

Cross-Linkage of *Canavalia ensiformis* Starch with Adipic Acid: Chemical and Functional Properties

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Adipic acid behavior was studied as a cross-linkage agent for *Canavalia ensiformis* native starch. A 2³ factorial design with two replicates of each treatment was utilized. Acid concentration (1 and 2%) and the chemical agent incorporation method (direct incorporation with an initial pH adjustment, direct addition with pH readjustment, and 3% NaOH simultaneous addition) were the factors utilized. Gel firmness and syneresis were used as response variables. Main factors and their interactions showed statistical influence ($P < 0.05$) over the cross-linkage level achieved; the most effective modification was obtained by reactant direct incorporation. Cross-linkage increased gel firmness (11.25 mm penetration) compared to native starch (14.75 mm penetration), diminished syneresis to 7.15 mL/50 mL, and reduced solubilization and swelling power to 18% and 28.04 g/g levels, respectively.

Keywords: *Cross-linked; starch; Canavalia ensiformis; adipic acid; functional properties*

INTRODUCTION

Starch is one of the most used raw materials in the food industry, but a limited number of vegetables are used for commercial production; corn is the main source worldwide (Wang et al., 1993).

Canavalia ensiformis, a legume known as jack bean, has its origins in western India (Thompson et al., 1986). The plant has an important agronomic potential under the climate and soil conditions of Yucatán, Mexico. Yields of 2580 kg/ha have been obtained in this area (Kessler et al., 1990). *Canavalia* seeds have been considered as food and feed due to their high contents of starch and protein: 36.7–50% and 29.7%, respectively (Kessler et al., 1990; Betancur et al., 1997). Moguel et al. (1996) developed a process to obtain starch and protein concentrates from *canavalia* seeds.

Despite the versatility of starches, some of the native ones have restrictions that limit their use at the industrial level, such as low tension resistance, thermic decomposition, high retrogradation, and syneresis (Fleche, 1985; Sívoli-Rodríguez and Pérez-Sira, 1996).

Modification of the starch by crossed-bond formation has been proposed to provide the pastes with more stability when overheated, more mechanical power, and pH changes, which are indispensable for food manufacture (Hoseney, 1986).

The cross-linkage reaction fortifies the starch granule structure through the natural bond reinforcement by the inter- and intramolecular chemical connection formation between the glucose and adjacent molecules that constitute starch (Yook et al., 1993; Wurzburg, 1986). Some of the chemical agents used in several studies include epichlorhydrin, phosphorus oxychloride, sodium trimetaphosphate, formaldehyde, and anhydride com-

binations (adipic acid and citric/acetic) (Kasemsuwan and Jane, 1993; Yook et al., 1993; Whistler and Daniel, 1990).

Amylose and amylopectine susceptibility studies on the addition of cross-linkage agents, particularly epichlorhydrin and adipic anhydride, indicate that the main linking places are located in the granule's amorphous region (Jane et al., 1992; An-Yeh and Su-Lang, 1993). This modification produces important changes in the starch functional properties. It increases or decreases the paste viscosity behavior depending of the derivation grade (Whistler and Daniel, 1990). This reaction produced reductions of the water absorption capacity, swelling power, and the derived starches' solubility compared to native starch (Pomeranz, 1991).

Cross-linked starches are employed mainly as thickening agents and stabilizers in baby food and high-acid food systems such as sauces and dressings for pizzas, spaghetti, jams, and pie fruit fillings (Luallen, 1985; Fleche, 1985). Due to their capacity to maintain a great amount of solids in suspension after cooking, these starches are essential in foods submitted to long periods of heating and/or agitation, such as bread-making products, glazed bread, aseptic processed products such as puddings and cheese sauces, preventing syneresis during storage (Cañizares, 1994; Whistler and Daniel, 1990).

The objective of this study was to determine the adipic acid behavior as a cross-linkage agent of *C. ensiformis* native starch, to evaluate the incorporation method and chemical agent concentration effects, and to develop the chemical and functional characterization compared to native starch.

MATERIALS AND METHODS

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

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Native Starch Extraction. Twenty-five kilograms of 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 levels, steeping the flour in an aqueous suspension (1:7 w/v) for 12 h at room temperature and pH 12.

Cross-Linkage of Starch. The adipic acid behavior as a cross-linkage agent in alkaline conditions was observed in a sample using a 2² bifactorial design with two replicates of each treatment. Two concentration levels (1 and 2%) and three adipic acid incorporation methods were evaluated.

Method I. Initial adjustment at pH 10 and adipic acid direct incorporation support crossed bond formation, according to Wurzburg (1986).

