Acetylation and Characterization of *Canavalia ensiformis* Starch

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Canavalia ensiformis native starch was acetylated, and its functional properties were evaluated. Reaction conditions assays included pH (7.0−7.5 and 8.0−8.5), acetic anhydride concentration (5 and 10%), and reaction time (30 and 60 min). Starch acetylated with 10% acetic anhydride at pH 8.0−8.5 for 30 min reached 2.34% acetyl value. Compared to native starch, these acetylated products showed an increasing pastes and gels clarity, solubility (38%), swelling power (48.4 g of water/g of starch), and viscosity (88.33 cP). The gelatinization temperature range was reduced to 67−72 °C, and retrogradation was eliminated.

Keywords: *Canavalia; starch; acetylation; functional properties*

INTRODUCTION

Canavalia ensiformis, a legume also known as Jack bean, has its origins in the western part of India and Central America (Thompson et al., 1986). This legume has important agronomic potential in the State of Yucatan, Mexico with yields of 2580 kg/ha per year (Kessler, 1985). The seeds can be used for food and feed, since they are a good source for starch and protein (Leon et al., 1991), 36.7−50% (Leon et al., 1989; Duke, 1981) and 29.7% (Kessler, 1985), respectively.

Native starches have been used since ancient times as raw material to prepare different products. They are employed in foods because of their good thickening and gelling properties. They are also a good texture stabilizer and regulator in food systems (Cousidine, 1982). However, they have limitations that reduce their use in industrial applications. They have low shear stress resistance, thermal decomposition, high retrogradation, and syneresis. These shortcomings can be overcome by starch modification (Fleche, 1985).

Starches can be modified by chemical, physical, and enzymatic methods (Agboola et al., 1991). Among chemically modified starches is the acetylated starch, which is obtained by the sterification of native starch with acetic anhydride (Jarowenko, 1989). The modified starches generally show better paste clarity, stability, increased resistance to retrogradation, and increased freeze−thaw stability (Agboola et al., 1991).

As in all chemical reactions, acetylation depends upon factors such as reactant concentration, reaction time, pH, and the presence of catalyst (Whistler and Daniel, 1990), which finally determine the number of acetyl groups incorporated into the molecule.

The objetive of this study was to investigate the effects of pH, time, and reagent concentration on the acetylation of the *Canavalia ensiformis* starch. Some chemical and functional properties of the modified starches were also evaluated.

MATERIALS AND METHODS

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

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

Starch Acetylation. A 2^3 factorial design was used with five replications of the central trial. Factors and their corresponding levels were concentration of the reactant (5 and 10%), pH (7.0−7.5 and 8.0−8.5), and reaction time (30 and 60 min). The procedure was performed according to Wurzburg (1964). A sample of 200 g of a starch suspension (40% w/w) in water was prepared in a flask that was placed in a thermostatic bath, Grant Model JB-3, at 30 °C. A mechanic stirrer (Caframo RZ1) with a stainless steel propeller operating at 300 rpm was used to make a uniform slurry.

Afterward, the pH was measured with a Cole Parmer Digi-Sense potentiometer and adjusted to 7.5 or 8.5 by adding drop-by-drop a 3% NaOH solution and acetic anhydride simultaneously. This operation was carefully performed to maintain the pH at the range of values previously set in the experimental design. After the reaction time had elapsed, the pH was adjusted to 4.5 with 0.5 N HCl and the slurry was vacuum-filtered through a piece of sailcloth. Modified starch was recovered and washed with 150 mL of distilled water. The slurry was filtered again to remove water. The washing step was repeated, and starch was dried at 60 °C in a Lab-line oven with mechanical convection. When the product was dry, it was milled in a Cyclotec Mill and passed through a 20-mesh. The response variables analyzed were the percent of acetyl groups and the reaction efficiency.

