

# Acid Hydrolysis and Characterization of *Canavalia ensiformis* Starch

A. D. Betancur\* and G. L. Chel

Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Av. Juárez No. 421 Cd. Industrial, C.P. 97288, A.P. 1266-A, Mérida, Yucatán, México

The physicochemical and functional properties of *Canavalia ensiformis* starch hydrolyzed with HCl were studied. The factors analyzed were HCl concentration (0.5–1.5%), temperature (45–55 °C), and reaction time (3–6 h). Alkali number and viscosity were the response variables. A 2<sup>3</sup> factorial design with five replicates of the central trial was used. Hydrolyzed starch with 1.5% HCl at 55 °C for 6 h reached an alkali number of 11.92. The hydrolysis reaction did not present important changes on the starch chemical composition except for an ash content reduction. A comparison between these hydrolyzed starches and the native starches showed a viscosity (36.4 cP), swelling power (21.86 g of water/g of starch), and retrogradation reduction. A 45.3% solubility increment was found. Gelatinization temperature did not vary (76–82 °C).

**Keywords:** *Canavalia*; starch; acid hydrolysis; functional properties

## INTRODUCTION

Starch is the second most abundant polysaccharide in plants, after cellulose. It is synthesized in the chloroplasts and chromatophores and stored as an energetic reserve substance in roots, seeds, and tubers as small particles known as granules (Whistler and Daniel, 1990).

Starches are the main components of food. They provide the energy needed in the human diet. In spite of their wide distribution in the vegetal kingdom, few vegetal sources are extensively used in commercial starch production. Cereal grains such as corn, wheat, rice, and sorghum, as well as tubers such as potato, cassava, and sweet potato, can be considered as important sources. Starch can also be found in leaves, legume seeds, and fruits (Gujska *et al.*, 1994; Zajac, 1989; Fuke and Matsuoka, 1984; Biliaderis *et al.*, 1981a). *Canavalia ensiformis*, a legume also known as jack bean, has its origins in the western part of India (Thompson *et al.*, 1986). It has been proved that it has important agronomic potential under typical weather and soil type conditions of the State of Yucatan, Mexico. Yields of 2580 kg/ha have been obtained in this area (Kessler *et al.*, 1985). *C. ensiformis* seeds have been considered for food and feed because they have high contents of starch and protein (Leon *et al.*, 1991), 36.7–50% (Leon *et al.*, 1991) and 29.7% (Kessler *et al.*, 1990), respectively.

Starches are employed in food because of their good thickening and gelling properties. They are a traditional ingredient in the manufacture of porridges, puddings, custards, biscuits, and sausages. It is important to point out that most of the starch production is mainly used in the elaboration of fructose syrups. This product has an important demand worldwide (Smith, 1992). However, native starches present limitations that reduce their use at the industrial level. They have low shear stress resistance, thermal decomposition, high retrogradation, and syneresis. These facts motivated the employment of modified starches (Fleche, 1985).

Starches can be modified by chemical, physical, and enzymatic methods (Agboola *et al.*, 1991). Hydrolyzed

starches can be found among those starches modified by chemical methods. Hydrolyzed starches are produced when a concentrated suspension of starch (30–40% solids) is treated with acid, at temperatures lower than those of gelatinization (30–60 °C), during one or many hours of reaction time. Mineral acids commonly used are hydrochloric acid and sulfuric acid (Pomeranz, 1991; Wurzburg, 1986; Fleche, 1985). The acid hydrolysis process diminishes the molar mass, increasing, consequently, the free aldehyde group content. It also decreases viscosity, increases the solubility of the granules, and minimizes the syneresis. Furthermore, this process increases the adhesiveness by reducing the elasticity and rigidity of the gels, and these modified starches have the capability of forming films (Whistler and Daniel, 1990; Wurzburg, 1986; Luallen, 1985; Fleche, 1985).

The objective of this study was to investigate the effects of hydrochloric acid, temperature, and reaction time on the hydrolysis of *C. ensiformis* starch. Some of the chemical and functional properties of the modified starches were also studied.

## MATERIALS AND METHODS

**Seeds.** *C. ensiformis* seeds were obtained from the February 1994 harvest at Campo Experimental Pecuario in the city of Tizimin, State of Yucatan, Mexico. All the chemicals were of reagent grade and were purchased from J. T. Baker (Phillipsburg, NJ).