Method II. Initial adjustment at pH 10, adipic acid direct incorporation, and readjustment at pH 10 maintain the alkaline conditions that support the cross-linkage during the whole reaction, according to Wurzburg (1986).

Method III. Simultaneous addition of a 2% adipic acid aqueous solution and 3% NaOH maintained the pH between 9.8 and 10.2. To obtain acetylated-crossed starches, the Valera principle cited by Cañizares (1994) was followed.

A sample of 100 g of starch was prepared in a 40% aqueous suspension (w/w) and placed in a Grant JB-3 thermostatic bath at 50 °C with constant agitation at 300 rpm provided by a Caframo RZR1 mechanical agitator. Afterward, the Cole Parmer Digi-Sense potentiometer electrode was inserted and the pH was adjusted to 10 with 3% NaOH. The adipic acid was incorporated as indicated by the corresponding treatment and left to react for 90 min.

Later, the pH was adjusted to 7.0 with either 0.2 N HCl or 3% NaOH. Starch was recovered by vacuum filtration in a Büchner funnel using cloth as a filtrating medium and washed three times with 150 mL of distilled water. The product was dried at 60 °C in a Lab-line mechanical convection oven and milled in a Cyclotec mill until cross-linked starch could pass through a 20 mesh. Gel firmness and retrogradation at acid conditions (pH 3) were determined and used as response variables to evaluate the experiment.

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 and cross-linked starches obtained with the best treatment. These analyses were made according to the AOAC (1990) official procedures. The nitrogen was determined with a Kjeltex system (Tecator, Sweden). The protein was calculated as 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 measured 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 reported by Gilbert and Sprag (1964). The residual canavanine level was measured by using the Natelson and Bratton (1984) colorimetric method.

Functional Properties. Gelatinization initial temperature was determined according to the colorimetric method as reported by Cañizares et al. (1993).

The viscosity was determined according to 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.

Gel firmness was evaluated according to the Tjahjadi and Breene (1984) technique, with Koehler model K19500 penetration equipment using 7% starch gels.

Retrogradation studies were performed according to the Tjahjadi and Breene (1984) method with 7% starch gels adjusted to pH 3 with 0.1 N acetic acid and kept under 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 at 90 °C patterns were determined using the Schoch (1964) method as modified by Sathe and

Table 1. Gel Firmness and Retrogradation Values of *Canavalia* Cross-Linked Starches

treatment	method	concn (%)	gel firmness ^a (mm)	retrogradation ^b (mL/50 mL)
1	I	1	12.95 \pm 0.07 ^b	7.60 \pm 0.10 ^b
2	I	2	11.25 \pm 0.21 ^d	7.15 \pm 0.05 ^d
3	II	1	15.61 \pm 0.10 ^e	10.2 \pm 0.02 ^e
4	II	2	17.00 \pm 0.14 ^f	11.8 \pm 0.00 ^f
5	III	1	13.92 \pm 0.04 ^a	8.75 \pm 0.05 ^a
6	III	2	13.50 \pm 0.05 ^c	8.17 \pm 0.02 ^c
native starch			14.75 \pm 0.05 ^g	9.95 \pm 0.03 ^g

^a Determined with the Koehler equipment by cone penetration no. k19900 on a 7% starch gel and stored at 4 °C for 24 h. Different letters on the same column indicate significant difference ($P < 0.05$). ^b Volume of water separated from gels prepared with 7% starch, adjusted to pH 3 with acetic acid, and formed at 4 °C for 24 h. Different letters on the same column indicate significant difference ($P < 0.05$).

Salunkhe (1981). Starch paste characteristics were evaluated according to the Wiesenborn et al. (1994) method, using a Brabender viscoamylograph. The consistency, breakdown, and setback were determined according to the AACC (1983), method.

All chemical analysis and functional property determinations were made in triplicate.

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

RESULTS AND DISCUSSION

Native Starch Cross-Linkage. Variance analysis for gel firmness and retrogradation at pH 3 indicated that the incorporation method, adipic acid concentration, and the interaction of these factors had significant influence ($P < 0.05$) on the native starch modification, as shown in Table 1. Starches obtained with direct incorporation of 2% adipic acid form gels with 11.25 mm penetration and 7.15 mL/50 mL of water expelled; higher penetration (12.95 mm) and syneresis (7.60 mL/50 mL) were found for gels obtained from starches prepared with 1% of acid.