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 AOAC official procedures (AOAC, 1990). The nitrogen was determined with a Kjeltec System (Tecator, Sweden). The protein was calculated by nitrogen × 6.25. Fat was obtained from a 4 h extraction with hexane. Ash was calculated from the weight remaining after heating the sample 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 by the iodometric method of Gilbert and Sprag (1964). Acetyl group content was

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Table 1. Percentage of Acetyl and Efficiency Values of Acetylated Starches of *Canavalia*[a]

treatment	acetic anhydride (% w/w)	pH	time (min)	acetyl (%)	efficiency (%)
1	5.0	7.0−7.5	30	0.94 ± 0[d]	45.43 ± 0[d]
2	10.0	7.0−7.5	30	1.61 ± 0.01[b]	39.85 ± 0.15[e]
3	5.0	8.0−8.5	30	1.50 ± 0.01[b,c]	72.48 ± 0.12[a]
4	10.0	8.0−8.5	30	2.34 ± 0[a]	58.09 ± 0[c]
5	5.0	7.0−7.5	60	1.00 ± 0.02[d]	48.49 ± 0.18[d]
6	10.0	7.0−7.5	60	1.86 ± 0.02[e]	45.97 ± 0.11[d]
7	5.0	8.0−8.5	60	1.40 ± 0.01[c]	67.63 ± 0.16[b]
8	10.0	8.0−8.5	60	2.48 ± 0[a]	61.47 ± 0[c]
9	7.5	7.5−8.0	45	1.66 ± 0.01[b]	54.32 ± 0.15[c]
10	7.5	7.5−8.0	45	1.69 ± 0[b]	55.01 ± 0[c]
11	7.5	7.5−8.0	45	1.68 ± 0[b]	54.66 ± 0[c]
12	7.5	7.5−8.0	45	1.69 ± 0[b]	55.01 ± 0[c]
13	7.5	7.5−8.0	45	1.69 ± 0[b]	55.01 ± 0[c]

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

% ACETYL = 0.4346 A + 0.2897 B + 0.04345 C + 0.5135 AB + 0.05268 AC − 0.0342 AC − 0.0342 BC + 1.6564

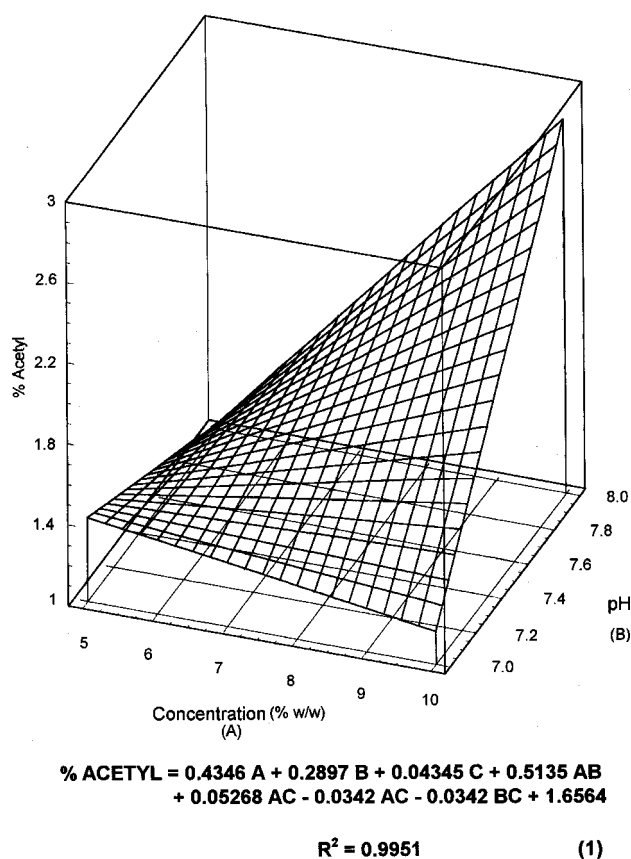**R^2 = 0.9951**   (1)

Figure 1. Surface response and mathematical model that illustrate the behavior of the acetyl groups (%) at 30 min of reaction time.

determined by using the Whistler and Paschall method (1967). Degree of substitution (D.S.) and reaction efficiency was determined according to Wuzburg (1964).

Functional Properties. Gelatinization temperature range was found according to the microscopic method of Watson (1964). The viscosity was determined with the method of Smith (1964) using a 2.5% starch suspension in a Brookfield viscometer LTV with spindle No. 2 at 30 °C and 100 rpm.

Retrogradation studies were performed according to the Tjahjadi and Breene (1984) method with 7% starch gels and kept in refrigeration (4 °C, 24 h). Retrogradation was measured as volume of water (mL) freed after centrifugation at 10 000 g (degree of syneresis).

