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PHYSICOCHEMICAL CHARACTERIZATION OF TWO CASSAVA (*Manihot esculenta* Crantz) STARCHES AND FLOURS

CARACTERIZACIÓN FISICOQUIMICA DE DOS ALMIDONES Y HARINAS DE YUCA (*Manihot esculenta* Crantz)

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Resumen

El almidón y la harina de yuca fueron obtenidos de raíces cultivadas en Colombia en dos condiciones ambientales específicas. Se evaluaron propiedades fisicoquímicas como tamaño y morfología del grano, contenido de amilosa, cristalinidad, propiedades térmicas y comportamiento al empastamiento. Las propiedades del almidón de yuca fueron altamente influenciadas por las condiciones ambientales durante el periodo de crecimiento de las raíces de yuca. El almidón extraído de las raíces de yuca cultivadas en una zona con temperatura promedio más alta presentó un tamaño de granulo más pequeño, mayor contenido de amilosa y mayor temperatura y entalpia de gelatinización, lo que está relacionado con una mayor temperatura de empastamiento y menor viscosidad. Las harinas de yuca presentaron diferencias con los almidones estudiados como una menor entalpia de gelatinización medida por calorimetría diferencial de barrido (DSC), mayor temperatura de empastamiento y menor desarrollo de viscosidad máxima. Este comportamiento posiblemente esta influenciado por la presencia de otros componentes diferentes al almidón en la raíz de yuca fresca.

Palabras clave: almidón, harina, yuca, DSC, rayos X, empastamiento

Abstract

Starch and flour were produced from cassava roots grown in two specific environmental conditions in Colombia. The physicochemical properties evaluated were granule size and morphology, amylose content, crystal form, thermal properties and pasting behavior. The properties of cassava starch were highly influenced by the environmental conditions during the growth of the roots. Starch extracted from roots cultivated in a warmer zone showed smaller granule size, higher amylose content and higher temperature and enthalpy of gelatinization. This starch also showed higher pasting temperature and lower peak viscosity. Cassava flours presented differences with their corresponding starch such as lower enthalpy of gelatinization measured by DSC, higher pasting temperature and lower peak viscosity on pasting. It is possible that this behavior is influenced by the presence of non-starch components from the fresh root.

Keywords**:** starch, flour, cassava, DSC, X-ray, pasting behavior.

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Introduction

Starch is the principal component of cassava (*Manihot esculenta* Crantz) roots which serve as an increasingly important source for different industries in places like Far East, Brazil and Colombia. One of its advantages is the possibility of being isolated in a pure form with least contamination of non-starch components [1]. The functional properties of this starch include, for example, low gelatinization temperature, non-cereal flavor, high viscosity, high water binding capacity, bland taste, translucent paste and a relatively good stability [2].

The effect of cultivar and growth season on the physicochemical properties of Colombian cassava starch has been studied by Asaoka [3, 4]. In these studies four cultivars of cassava were grown in a specific environmental zone (average temperature 23-25 ^{o}C) and harvested at different time. These starches presented minor variation in the physicochemical properties, although a considerable difference was detected in the organoleptic qualities of cooked cassava roots. Fernández-Quintero [5] studied the physicochemical properties of cassava starches extracted from four cultivars planted in two zones of different environmental conditions, Zone A (Average temperature 23-25 ºC, total rainfall of 644 cm; located in an experimental field at Valle del Cauca) and Zone B (Average temperature 31-35 ºC, total rainfall 807 cm; located on the northern coast of Colombia). From this study was concluded that although there was an effect of the cultivar in the functional characteristics of starch, the environmental conditions during the growth of the roots exerted a major effect over the properties of the starch. There was a trend in the behavior of the starch from roots grown in the same conditions that appeared to be associated with the molecular structure and the architecture of the starch granule.

Sriroth [6] studied four Thai cassava cultivars planted at Rayong Field Crops Research Center; they found slight differences between the cultivars concerning size, distribution of the granules, gelatinization temperature and enthalpy. Tester [7] reported that the biosynthesis of starch is affected by environmental factors; high temperatures depressed starch deposition resulting in reduced yields as a consequence of fewer and small granules being synthesized.