These values were smaller than those obtained from *C. ensiformis* native starch, with a 14.75 mm gel penetration and 9.95 mL/50 mL syneresis. On the other hand, with 2% adipic acid direct addition and pH readjustment, levels achieved were 17.0 mm and 11.8 mL/50 mL for gel firmness and syneresis, respectively, higher than those obtained by employing 1% acid (15.6 mm penetration and 10.2 mL/50 mL of expelled water). Meanwhile, with the simultaneous addition of NaOH and 2% acid, gels were obtained with 13.5 mm penetration and 8.17 mL/50 mL syneresis; these values are lower than the ones observed using 1% adipic acid (13.92 mm and 8.75 mL, respectively).

The gel firmness of the products increases, evaluated as the penetration and retrogradation reduction, which indicates granules with more stability and resistance to thermic, mechanic, and acid processes, which are fundamental characteristics of cross-linked starches. As cited by Yook et al. (1993), this indicates the starch fraction covalent bonds were reinforced by the chemical bond formation between the adjacent molecules that fortify the granule structure.

Mean comparison analysis indicated significant differences ($P < 0.05$) among all treatments (Table 1). The best treatment presented the highest gel firmness and lowest retrogradation at pH 3 by direct incorporation

Table 2. Native and Cross-Linked *Canavalia* Starch Functional Properties

treatment	method	concn (%)	viscosity ^a (cP)	solubility ^b (%)	swelling power ^b (g of water/g of starch)
1	I	1	68.6 ± 0.4	20.05 ± 0.15	39.39 ± 0.37
2	I	2	64.8 ± 0.0	18.08 ± 0.18	28.04 ± 0.18
3	II	1	60.6 ± 0.2	31.90 ± 0.35	35.54 ± 0.22
4	II	2	60.0 ± 0.0	32.65 ± 0.25	32.23 ± 0.19
5	III	1	71.1 ± 0.3	22.92 ± 0.10	33.56 ± 0.18
6	III	2	68.6 ± 0.2	22.65 ± 0.15	29.74 ± 0.20
native starch			64.7 ± 0.3	27.65 ± 0.35	33.92 ± 0.50

^a Measured at 2.5% of starch with a Brookfield viscometer, splin no. 2, at 30 °C and 100 rpm. ^b Determined at 90 °C.

of 1 and 2% adipic acid for the *Canavalia* native starch cross-linked modification.

The direct addition of adipic acid followed by pH readjustment to 10 suggests a starch soft hydrolysis, distinguished by a gel firmness and retrogradation decrease of the pastes and gels, as shown in Table 1.

Functional Properties. The results showed that adipic acid direct incorporation and simultaneous addition of 3% NaOH lead to cross-linkage, while incorporation with pH readjustment induces a light hydrolysis. The changes produced in the derivative's viscosity, solubility, and swelling power, compared to native starch, were evaluated to confirm those results.

Data shown in Table 2 indicate the cross-linkage reinforcement of the starch granule structure establishing both inter- and intramolecular bonds (Cañizares, 1994). This effect was reflected in the native starch solubility (27.65%) and swelling power (33.92 g/g) decrease to 18.08% and 28.04 g/g levels, respectively, in cross-linked starches prepared by adipic acid direct addition. These properties diminished as a function of the cross-linkage level: the highest modification showed the lowest solubility and swelling power.

As reported for native starches, low hydrolysis modification with HCl depolymerization (Wurzburg, 1986; Fleche, 1985; Radley, 1976) leads to starch pastes having a solubility increase and viscosity and swelling power decreases, which confirms that the treatment with adipic acid and pH readjustment induces native starch low hydrolysis, being lower than the one obtained with HCl. This is due to the fact that the adipic acid is a weak acid with a low ionization grade ($pK_{a1} = 4.42$ and $pK_{a2} = 5.41$) compared to HCl, which is a strong acid, considered totally ionized when reacting (Newton, 1973). With this method and compared to native starch, solubility increased from 27.65 to 32.65%, viscosity decreased from 64.7 to 60.0 cP, and swelling power was slightly reduced from 33.92 to 32.23 g/g (Table 2).

Wurzburg (1986) states that cross-linkage affects corn starch paste viscosity behavior, depending on the intensity. At low cross-linkage values (≈ 1 bond for each 1300 units of anhydride glucose) the starch dispersion viscosity increases. If the substitution level is moderated, the cross-linkage interferes with granule swelling and reduces the viscosity. This behavior is similar for derivatives obtained from starch (Table 2): viscosity values of 71.1 cP were obtained with 1% adipic acid, but the value decreased to 64.8 cP when 2% of the reactant was added.