**Native Starch Extraction.** A sample of 25 kg seeds was used. Impurities and damaged seeds were discarded. Sound seeds were milled in a Mykros impact mill, and the flour was passed through a 20 mesh. To extract the starch, a previous process developed by Moguel *et al.* (1996) was scaled up to pilot plant. This process consists of steeping the flour in an aqueous suspension (1:7 w/v) for 12 h at pH 12 and room temperature.

**Starch Hydrolysis.** A 2<sup>3</sup> factorial design with five replicates of the central trial was used. The factors and their corresponding levels analyzed were HCl concentration (0.5 and 1.5%), temperature (45 and 55 °C), and reaction time (3 and 6 h). A modification of the method of Wurzburg (1986) was followed to depolymerize these starches. In this process an aqueous starch suspension was mildly treated with hydrochloric acid at low concentrations. A sample of 200 g of a starch suspension (40% w/w) was prepared in a flask with HCl

\* Author to whom correspondence should be addressed (e-mail cguerrer@tunku.uady.mx).

at two different concentrations (0.5 or 1.5%) based on starch weight. The slurry was placed in a thermostatic bath, Grant Model JB-3, at 45 or 55 °C. A mechanic stirrer (Cafrao RZ1) with a stainless steel propeller operating at 300 rpm was used to obtain a uniform slurry. Reaction time was 3 or 6 h. After the reaction time had elapsed, the suspension of hydrolyzed starch was neutralized to pH 7.0 by using a 10% (w/v) sodium carbonate solution. The modified starch was recovered by vacuum filtration in a Büchner funnel. A piece of sailcloth was used as filter, and the recovered starch was washed twice with 150 mL of distilled water. The starch was dried at 60 °C in a Lab-line oven with mechanical convection. Afterward, it was milled in a Cyclotec mill to obtain the powdered hydrolyzed starch. The response variables analyzed were the alkali number and the viscosity.

**Chemical Analysis.** Nitrogen (Method 954.01), fat (Method 920.39), ash (Method 923.03), crude fiber (Method 962.09), moisture (Method 925.09), and starch contents were determined for the native starch and modified starches obtained with the best treatments. These analyses were made according to the official AOAC procedures (AOAC, 1990). The nitrogen was determined with a Kjeltac system (Tecator, Sweden). The protein was calculated by nitrogen  $\times$  6.25. Fat was obtained from a 4 h extraction with hexane. Ash was calculated from the weight remaining after the sample was heated at 550 °C for 2 h. Moisture was from the weight loss after oven drying at 110 °C for 2 h. Starch content was estimated as nitrogen-free extract.

Apparent amylose content was analyzed according to the iodometric method of Gilbert and Sprag (1964). The alkali number was determined with the method of Schoch (1964).

**Functional Properties.** Gelatinization temperature range was found according to the microscopic method of Watson (1964) with a Seiwa Optical microscope.

The viscosity was determined with the method of Smith (1964) using a 2.5% starch suspension in a Brookfield viscometer LTV with spindle 2 at 30 °C and 100 rpm.

Retrogradation studies were performed according to the Tjahjadi and Breene (1984) method with 7% starch gels kept in refrigeration (4 °C for 24 h). Retrogradation was measured as volume of water (milliliters) freed after centrifugation at 10000g (degree of syneresis).

Solubility and swelling patterns were determined using the Schoch (1964) method as modified by Sathe and Salunkhe (1981). All of the chemical analyses and the functional properties determinations were made in triplicate.

**Statistical Analysis.** Analysis of variance of the results was made according to the methods reported by Montgomery (1991). The method of Duncan was used to obtain the means comparison. Regression analysis and the other analyses were made with Statgraphics 5.0 computer software.

## RESULTS AND DISCUSSION

The viscosity reduction and the increment of the alkali number both indicate the existence of a high number of end aldehyde groups. This confirms the breaking of the glucoside bonds in the amylose and amylopectin chains (Radley, 1976; Wurzburg, 1986), illustrated in Table 1, as well as the hydrolysis with 0.5% HCl at 45 °C for 3 h (treatment 1), which was very low. Viscosity values of 63.4 cP and alkali number of 5.99 were obtained, and both were similar to the native starch values (64.7 cP and 5.76, respectively). The opposite results were observed in treatment 8 (1.5% HCl at 55 °C for 6 h). In this case hydrolysis was more severe. The viscosity was reduced to 36.4 cP and the alkali number increased to 11.92, compared to the native starch.