Cassava flour is made by slicing or chipping peeled roots, then drying and milling them [8, 5]. The physicochemical and functional properties of cassava flour from different crops have been reported elsewhere [9, 10, 11, 5, 12, 8]. Cassava bran, a by-product of cassava starch industrial production which contents the fiber presented in the peel of the roots, is often dried and sold as flour [13]. This material also could be a new fiber source for food use and its functionality needs to be investigated [14].

Functional and pasting properties of flour and starch are important for their use in the food industry, as they greatly influence the characteristics of new products [11]. However, despite the studies on the characteristics of cassava starches and flours, the literature regarding the physicochemical and functional properties of these materials industrially obtained is scarce.

The aim of this paper was to characterize the physicochemical and functional properties of two Colombian industrial starches and their corresponding flours, extracted from roots cultivated in specific environmental conditions.

Materials and Methods

Raw Material. Cassava roots were cultivated in two geographical zones in Colombia. Zone A is situated in Valle del Cauca at 1000 m over the sea level with an average daily temperature between 23-25 °C. Zone B is located on the northern coast of Colombia at 50 m over the sea level with an average temperature higher than 30 °C. These zones are the same ones reported by Fernández-Quintero [5]. All the roots were harvested between 8-10 months.

Roots grown in Zone A produced cassava starch and flour. Cassava Starch A was obtained in a small factory, the roots were grated on the sharp surface of a rotary solid cylinder and then the disintegrated mass was filtered in excess of water by sieving it through cloths. The fiber-free starch slurry was poured into tanks and left to sediment. Cassava flour A was processed in Clayuca-CIAT, this flour was obtained using a traditional process where roots were washed and chipped, then dried and milled, then the material was sieved to a particle size of less than 150 µm.

Roots grown in zone B produced cassava starch and flour. Cassava starch B was supplied by Industrias del Maiz, S.A. (Cali, Colombia). To obtain this starch, a conventional industrial process was used, where the roots were smashed and separated from the fiber using cyclones. Cassava flour B was supplied by the same industry, this flour is a by-product from cassava starch industrial extraction process. All laboratory reagents were from Sigma (UK) except for amylose standard, which was from MP Biomedicals INC. (USA).

Proximate Analysis. The proximate composition (ash, protein, crude fiber and fat) of cassava starch and

flour was determined by the Standard Methods of AOAC [15]. Total starch in the samples was determined using a commercial assay kit from Megazyme (Ireland). The samples were dissolved in DMSO 95 % (v/v) and treated with amyloglucosidase for hydrolysis to glucose. Glucose was determined using the glucose/peroxidase (GOP/POD) assay [16].

Amylose Content. Calorimetric determination of amylose content was performed with modifications of the methods proposed by Gérard et al. [16] and Mestres et al. [17]. Amylose content was studied using a Perkin-Elmer DSC-7 (Perkin-Elmer, UK). A sample of 8 mg starch (d. b) was weighed in a stainless steel pan (50 µl), then 40 µl of LPC (Lysophosphatidylcholin solution; 2 % w/w in water) were added directly into the pan before hermetic sealing. Samples were heated from 35 °C to 165 °C at 15 °C min^{-1}, kept at 165 °C for two minutes and cooled at 10 °C min^{-1} until 45 °C. A pan filled with 40 µl of distilled water was used as a reference. The exotherm of complex formation during cooling was measured and compared with the enthalpy of pure amylose (28 J g^{-1}). Amylose content was deduced from the ratio of sample enthalpy to that of amylose standard.

Polarized Light Microscopy. A small amount of starch was suspended in distilled water and examined at room temperature using a Leitz-Diaplan light microscope (LeitzWetzlar, Germany). The micrographs were acquired under normal and polarized lights [18]. Granule shapes and sizes were analyzed from the micrographs using the program Image–Pro Plus 5.0 (Media Cybernetics, Inc).

