, Corn, and Potato Starches Measured from SEM Microphotographs

starch	amylose content (%)	minor axis (μm)	major axis (μm)
achira, Sandia	33.1 ± 3.7	45.8 ± 12.4	33.2 ± 6.1
achira, San Gabán	39.4 ± 4.7	52.0 ± 15.2	34.7 ± 11.6
corn[a]	23.8 ± 0.9	17.0 ± 1.8	
potato	27.3 ± 1.1	34.9 ± 18.6	27.6 ± 11.4

[a] Only one dimension defines size of granule.

Effect of Acid Conditions. The gelatinization of starch in acidified conditions significantly reduced apparent viscosity in all samples, except for corn starch, for which viscosity increased and decreased slightly at pH 4 and 3, respectively. Consistency coefficient values obtained reflect this response to acid conditions (**Table 3**), whereas the flow behavior index values increased with pH reduction (**Table 3**). This decrease in viscosity under acidified conditions during heating (gelatinization) is typical of starch from different botanical sources (*16*). Hydrolysis of starch glucosidic linkages has been attributed as the cause for observed apparent viscosity reduction (decreased consistency coefficient) and for the decrease of pseudoplastic behavior (increased flow behavior index) (*2*).

Effect of High-Temperature (*Autoclave*) *Conditions*. Subjecting the different starch solutions to autoclaving conditions (121 °C for 20 min) resulted in various final viscosity responses. San Gabán achira starch exhibited a significant increase in viscosity as could be observed from the increase of the consistency coefficient values (**Table 3**). In contrast, Sandia achira starch showed a significant viscosity (consistency coefficient) decrease (**Table 3**). These differing results in starch behavior for the two ecotypes of achira stress the importance of investigating other ecotypes for unusual starch properties; especially interesting are those with high amylose content. On the other hand, potato starch showed a dramatic decrease in viscosity, whereas the viscosity of corn starch was essentially unchanged due to high-temperature conditions (**Table 3**).

The flow behavior index values of all starch solutions subjected to the thermal treatment also increased, but to a lesser degree than the other treatments (**Table 3**), suggesting the occurrence of less damage to starch structure compared to other treatments.

The different viscosity behaviors observed for the various starch solutions subjected to high-temperature conditions could be due in part to the organization of the granule structure and to the amylose/amylopectin ratio. For example, the high amylose content (39%) in San Gabán achira starch could be responsible for the observed viscosity increase at high temperatures. Apparently, San Gabán achira starch did not fully gelatinize during preparation of starch solutions (95−98 °C for 20 min). It took further heating at a higher temperature (autoclaving at 121 °C for 20 min) to fully gelatinize the starch. Past studies have reported a correlation between amylose content and granule integrity during thermal treatment of starch solutions (*17*).

On the other hand, the viscosity of corn starch was not most affected by high-temperature thermal treatment. Possibly, a poor-swelling tightly bound outer granule surface structure of corn starch resisted granule rupture until high temperatures were reached (121 °C), thereby preventing the