The analysis of variance of the alkali number and the viscosity indicated that HCl concentration, temperature, and reaction time, and their higher interactions, had significant influence ( $P < 0.05$ ) on the native starch modification. In a similar way, the importance of the

**Table 1. Alkali Number and Viscosity of Native and Hydrolyzed Starches of *C. ensiformis***

treatment	HCl (% w/w)	temp (°C)	time (h)	alkali no. <sup>a</sup>	viscosity <sup>a</sup> (cP)
1	0.5	45	3	5.99 $\pm$ 0.1 <sup>c</sup>	63.4 $\pm$ 0.2 <sup>c</sup>
2	1.5	45	3	7.95 $\pm$ 0.0 <sup>f</sup>	59.8 $\pm$ 0.2 <sup>f</sup>
3	0.5	55	3	6.95 $\pm$ 0.2 <sup>de</sup>	61.4 $\pm$ 0.2 <sup>de</sup>
4	1.5	55	3	9.54 $\pm$ 0.2 <sup>b</sup>	51.2 $\pm$ 0.0 <sup>b</sup>
5	0.5	45	6	6.86 $\pm$ 0.1 <sup>cd</sup>	62.2 $\pm$ 0.5 <sup>cd</sup>
6	1.5	45	6	9.58 $\pm$ 0.1 <sup>b</sup>	51.6 $\pm$ 0.0 <sup>b</sup>
7	0.5	55	6	7.73 $\pm$ 0.2 <sup>ef</sup>	60.2 $\pm$ 0.0 <sup>ef</sup>
8	1.5	55	6	11.92 $\pm$ 0.2 <sup>a</sup>	36.4 $\pm$ 0.2 <sup>a</sup>
9	1.0	50	4.5	7.00 $\pm$ 0.2 <sup>d</sup>	58.2 $\pm$ 0.2 <sup>f</sup>
10	1.0	50	4.5	7.18 $\pm$ 0.0 <sup>d</sup>	58.6 $\pm$ 0.2 <sup>f</sup>
11	1.0	50	4.5	7.18 $\pm$ 0.0 <sup>d</sup>	58.6 $\pm$ 0.0 <sup>f</sup>
12	1.0	50	4.5	7.09 $\pm$ 0.1 <sup>d</sup>	58.5 $\pm$ 0.5 <sup>f</sup>
13	1.0	50	4.5	7.18 $\pm$ 0.1 <sup>d</sup>	58.3 $\pm$ 0.3 <sup>f</sup>
native				5.76 $\pm$ 0.04 <sup>c</sup>	64.7 $\pm$ 0.3 <sup>c</sup>

<sup>a</sup> Means with the same superscript do not differ significantly from one another ( $P > 0.05$ ).

HCl concentration to obtain acid-hydrolyzed products from yucca starch were reported by Osunsami *et al.* (1991). The importance of the reaction time was also noticed by Biliaderis *et al.* (1981b) in the starch acid hydrolysis of different legumes. These authors analyzed adzuki bean, mung bean, pea, and lentil starches. When they treated 2.5% suspensions of these purified starches with 2.2 N HCl at 35 °C, they found that a fast hydrolysis was present in the amorphous region of the granules, followed by the starch molecule crystalline region's slow degradation that depended on the reaction time.