Wide-angle X-ray Diffraction. Samples of starch were stored over NaCl saturated solution (75 % RH) at 5 ºC for 2 weeks. X-ray spectra were recorded for 2θ between 4º and 34º at 0.05º (1 s exposure) intervals using a Bruker D5005 diffractometer (Bruker AXS, UK) equipped with a copper tube operating at 40 kV and 50 mA producing CuKα radiation of 1.54 Å wavelength. The levels of crystallinity in the starch granules were calculated by the separation and integration of the relative areas of the crystalline peaks [18].

Differential Scanning Calorimetry (DSC). Starch and flour gelatinization was studied using a Perkin-Elmer DSC-7 (Perkin-Elmer, UK). The samples were weighted (10 mg, d. b), and distilled water was added to create water-excess conditions (proximate ratio 1:3). The pans were hermetically sealed and placed on mixing rollers and left overnight to allow homogeneous hydration of the sample before carrying out the analysis. The thermograms were acquired between 10 to 120 ºC at a heating rate of 10 ºC min^{-1}. An empty pan was used as a reference [18].

Pasting Profiles. The pasting profiles were studied using a Rapid ViscoAnalyser (RVA) series 4 (Newport Scientific, NSW, Australia). 2.5 g (d.b) of the sample were mixed with 25 g of distilled water to give a starch concentration of 10 g kg^{-1}, after correcting the original moisture content of the starches, the time-temperature profile used was as follows: the system was held at 50 ºC for 1 min, heated from 50 to 95 ºC in 3 min and 45 s, then held at 95 ºC for 2 min and 30 s; the sample was subsequently cooled to 50 ºC over a period of 3 min and 45 s, followed by a period of 2 min where the temperature was controlled at 50 ºC [18].

Statistical Analysis. The values reported are the mean of three replicates. The significance of differences was assessed using the analysis of variance (ANOVA) performed by the analysis tool of Microsoft Excel ®.

Results and discussion

The results from proximate analysis composition are shown in Table 1. Values were in the expected range for cassava starches [4, 19]. The starch content and the crude fiber varied greatly between flours, and chemical composition was significantly affected by the procedure for obtaining the flours. Flour B a by-product of starch extraction, it presented less starch content and more crude fiber; higher values of crude fiber have been reported before by Leonel and Cereda [20] for Brazilian cassava bran. Also, the fat content in the flours was higher than the levels found in the starches; this behavior was also reported by Moorthy et al. [12].

Table 1. Proximate analysis cassava starches and flours.[1]

	Starch A	*Flour A*	*Starch B*	*Flour B*
Protein (%)	0.08 ± 0.01	1.2 ± 0.04	0.08 ± 0.02	1.42 ± 0.18
Ash (%)	0.06 ± 0.02	1.73 ± 0.26	0.13 ± 0.05	1.25 ± 0.24
Fat (%)	0.05 ± 0.001	0.35 ± 0.03	< 0.01	0.5 ± 0.01
Starch (%)	94.34 ± 0.17	81.2 ± 0.28	95.51 ± 0.15	65.5 ± 0.17
Crude fiber (%)	N.D.	2.98 ± 0.4	N.D.	12.43 ± 0.10

[1]Values are expressed on a dry weight basis and each one represents an average and a standard deviation. N.D: non-determined.

The values of amylose content in the starches were statistically different ($P < 0.01$). The amylose content of starch B (20.09 ± 0.14 g kg^{-1}) was higher compared to starch A (18.17 ± 0.02 g kg^{-1}). Higher values of amylose content for rice starch synthesized at elevated environmental temperature have been observed by Asaoka et al. [21]. The results of amylose content were within the range 16-20 g kg^{-1} reported for Colombian cassava varieties by Asaoka et al. [3] using fractionation by columns after debranching of the starch. Whereas Fernández-Quintero [5] stated higher values (20-26 g kg^{-1}) using a colorimetric iodine method. Sriroth et al. [6] and Charles et al. [1] reported values of amylose content between 18-25 g kg^{-1} for Thai cassava starch. The amylose content for Indian cassava starch was within the range of 22-26 g kg^{-1} [12].