Solubility and swelling patterns were determined using the Schoch (1964) method modified by Sathe and Salunkhe (1981).

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 acetyl groups content values (%) and reaction efficiencies obtained from the different treatments are shown in Table 1. As illustrated in the table, the best products (according to the highest amount of acetyl groups 2.5% allowed by the FDA in foods) were the ones obtained in treatments 3 and 4 in which starch was treated for minutes with 5% acetic anhydride at pH 8.0−8.5 and acetylated with 5% and 10% acetic anhydride, respectively. Although treatment 4 is statistically the same as treatment 8 ($P > 0.05$), the former was selected because less time of reaction was needed.

Acetylated starch from treatment 3 showed 1.5% acetyl groups, considered acceptable based on the reaction efficiency (72.48%). This value is slightly higher than the 70% reported by Jarowenko (1986). This investigator used acetic anhydride to modify granular starch in water. The former efficiency is statistically different ($P < 0.05$) from those reached with the other treatments. Intermediate levels of acetylation would allow the use of modified starch obtained in products that require very specific functional properties.

Nieto (1993) reported similar patterns in the acetylation reaction in the aqueous phase of *Xanthosoma violaceum* starch granules. This researcher noticed that pH and acetic anhydride concentration had influence over the acetyl groups bondings and reaction efficiency. Higher values were obtained with moderated acetic anhydride concentration (7.2%) at pH 8.0−8.4.

Regression analysis for each response variable was performed. It suggested that experimental data fit adequately to a first-order polynomial model ($P < 0.05$). The mathematical models and surface response illustrate the behavior of acetyl groups content (%) and reaction efficiency of the products obtained as a function of the amount of the modifying agent (A), pH (B), and reaction time (C) represented by eqs 1 and 2 and shown in Figures 1 and 2.

As illustrated, all the main factors and their double interactions show positive effects toward the acetyl content except for the interaction pH−time (Figure 1). This means that high levels of these factors increase the number of hydroxyl groups substituted. Regression coefficients for these equations showed the strongest effect of acetic anhydride concentration over the acetyl groups percent, followed by the impact caused by pH and the reaction time. Definitely, the acetylation reaction is favored by using high levels of these factors.

From Table 1, it can be noticed that a 72.48% efficiency was achieved with treatment 3 (5%/8.0−8.5/30 min). This value is higher than the one obtained with treatment 4 (10%/8.0−8.5/30 min), 58.09%. This was probably due to the higher acetic anhydride concentration, which induced the occurrence of hydrolysis reactions. These reactions are commonly found in these kinds of modifications. Regarding the efficiency (eq 2), it is noticeable that this value decreased as the acetic anhydride concentration increased. In addition, the efficiency was independent of the acetyl groups value achieved. However, pH and the pH−concentration combination were the factors that had the highest influence over the acetylation reaction efficiency.

Determination coefficients (R^2) show an excellent experimental result fitting to the mathematical models proposed to explain the acetylation reaction pattern.
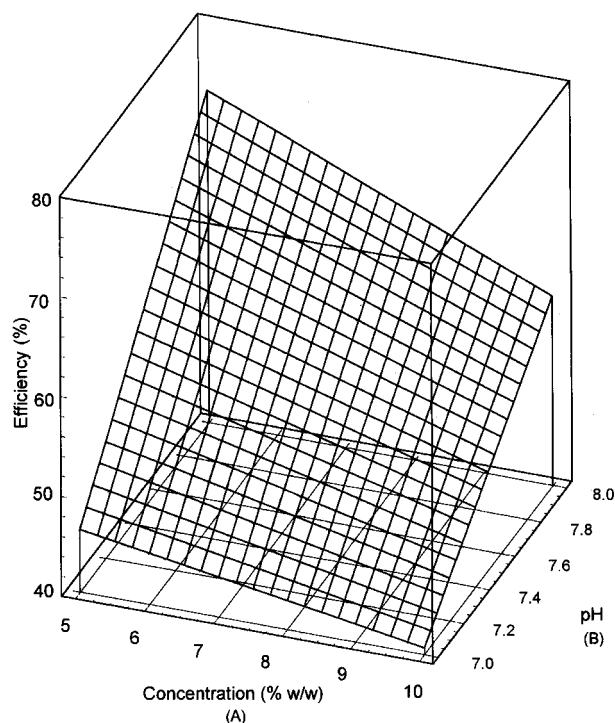
$$\text{EFFICIENCY (\%)} = -3.5789\ A + 9.9911\ B + 0.0965\ C - 1.5580\ AB$$
$$+ 1.4118\ AC - 1.3305\ BC + 0.6475\ ABC + 54.8770$$

$$R^2 = 0.9988 \qquad\qquad (2)$$

Figure 2. Surface response and mathematical model that illustrate the behavior of the efficiency (%) at 30 min of reaction time.