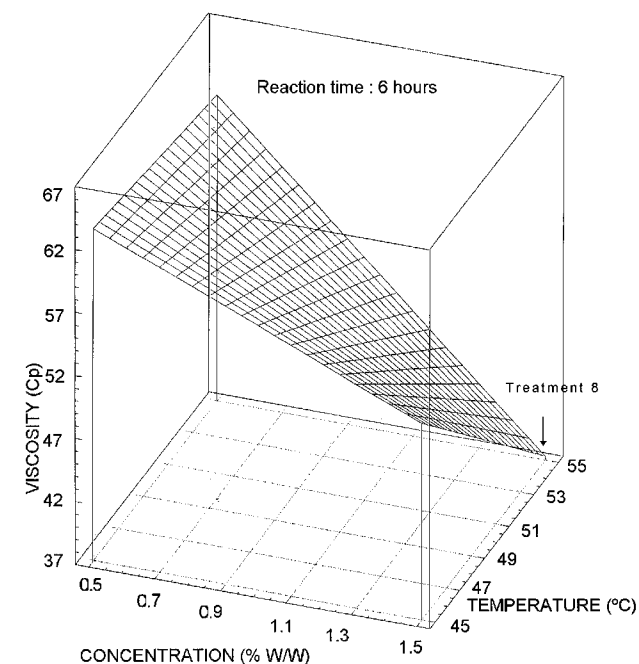
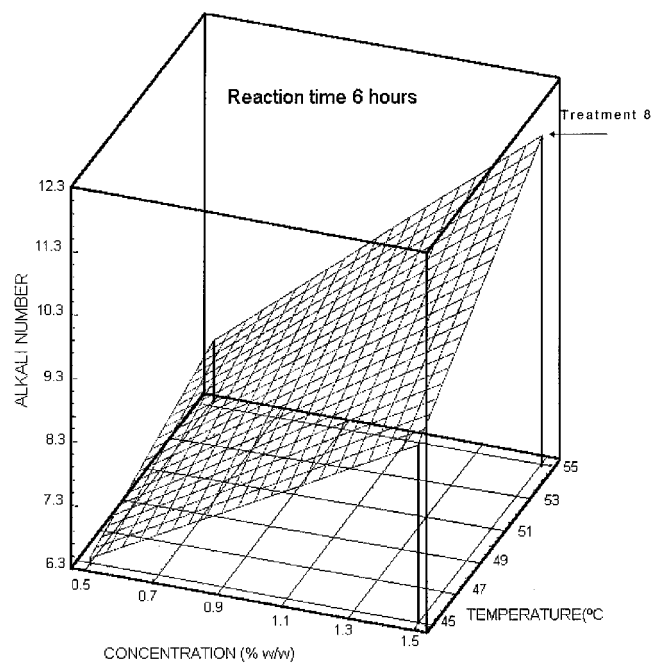
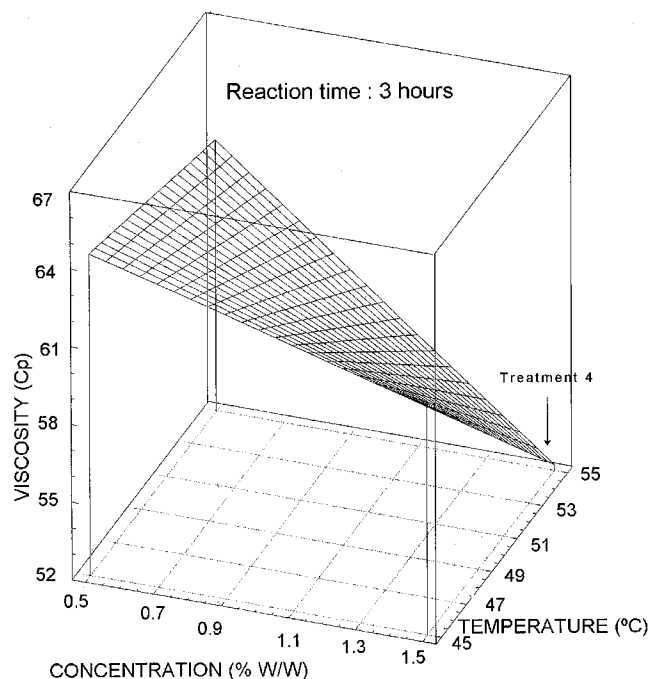
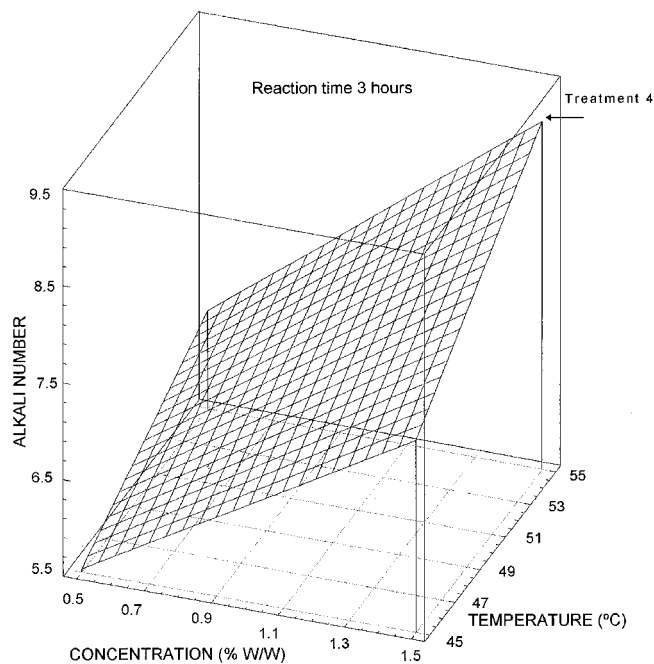
Regression analysis for each response variable was performed; it suggested that the experimental data fit adequately a first-order polynomial model ( $P < 0.05$ ).