Treatments with adipic acid did not influence the gelatinization initial temperature, which was the same for all of the derivatives obtained (77 °C) and similar to that of native starch. A similar effect was observed by Nieto (1993) when cross-linking malanga starch (*Xan-*

Table 3. Chemical Composition and Amylose Content of Native and Cross-Linked *Canavalia* Starches (DB %)

component	starch type	
	native	cross-linked
moisture	(10.16 ± 0.09)	(7.55 ± 0.10)
protein	0.34 ± 0.006	0.32 ± 0.01
fat	0.14 ± 0.01	0.17 ± 0.01
crude fiber	0.11 ± 0.02	0.11 ± 0.01
ash	0.40 ± 0.01	0.18 ± 0.01
starch	99.01 ± 0.14	99.13 ± 0.13
apparent amylose	37.50 ± 0.25	50.16 ± 0.14
canavanine	0.047 ± 0.004	0.024 ± 0.001

thosoma violaceum) with 0.1% phosphorus oxychloride at pH 10.5 for 60 min: he obtained a gelatinization initial temperature of 79 °C, the same for this tuber native starch. This behavior has been reported by Sívoli-Rodríguez and Pérez-Sira (1996) and Hosney (1986), indicating that at low modification levels, corn cross-linked starches developed high viscosity, affecting very little the gelatinization initial temperature. However, this is as a function of the incorporated bonds. Whistler and Daniel (1990) reported that corn cross-linked starch gelatinization temperature rank increased as the cross-linkage grade did, due to the formed chemical bonds' interference with the swelling and therefore with the gelatinization process.

Chemical Composition. The chemical composition of *canavalia* native and cross-linked starches (Table 3) prepared with the best treatment (number 2) indicated that *Canavalia* native starch presented a high purity grade (99.01%), estimating the nitrogen-free extract content as starch, due to the fact that the chemical component levels grouped as impurities are <1%. Its low protein content (0.34%) makes it industrially useful for syrup elaboration with a high glucose content. This protein level is <0.35%, the standard indicated by the FDA for corn starches to avoid the undesirable formation of dark syrups as a result of the Maillard reaction during the process (Zajac, 1989). The apparent amylose content (37.5%) is high, compared to that of some common cereal native starches such as corn (28%), wheat (28%), rice (17%), and sorghum (28%) as well as tubers such as cassava (17%) and potato (21%) (Swinkels, 1985) and a bit higher than the ones indicated by Gujska et al. (1994) for legumes such as pinto bean (32.2%), navy bean (32.1%), and field pea (34.2%) but lower than the ones reported by Biliaderis et al. (1981) for some legumes with values between 55 and 65%. The cross-linkage reaction did not induce important changes in the obtained derivatives' composition; the only outstanding fact is the ash content reduction due to the chemical agent's capability to solubilize minerals during the reaction. Agboola et al. (1991) reported a similar reduction of the ash content by the effect of the acetic acid and HCl treatment used in the cassava starch modification.

The presence of some crossed bonds interferes with the reassociation of amylose and amylopectin fractions during the cooling of starch molecules that have been submitted to a gelatinization process (Wurzburg, 1986; Pomeranz, 1991; Whistler and Daniel, 1990); this facilitates the absorption of a great amount of iodine and can explain the apparent amylose increase in the cross-linked starch compared to native starch.

The native starch extraction process induced a considerable reduction of the 33% canavanine level present in the *canavalia* flour: 0.047% in the recovered native