Table 2. Chemical Composition of Native and Acetylated Starch of *Canavalia* (% Dry Basis)

	starch		
component	native	acetylated treatment 3[a]	acetylated treatment 4[b]
moisture	(10.16 ± 0.09)	(5.08 ± 0.14)	(5.40 ± 0.08)
protein	0.34 ± 0.006	0.32 ± 0.008	0.32 ± 0.01
fat	0.14 ± 0.01	0.16 ± 0.01	0.17 ± 0.01
fiber	0.11 ± 0.02	0.17 ± 0.01	0.20 ± 0.02
starch	99.01 ± 0.14	99.19 ± 0.18	99.13 ± 0.13
apparent amylose	37.50 ± 0.25	37.96 ± 0.12	42.85 ± 0.14

[a] 5% acetic anhydride, pH 8.0−8.5, 30 min. [b] 10% acetic anhydride, pH 8.0−8.5, 30 min.

Chemical Composition. The acetylation reaction does not induce important changes in the derivatives composition produced according to the best treatments obtained compared with the native starch (Table 2). The only important fact is an ash content reduction detected due to the capability of the chemical agent to solubilize minerals during the reaction.

Low substitution degree starch acetates produce stable suspensions. However, the presence of some acetyl groups interferes with the regrouping of amylose and amylopectin fractions during the cooling of the starch molecules that have been subjected to gelatinization process (Whistler and Daniel, 1990). Based on this, the creation of more lineal segments eases the absorption of a higher amount of iodine, reflected in the apparent amylose content obtained, which depends on the number of acetyl groups incorporated.

Functional Properties. Functional properties of the native and modified starches are presented in Table 3. The results of gelatinization temperature show that these values are lower in acetylated derivates than in *Canavalia* native starches and diminished as the acetyl groups content increases. This characteristic is one of the many advantages achieved with acetylation. It allows the suggestion of employing these derivates in processes where a thickening agent must gel at lower temperatures, or simply to reduce energy costs during the manufacture of products where these derivates are used.

Starch acetylation normally reduce the viscosity of pastes (Jarowenko, 1986; Rutenberg and Solarek, 1984). However, this can be increased or reduced depending on the starch source and the sterification method (Agboola et al., 1991). In *Canavalia* acetylated starches this reaction induced an increase of viscosity as the number of linked acetyl groups increased. This result can be achieved by controlling the reaction conditions, particularly pH. This allows certain reduction of the secondary hydrolysis reactions and the incorporation of acetyl groups that facilitate the capture and retention of water molecules and the development of more organized structures, which show higher resistance to deformation. In a similar way, Sathe and Salunkhe (1981) report an increment of viscosity in *Phaseolus vulgaris* L. native starch pastes, 225 BU (Brabender units) up to 355 BU when acetylated with 10.2% acetic anhydride at pH 8.0 for 30 min. This pattern was also observed by Agboola et al. (1991) in the *Cassava* native starch acetylation with acetic anhydride and 1% sulfuric acid as the catalyzer. In this study, an increment of viscosity was found by comparing native starch with acetylated, giving 27 and 32 cP, respectively. The result is desirable, since it provides higher viscosities, clarity, and stability at low temperatures to the pastes manufactured with this starch.

The incorporation of acetyl groups drastically diminished and even eliminated syneresis problems found in gels prepared with 7% native starch. This phenomenon was relatively insignificant at higher degrees of substitution (D.S.). It gave better results than those presented by Pomeranz (1991) with corn acetylated starches. That suggests a total reduction of syneresis at 0.2 D.S. This was achieved with the acetylated *Canavalia* starch at 0.091 D.S.