The mathematical models and surface response that illustrate the behavior of these phenomena are shown in Figures 1 and 2 and are represented by eqs 1 and 2. These models represent the behavior of the alkali number and the viscosity of the products obtained, expressed in terms of the acid concentration ( $A$ ), temperature ( $B$ ), and reaction time ( $C$ ).

In these equations all of the factors and their higher interactions had effects with the same trend on the modification. In other words, all of them have positive effects on the alkali number and negative effects on the viscosity. Therefore, the stronger the treatment, the higher the hydrolysis, which produces higher alkali numbers and product viscosity reduction. On the basis of the regression coefficients, the HCl concentration was the factor that most affected the hydrolysis of *C. ensiformis* native starch. This was also expected. On the other hand, the temperature and the reaction time have the same influence on the viscosity and alkali number. However, they are less important than the HCl concentration.

The results of the analysis suggested that there are two adequate treatments which can be used to obtain *C. ensiformis* hydrolyzed starches, based on the fact that most of the hydrolyzed derivatives from potato and corn usually employed in the industry have alkali numbers between 10 and 40 (Radley, 1976). The treatments selected were 1.5% HCl at 55 °C for 6 h and 1.5% HCl at 55 °C for 3 h, treatments 8 and 4, respectively. Treatment 4 was chosen because its reaction time is shorter than that of treatment 6; in terms of the viscosity and alkali number, means analyzed separately are statistically the same ( $P > 0.05$ ).

**Chemical Composition.** The chemical composition and the amylose content of *C. ensiformis* hydrolyzed



**Figure 1.** Effect of the concentration of hydrochloric acid (A), temperature (B), and reaction time (C) on alkali number value during the course of the hydrolysis of the starch of the *Canavalia*. Alkali no. =  $7.8586 + 1.4317A + 0.7228B + 0.7082C + 0.2605AB + 0.2955AC + 0.0834BC + 0.1051ABC$ ;  $R^2 = 0.9105$  (eq 1).

**Figure 2.** Effect of the concentration of hydrochloric acid (A), temperature (B), and reaction time (C) on the viscosity during the course of the hydrolysis of starch of *Canavalia*. Viscosity (cP) =  $56.80 - 6.025A - 3.475B - 3.174C - 2.475AB - 2.575AC - 0.825BC - 0.825ABC$ ;  $R^2 = 0.9124$  (eq 2).

starches obtained in the best treatments are shown in Table 2. These starches do not show significant changes in their composition, compared to native starch, except in the ash content. The chemical hydrolysis induced an ash content reduction due to the solubilization effect on minerals caused by HCl. Agboola *et al.* (1991) reported an ash content reduction from 0.21 to 0.12–0.16% of yucca starch treated with 1%  $H_2SO_4$ .

Acid hydrolysis generally takes place when the acid attacks the amorphous regions of the granule. Consequently, the amylopectin fraction depolymerization is faster than that of the amylose fraction. This generates a higher number of linear polymer fractions (Biliaderis *et al.*, 1981b; Wurzburg, 1986; Pomeranz, 1991; Whistler

and Daniel, 1990). The hydrolyzed starch apparent amylose content increment, according to the treatment used, ranged from 50.24 to 58.26%.