The granule morphology of the cassava starch showed the characteristic rounded shape. The granule size of cassava starch was in the range of 2.5-28 µm, which was within the range reported in other parts [11, 6, 22, 5, 3]. The analysis of granule size and distribution from the micrographs showed a significant difference ($P < 0.001$) between starches. As shown in Figure 1, Starch A presented a distribution with a peak at 13 µm and a major presence of granule size higher than 10 µm; Starch B showed a higher presence of granules between 7.5-15.5 µm with peak at 9.5 µm.

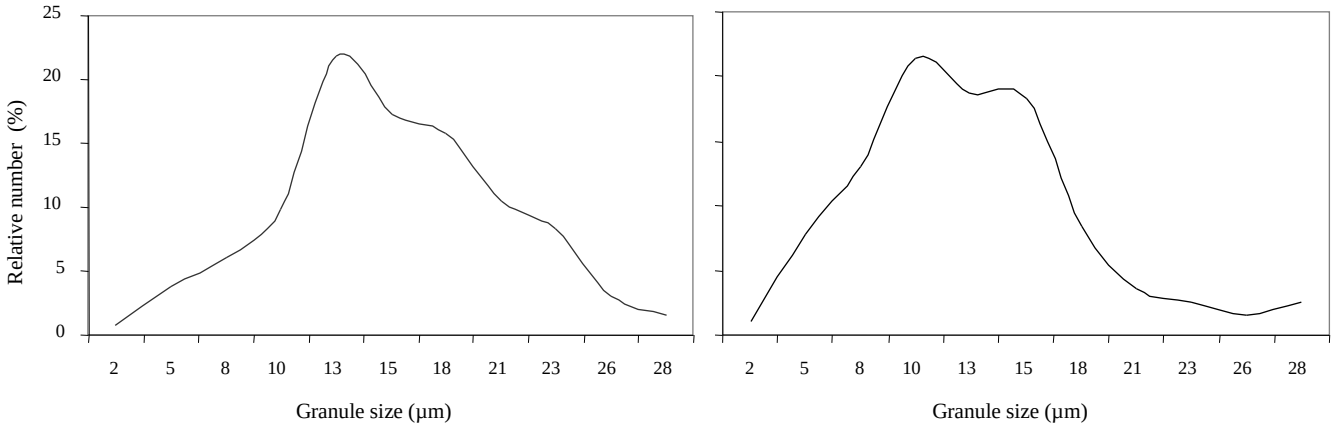

Figure 1. Granule size distribution in cassava starches. Starch A (left) and Starch B (right).

This difference in granule size distribution was also observed by Fernández-Quintero [5], who found that cassava starch from Zone A presented a distribution of granules with peak between 14.2-17.88 µm, whereas starch extracted from roots cultivated in Zone B, presented the peak at 9 µm. In this study, the molecular weight of starch was measured by size exclusion chromatography (SE-HPLC), assisted by multi-angle laser light scattering and a refractive index (MALLS-RI) system. The analytical results suggested that amylose of low molecular weight and amylopectin with reduced branching were characteristic of starches with small size granule populations.

The wide–angle X-ray diffractograms obtained for both starches are shown in Figure 2. Both starches presented a similar spectrum characteristic of crystallinity A Type, with peaks at 15.3°, 17.3°, 18° and 23.3° as reported elsewhere [5, 3]. The values of percentage of crystallinity were in the range of 20-23 %. These values were higher compared to those reported by Asaoka et al. [3], (15-17 % level of crystallinity), but lower than those reported by Fernández-Quintero [5] and Zobel [22], (35-38 %). Discrepancies in crystallinity could be related to differences in the analysis methodology.