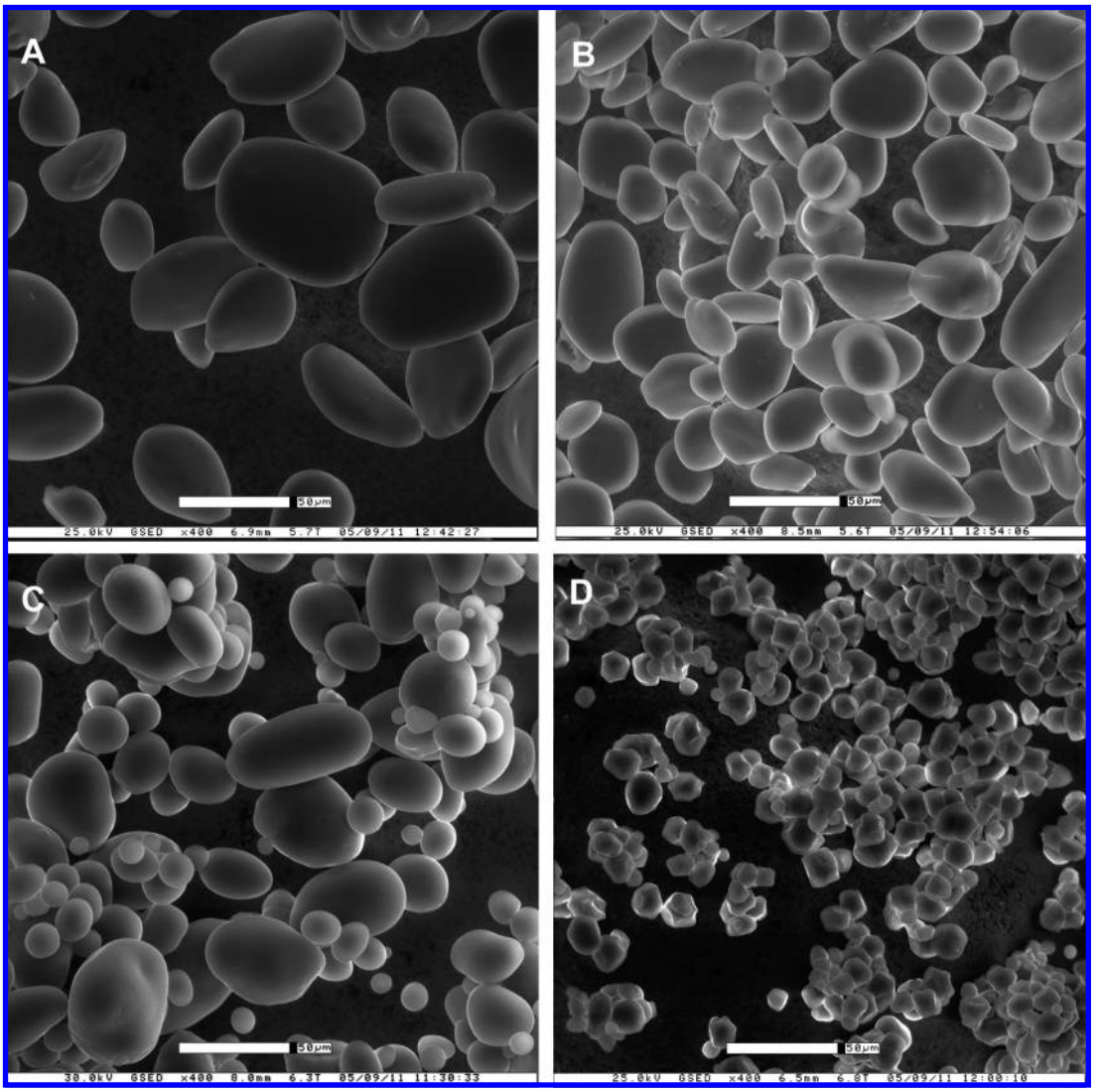

Figure 2. SEM microphotographs of achira starch granules (magnification ×400): (**A**) achira, San Gabán; (**B**) achira, Sandia; (**C**) potato; (**D**) corn. Potato and corn starch granules are shown for comparison.

mass release of amylopectin and amylose molecules into the solution at lower gelatinization temperatures (e.g., 98 °C) (*18*). This prevented a steep viscosity increase during gelatinization at low gelatinization temperatures, but rather a gradual one. The low viscosity developed by corn starch during gelatinization could also be attributed to this resistant outer granule surface, which could limit granule swelling during gelatinization (*19*).

Effect of High-Shear Conditions. The viscosities of all starch samples were greatly affected by the high-shear conditions of the blender, as seen from the very low consistency coefficient values shown in **Table 3**. This is an indication of the extent of damage to the starch molecule and/or granule (*1*, *17*).

The flow behavior index values increased significantly to approximately 0.8 for achira and potato starch. However, corn starch showed a flow behavior index increasing to approximately 1; that is, it virtually became a Newtonian liquid (**Table 3**). Shear treatment of starch produced the largest increase of flow behavior index of all the treatments, confirming significant damage to the starch structure.

From these rotational viscometry results, it seems that the application of high shear to starch solutions causes extensive starch granule disintegration and starch molecule fragmentation, regardless of starch botanical origin.