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

	starch		
functional properties	native	acetylated treatment 3 (5%/8−8.5/30)	acetylated treatment 4 (10%/8−8.5/30)
% acetyl	0	1.50 ± 0.01	2.34 ± 0
D.S.	0	0.057 ± 0.002	0.091 ± 0
gelatinization temp (°C)[a]	(77−83) ± 0	(68−74) ± 0	(66−72) ± 1 0
apparent viscosity (cP)[b]	64.7 ± 0.3	84.26 ± 0.3	88.33 ± 0.2
retrogradation(mL/50 mL)[c]	9.8 ± 0.05	2.85 ± 0.05	0.0 ± 0

[a] Method of Watson (1964). [b] Measured to 2.5% starch suspension in a Brookfield viscometer, spindle number 2, 30 °C, and 100 rpm. [c] Water freed in gels prepared with 7% starch and kept at 4 °C for 24 h.
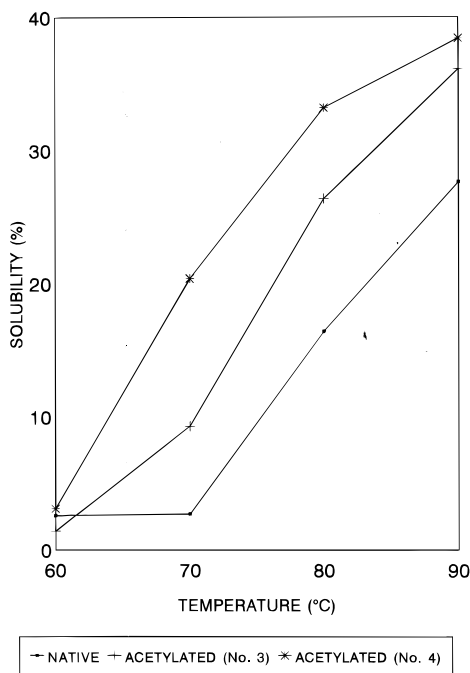
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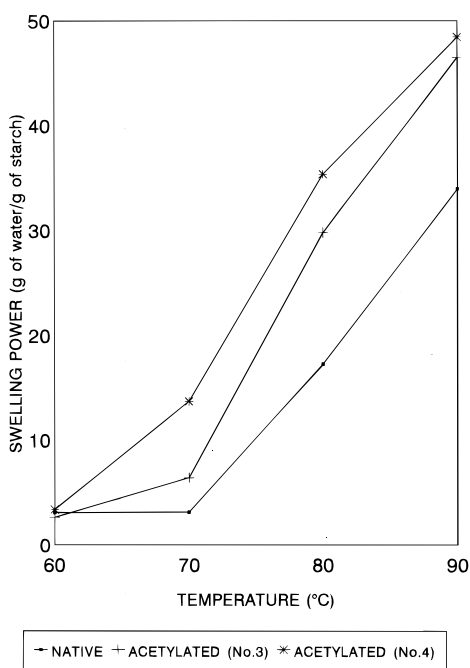**Figure 3.** Solubility of native and acetylated starch of *Canavalia ensiformis.*

Figure 4. Swelling power of native and acetylated starch of *Canavalia ensiformis.*

Acetylation considerably increased solubility (Figure 3) and the swelling power of the granule (Figure 4). These increases in solubility and swelling power confirm viscosity increment results, since better dispersion of starch in aqueous systems and water absorption were obtained. These facts can be explained by the introduction of hydrophilic substituting groups that allow the retention of water molecules because of their ability to form hydrogen bonds. This ensures high retention of water that enters the granule, increasing the swelling power and favoring gelatinization. Increasing swelling power favored the clarity of pastes and gels. This is a useful property in the manufacture of some confectionery products.

CONCLUSIONS

Incorporation of acetyl groups to *Canavalia ensiformis* native starch is widely favored by high acetic anhydride concentrations and pH 8.0−8.5. Acetylation induces gelatinization temperature reduction (66−72 °C). A 0.091 degree of substitution eliminates retrogradation and increases viscosity to 88.3 cP, solubility to 38.4%, and swelling power to 48.4 g/g of starch. These give pastes and gels a better thickening capacity, clarity, and stability at low temperatures and acidic pH. Owing to these properties, starch acetates might be used as thickening and stabilizing agents in ice creams, fruit jellies, baked products, sauces, and frozen foods.

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