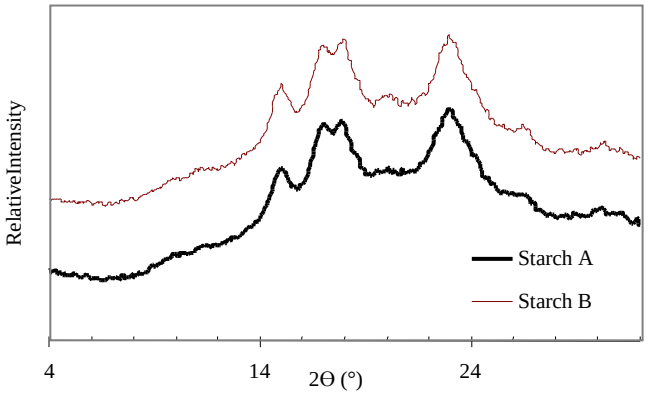

Figure 2. X–ray diffractograms of cassava starches A and B.

The results of thermal properties for cassava samples are summarized in Table 2. The starches presented significant differences ($P < 0.001$) in values of temperature of gelatinization and enthalpy. Starch B showed the highest values; in other crops, it has been observed that the gelatinization temperature is affected by the environmental temperature during

which the starch granules are synthesized [21, 7]. Besides, Noda et al. [23] have reported, in studies on sweet potato and wheat starches, that low values of gelatinization onset, peak and conclusion temperature measured by DSC, reflected the presence of abundant short amylopectin chains.

The values of temperature and enthalpy of gelatinization determined in this study were higher than the values reported by Asaoka et al. [4] for Colombian varieties, temperature of gelatinization 50.7-57 ^{o}C and enthalpy 7-9 J g^{-1}. The enthalpy of gelatinization for starch A was in agreement with the value reported by Charles et al. [1] for Thai cassava starch and for Indian starch [12]. Higher value of enthalpy as starch B has been reported by Abera and Rakshit [24].

Table 2. DSC thermal properties of cassava starches and flours.[1]

Material	*DSC onset (°C)*	*DSC peak (°C)*	*DSC end (°C)*	*Enthalpy Δ H (J g^{-1})*
Starch A	60.69 ± 0.53	65.44 ± 0.51	71.36 ± 0.98	13.96 ± 0.06
Starch B	66.51 ± 0.24	72.00 ± 0.44	79.26 ± 0.33	15.50 ± 0.74
Flour A	60.99 ± 0.71	66.14 ± 0.33	69.91 ± 0.14	7.75 ± 0.07
Flour B	66.01 ± 0.41	71.00 ± 0.22	77.22 ± 0.59	8.11 ± 0.64

[1] The results are means of three experiments and standard deviation.

The results of gelatinization temperature for the flours were closer to the values determined for their corresponding starch, this contrasts with the results of Moorthy et al. [12] and Defloor et al. [25]. They postulated that the presence of non-starch components, which competed for the available water, delayed the gelatinization. Despite the high crude fiber content for flour B, the temperature of gelatinization was the same as in the starch. These differences could be related to crude protein content as well as environmental conditions. Pereira and Beleia [10] reported that cassava flours from peeled roots presented higher protein content which decreased with the age of the roots. Besides, Defloor et al. [23] stated that gelatinization temperature of cassava flour increased as roots were grown in dry conditions. Root age at harvest, protein content and moisture stress were not reported by Moorthy et al. [12]. The enthalpy of gelatinization for flour samples presented significant differences ($P < 0.001$) with the enthalpy of the starch. This behavior was reported before by Moorthy et al. [12]. Cassava flour gelatinization enthalpy was in the range of 7-8 J g^{-1}, these values were lower than those reported for Indian varieties (9-10 J g^{-1}).

The RVA results are presented in Table 3. Peak viscosity and final viscosity values of cassava starches showed significant differences ($P<0.001$). Starch A showed a lower pasting temperature and developed higher viscosity. Differences in pasting behavior between starches were observed before by Fernández-Quintero [5], who stated that starches from plants of zone B presented higher initial pasting temperature and exhibited lower viscosities on pasting.