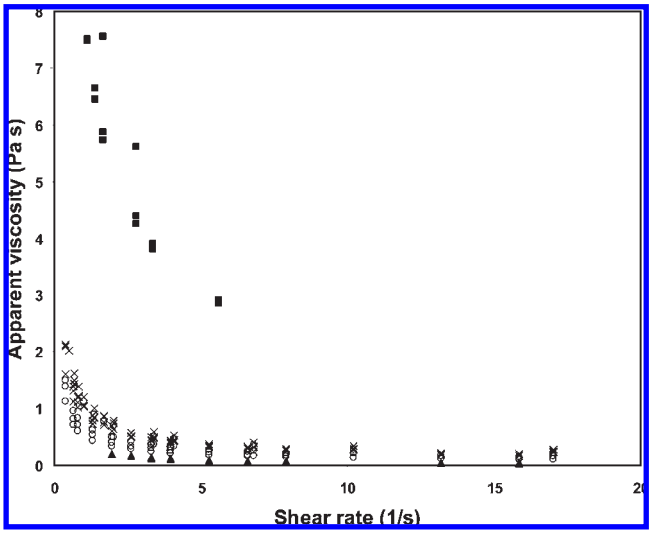

Figure 3. Apparent viscosity of starch solutions (2%) as a function of shear rate: (×) achira, San Gabán; (○) achira, Sandia; (■) potato; (▲) corn.

RVA Studies. The RVA viscograms of San Gabán and Sandia achira starches and those of potato and corn starches are shown

Table 3. Power Law Model Parameters (Consistency Coefficient, *k*, and Index Flow Behavior, *n*) of Starch Solutions (2%) Subjected to Various Treatments[a]

starch	parameter	pH 5	pH 4	pH 3	autoclaved	sheared
achira, San Gabán	*k* (Pa s^n)	1.08 ± 0.07	0.13	0.02 ± 0.01	2.14 ± 0.40	0.03 ± 0.00
	n	0.42 ± 0.01	0.61	0.79 ± 0.13	0.43 ± 0.05	0.82 ± 0.01
achira, Sandia	*k* (Pa s^n)	0.66 ± 0.14	0.05	0.01 ± 0.00	0.36 ± 0.03	0.08 ± 0.00
	n	0.47 ± 0.02	0.73	0.89 ± 0.04	0.55 ± 0.01	0.76 ± 0.01
corn	*k* (Pa s^n)	0.17	0.25	0.11	0.19	0.00
	n	0.47	0.47	0.56	0.53	1.00
potato	*k* (Pa s^n)	9.15 ± 0.94	0.31 ± 0.02	0.01 ± 0.00	1.82 ± 0.54	0.07 ± 0.01
	n	0.32 ± 0.01	0.52 ± 0.02	0.85 ± 0.01	0.59 ± 0.02	0.82 ± 0.02

[a] Data with standard deviations correspond to triplicates.