**Functional Properties.** The hydrolyzed starch functional property results of treatments 4 and 8 are illustrated in Table 3. The hydrolysis treatments do not have a strong influence on the gelatinization temperature, since a slight reduction of these values was observed when compared to native starches. This was probably due to the low levels of hydrolysis reached. The treatments were not intense enough to disintegrate the granules before they swelled in the aqueous medium. In addition, they were slightly damaged but the hydrolysis did not drastically affect the starch molecule crystalline region. This region is responsible of the

**Table 2. Chemical Composition of Native and Hydrolyzed Starches of *Canavalia* (Percent Dry Basis)**

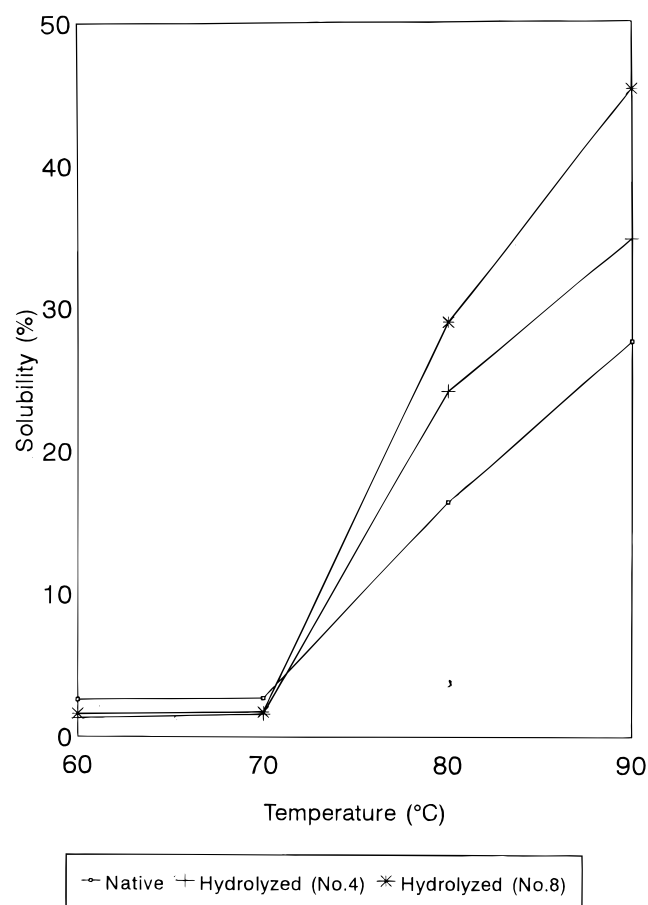
component	starch		
	native	hydrolyzed treatment 4 <sup>a</sup>	hydrolyzed treatment 8 <sup>b</sup>
moisture	(10.16 ± 0.09)	(3.77 ± 0.11)	(6.16 ± 0.12)
protein	0.34 ± 0.01	0.33 ± 0.01	0.32 ± 0.01
fat	0.14 ± 0.01	0.16 ± 0.01	0.17 ± 0.01
fiber	0.11 ± 0.02	0.11 ± 0.01	0.11 ± 0.01
ash	0.40 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
starch	99.01 ± 0.14	99.23 ± 0.15	99.22 ± 0.17
apparent amylose	37.50 ± 0.25	50.24 ± 0.22	58.26 ± 0.25

<sup>a</sup> 1.5% HCl, 55 °C, 3 h. <sup>b</sup> 1.5% HCl, 55 °C, 6 h.

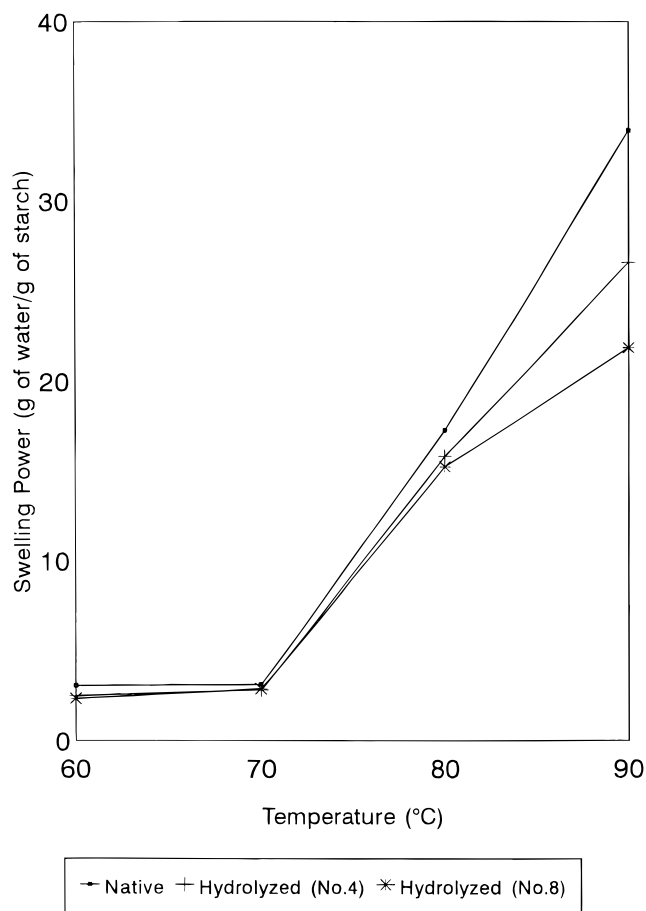
**Table 3. Functional Properties of Native and Hydrolyzed Starches of *Canavalia***