Table 3. RVA pasting results for cassava starches and flours.

Material	*Peak viscosity (m·Pa·s)*	*Trough viscosity (m·Pa·s)*	*Final viscosity (m·Pa·s)*	*Onset temperature (°C)*
Starch A	5716	2367	2697	67.85
Starch B	4862	2670	3085	74.35
Flour A	4325	2640	3080	71.25
Flour B	3100	2580	3210	75.95

The size and size distribution of starch granules might contribute in the pasting behavior and the rheological response of starches and swelling of granules [26, 27]. Starch from Zone B presented a higher proportion of small granules than starch from zone A. The differences in the granule size between the samples could partially explain their different behavior during pasting. Starch B also presented a higher value of final viscosity. Charles et al. [1] and Sriroth et al. [6] reported that starch with high amylose content developed high final viscosity and setback on pasting.

Pasting profiles for cassava starches and flours are plotted in Figure 3 and Figure 4. There were significant differences ($P < 0.001$) in the pasting profiles between starches and their corresponding flours. Lower peak viscosity values and higher pasting temperatures were obtained for both flours. This lower viscosity values could be partly attributed to lower starch content. It is also possible that the minor components (protein and fiber) influenced the values of viscosity on pasting [11, 12].

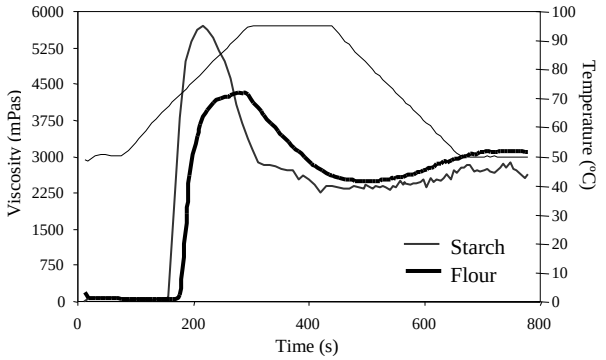

Figure 3. Pasting behavior for cassava starch and flour A.

Pasting profiles for cassava starches and flours are plotted in Figure 3 and Figure 4. There were significant differences ($P < 0.001$) in the pasting profiles between starches and their corresponding flours. Lower peak viscosity values and higher pasting temperatures were obtained for both flours. This lower viscosity values could be partly attributed to lower starch content. It is also possible that the minor components (protein and fiber) influenced the values of viscosity on pasting [11, 12].

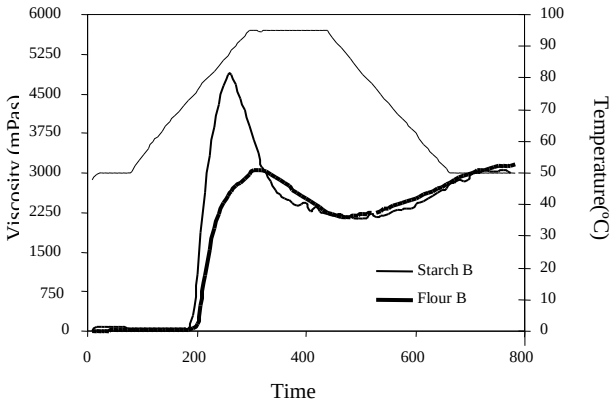

Figure 4. Pasting behavior for cassava starch and flour B.

Conclusions

The physicochemical properties of starches were highly influenced by the environmental conditions during the growing period of the plants. Small granule size, high amylose content, high temperature and enthalpy of gelatinization were characteristics of starch extracted from roots cultivated at high temperatures. These physicochemical properties are also related to functional properties as high pasting temperature and low peak viscosity. Therefore, it was confirmed that there are trends in the behavior of cassava starch from roots grown in a specific environmental condition.

Physicochemical properties of flour were influenced by chemical composition, which was a consequence of the procedure for obtaining the flours. The presence of non-starch components in the flours decreased the values of enthalpy of gelatinization and increased pasting temperature but decreased peak viscosity.

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