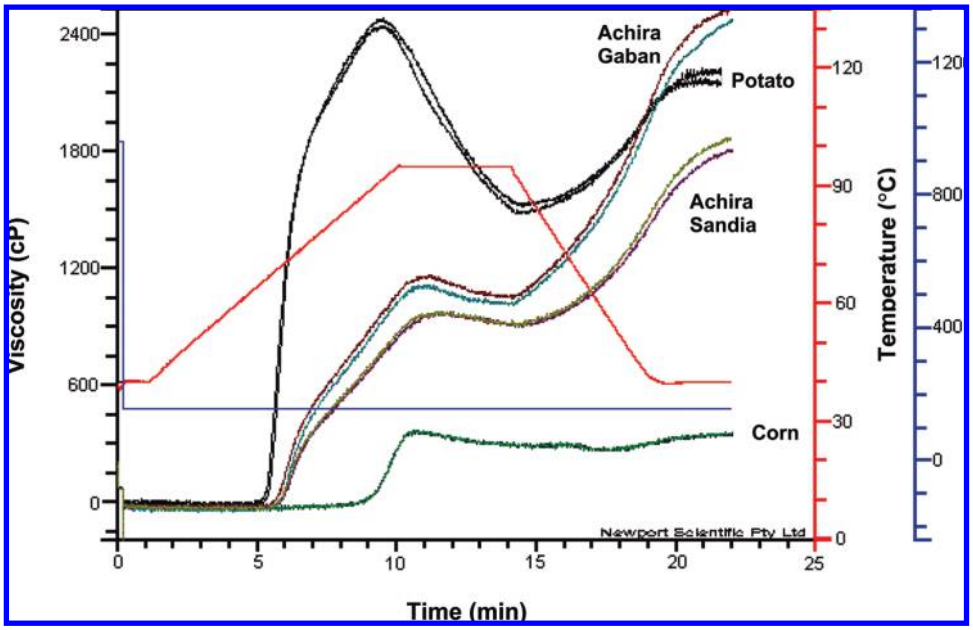

Figure 4. RVA curves for starch pastes (5%) of San Gabán achira and Sandia achira compared to those of potato and corn starches.

Table 4. RVA Viscogram Parameters of Aqueous Starch Pastes (5%)

starch	gelatinization temperature (°C)	peak viscosity (cP)	time to peak viscosity (min)	channel viscosity (cP)	final viscosity (cP)	stability[a] (cP)	peak retrogradation (cP)	channel retrogradation (cP)
achira, Sandia	67.8	979 ± 000	12.0 ± 0.1	900 ± 00	1822 ± 56	79 ± 00	843 ± 28	922 ± 28
achira, San Gabán	67.0	1130 ± 037	14.0 ± 0.2	1032 ± 25	2501 ± 44	98 ± 31	1371 ± 40	1469 ± 34
corn	85.6	362 ± 000	12.0 ± 0.0	284 ± 00	344 ± 00	78 ± 00	−18 ± 00	60 ± 00
potato	65.2	2457 ± 031	10.0 ± 0.1	1487 ± 06	2179 ± 49	970 ± 18	−278 ± 24	692 ± 28

[a] A positive sign in stability means a decrease in viscosity and a negative sign, an increase in viscosity.

in **Figure 4**. The RVA curves for achira starches are very different from those of potato and corn starches. As in rotational viscometry, in this method achira starches also exhibited intermediate viscosities between those of potato and corn starches under normal gelatinization conditions. Achira starches do not develop a prominent peak viscosity as potato starch does. However, upon cooling, the viscosity of achira starches is comparable with that of potato starch. Indeed, San Gabán achira starch shows a higher setback viscosity than potato starch; furthermore, an increasing trend is observed as opposed to that of potato, which leveled off. The high-amylose content of achira starches, especially that of San Gabán, could be responsible for the sharp increase in the setback viscosity observed in the viscogram (*8*).

RVA parameters obtained from viscograms are presented in **Table 4**. One such parameter is starch gelatinization temperature, which had similar values for both achira ecotypes, 67.0 and 67.8 °C for San Gabán and Sandia, respectively, but were slightly higher than that for potato starch, 65.2 °C, and much lower than that for corn starch, 85.6 °C (**Table 4**).

Texture Analysis. The hardness values of the starch gels from San Gabán achira and Sandia achira were 34.6 and 8.0 N, respectively. Compared to the hardness of starch gels from potato and corn, 27.6 and 20.4 N, respectively, it is interesting to observe that achira starches generated firmer and softer gels depending on ecotype. This underscores the need to search for other little-known Andean achira ecotypes for unusual starch properties.

Texture analyses of achira starch gels (8% starch) follow the same trend as Rapid Visco Amylography (5% starch) and rotational viscometry (2% starch) in that San Gabán achira produces stronger starch gels than the Sandia achira ecotype. The cause for this difference in gel strength could lie in the greater amylose content of San Gabán achira starch compared to Sandia achira. Previous studies have found a positive relationship between amylose content and starch gel strength/viscosity for some botanical species (*20*).

In summary, achira starch showed some unusual properties, such as very large granules and relatively high amylose content. San Gabán achira ecotype formed high-consistency gels upon cooling, as shown in RVA study (5% starch) and in texture analysis (8% starch), compared to the other starch gels studied. This ecotype also exhibited higher thermal resistance to viscosity breakdown at elevated temperatures (121 °C).

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