functional properties	starch		
	native	hydrolyzed treatment 4 (1.5%/55 °C/3 h)	hydrolyzed treatment 8 (1.5%/55 °C/6 h)
gelatinization temp <sup>a</sup> (°C)	(77–83) ± 0	(76–82) ± 0	(76–82) ± 0
viscosity <sup>b</sup> (cP)	64.7 ± 0.3	51.2 ± 0	36.4 ± 0
retrogradation <sup>c</sup> (mL/50mL)	9.8 ± 0.05	9.55 ± 0.05	5.95 ± 0.05
alkali no.	5.76 ± 0.1	9.54 ± 0.2	11.92 ± 0.2

<sup>a</sup> Method of Watson (1964). <sup>b</sup> Measured to 2.5 starch suspension in a Brookfield viscometer, spindle 2, 30 °C, and 100 rpm. <sup>c</sup> Water freed in gels prepared with 7% starch and kept at 4 °C for 24 h.

**Figure 3.** Effect of temperature on solubility of native and hydrolyzed starches of *Canavalia*.

hydrated micelle formation since it has the capability to link water molecules. Similar results were reported by Escobar (1993) in the hydrolysis of *Xanthosoma violaceum* starch. This research worker used the fol-

**Figure 4.** Effect of temperature on swelling power of native and hydrolyzed starches of *Canavalia*.

lowing conditions: 0.5 and 0.4% HCl at 50 °C for 6 h. Compared to the native starch values, no significant changes occurred in the gelatinization temperature range (74–80 °C).

As expected, the alkali number increased due to the breaking of the glucoside bonds, and there was a formation of a high number of end aldehyde groups in the starch fractions. The glycoside link rupture induced a viscosity reduction as the degree of hydrolysis increased. This process produces short molecular segments that are more soluble and therefore absorb less water and have a smaller swelling power.

The retrogradation, measured as the syneresis degree of the acid derivatives obtained, diminished as the final degree of hydrolysis was reached. The values were 9.8 mL/50 mL for native starch and 5.95 mL/50 mL for hydrolyzed starch produced with treatment 8. These results confirm reports of Cañizares (1994), Fleche (1985), and Rohwer and Klem (1984), indicating that the starch acid modification minimizes gel and paste retrogradation.

The solubility increased (Figure 3) and the swelling power diminished (Figure 4) at the same rate the modification intensity did, as a result of the low molecular weight linear fraction increment with hydroxyl groups that facilitated solubilization in warm water. Theoretically, these hydroxyl groups can retain water molecules by means of hydrogen bonds, increasing the swelling power. However, this does not happen to hydrolyzed starches, because their granules are fragmented in a radial way when they are heated and then cooled without reaching an organized structure. There-

fore, they cannot retain water inside the structure, as a result of the hydrolysis.

**Conclusions.** The acid hydrolysis of *C. ensiformis* starch depends on the treatment intensity. The acid concentration is the most important factor. The starch depolymerization with 1.5% HCl, at 55 °C for 6 h, leads to a 45.3% solubility increment and a granule swelling power reduction (21.8 g/g of starch). It also diminishes the viscosity to 36.4 cP and decreases paste and gel retrogradation. The modified starch characteristics allow these derivatives to be used in the candy industry in the manufacture of syrups and jellies, soft candies, compressed candies, and gum products. They can also be employed as stabilizers in sausages and dressings. These modified starches provide new options in the manufacture of parenteral food and in the elaboration of instant beverages.

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