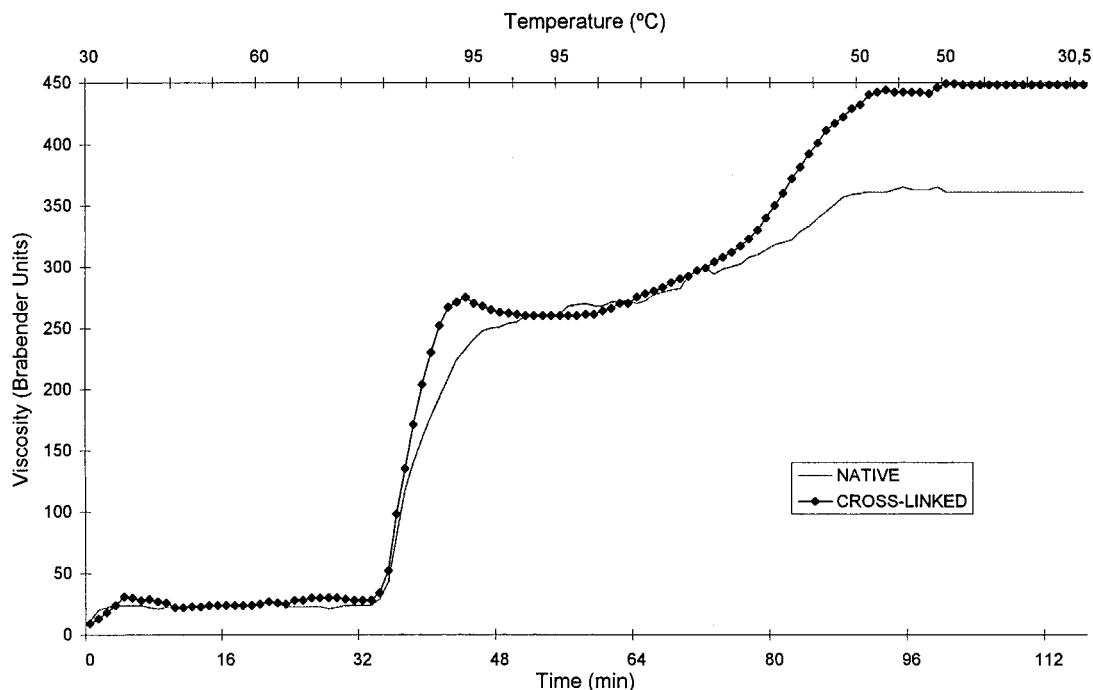


Figure 1. Brabender pasting curves for native and cross-linked canavalia starches.

Table 4. Native and Cross-Linked *Canavalia* Starch Paste Characteristics

parameter	starch type	
	native	cross-linked
gelatinization initial temp (°C)	82.0	82.5
peak viscosity (UB)	260	274
viscosity at 95 °C (UB)	240	254
viscosity at 95 °C × 15 min (UB)	260	262
viscosity at 50 °C (UB)	354	420
viscosity at 50 °C × 15 min (UB)	364	444
consistency ^a	94	146
breakdown ^b	0	12
setback ^c	94	158

^a Consistency: viscosity at 50 °C (UB) – peak viscosity (UB).

^b Breakdown: peak viscosity (UB) – viscosity at 95 °C × 15 min (UB). ^c Setback: viscosity at 50 °C (UB) – viscosity at 95 °C × 15 min (UB).

starch and decreasing more in the HCl treatments (0–0.205%) compared to acetylation (0.025%) (Betancur, 1996). Since canavanine has an LD₅₀ of 5530 mg/kg (Ramírez, 1985) and it is found naturally in red onion at 0.53% level (Sánchez et al., 1987) and in alfalfa at 1–1.5% (Natelson and Bratton, 1984), the canavanine levels in the obtained starches are insignificant and allow their incorporation in food elaboration without risk.

Paste Characterization. From the amylogram interpretation, the canavalia native and cross-linked starches' cooking characteristics were obtained for the best treatment (Table 4). As indicated before, the gelatinization initial temperature did not vary by the effect of the modification: 82 °C for the Brabender viscoamylograph method, compared to the Cañizares et al. (1993) colorimetric method value of 77 °C. This variation was due to the different operation conditions and principles on which these techniques are based. As shown in Figure 1, native and modified starches had similar behaviors, indicating that the number of crossed bonds incorporated was low and did not present a maximum gelatinization peak. However, there was a maximum viscosity increase as a treatment effect,

compared to native starch pastes prepared at the same concentration. Similar behaviors have been reported by Sívoli-Rodríguez and Pérez-Sira (1996) for corn cross-linked starch with sodium tripolyphosphate and by An-Yeh and Su-Lang (1993) for modified corn and rice starches with POCl₃. Consistency also was affected with increasing modification, as well as fragility or breakdown, although in less proportion. The setback values indicate canavalia cross-linked starch had more stability than native starch, which presented good resistance to heating–cooling processes. With these properties, canavalia cross-linked starch pastes present a great granule stability, contributing to the product's structures to be incorporated; as a consequence of the paste, less mixing effort is required, which may increase the product's shelf life. It should also be mentioned that pastes were capable of forming very transparent and translucent plastic films when dried.

Conclusions. Cross-linked starches were obtained with appropriate functional properties for incorporation into food formulations. The *Canavalia* natural starch cross-linkage can be developed adequately using adipic acid as modifying agent. The obtention of these derivatives depends on the incorporation method and the reactant concentration; adipic acid direct addition was the easiest and most effective method for this modification. The cross-linkage provided canavalia native starch with more granule stability than starch subjected to thermic, mechanical, and acid treatments; it decreased solubility to 18.08% and swelling power to 28.04 g/g. The method reduced syneresis and gave more gel firmness compared to native starch. Canavanine levels were also diminished to 0.025%, allowing its inclusion in food without risk.

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