

Properties of maize (amidex) starch crosslinked by pregelatinisation with sodium trimetaphosphate: i. spectral and thermogravimetric characteristics

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Abstract

The moisture content of maize starch (native Amidex 3001 starch) was determined. The starch was then cross-linked with sodium trimetaphosphate by gelatinisation at 90°C for 45 min. The resulting slurry was treated subsequently with varying amounts (0, 0.4, 0.8 and 1.2 g) of sodium trimetaphosphate, and sodium sulphate at pH 11. Spectral properties (¹³CNMR and Infra red) of the crosslinked and native starches were studied using relevant standard procedures. Thermographic analysis (TGA) were carried out on the native starch as well as the starch cross-linked with 0.4 g of STMP using a Perkin Elmer TGA 7 Analyzer (Perkin Elmer, USA). The ¹³CNMR spectroscopy showed that cross-linking resulted to a reduction in the chemical shifts of the starch. Cross-linking introduced more peaks in the IR spectra due to the incorporated phosphoryl groups. The results of the thermogravimetric analysis of the starches showed that cross-linking reduced the total organic matter present, while increasing the peak degradation temperature and total inorganic matters.

Keywords: ¹³CNMR, Infra red, Mass spectra, thermogravimetry, maize starch

Introduction

Starches are carbohydrate polymeric substances, synthesized within the plant by the chemical interlinking of hundreds and thousands of individual glucose units to form long-chain molecules (1, 2). Treatment with acid or enzymes can break the starch down to its constituent glucose molecules. Starches form the major food reserve in plants especially in the seeds and tubers, sustaining them through winter dormancy and nurturing the new plant during germination (3).

Starches have found wide uses in various segments of the industry especially agricultural, pharmaceutical, food and chemical sectors and is reportedly the most important hydrocolloid both on a weight and money basis (4).

Corn starch is the cheapest of the common starches and enjoys a huge market where cost is the prime consideration. When corn starch is heated in water, the solution is cloudy, noncohesive, and upon cooling gels or sets back to form viscous, relatively short, opaque pastes. Its pastes however have a pronounced tendency to retrograde under freeze-thaw conditions (4).

It is possible to structurally modify starch to improve its functionality (5 - 7). Such modifications impart new properties, improve some of the inherent properties, or repress and modify some of their other properties. Properties of starch that are important during

Processing are gelatinization (pasting) temperature, rate of thickening, time and temperature of maximum viscosity, and general flow properties.

Several techniques have been employed in modification of starches including heating, hydrolysis (acid or alkali), oxidation, addition of chemicals that will result in the introduction of new chemical groups and/or changes in the size, shape, and structure of the starch molecules, extrusion-cooking and cross-linking (8 - 11).

Cross-linking as one of the methods of starch modification involves re-inforcing the hydrogen bonds on the granules with chemical bonds that act as bridges between the starch molecules. As a result, the cross-linked starches are resistant to high temperature, low pH, high shear, and have improved viscosity and textural properties of the native starch (12). Cross-linking can also be accomplished by adding multifunctional reagents capable of forming ether or ester linkages with the hydroxyl groups in the starch (13).

A number of bifunctional reagents (cross-linkers) have been used in the past to cross-link various starches with varied degrees of success (14). These agents include monosodium phosphate, sodium trimetaphosphate, formaldehyde, organic dihalides, sodium tripolyphosphate, epichlorohydrin, phosphoryl chloride, adipic/acetic anhydride mixtures and succinic anhydride/vinyl acetate mixture. These cross-bonding reactions apparently take place on or near the surface of the granules, and although the degree of cross-linking is very low - approximately one cross-link per 200-1000 glucose units, the effect on the dispersion characteristics are quite pronounced (14).

Sodium trimetaphosphate has earlier been employed as a non-toxic cross-linker in the cross-linking of several starches including cassava starch and wheat starch (15 - 17).

In this present study, a commercial product of corn starch, Amidex 3001, was modified by cross-linking with sodium trimetaphosphate using pregelatinisation and the spectral as well as thermogravimetric properties of the cross-linked starches were evaluated.

Materials and methods

Materials

The following reagents were employed in the study: ethanol, sodium chloride, sodium hydroxide and hydrochloric acid all from Vetec, Industria Brasileira, Brasil; sodium trimetaphosphate (Sigma, England) and sodium sulphate (Merck, Darmstadt). Amidex 3001 was kindly donated by Corn Pds, Brazil.

Moisture content of native starch

Moisture content of the starch samples was determined by the AOAC 1995 method (18). A 1 g quantity of the native Amidex 3001 was dried in the oven (Temp-Therma, Brazil) at 105°C for 3 h. The weight of the sample compared to the original weight was noted. The heating was continued and the weights measured at hourly intervals until constant. Three replicate determinations were taken.

Cross-linking

A slight modification of the method of Woo (1999) (19) was employed. A 3% dispersion of the Amidex 3001 was pregelatinised by heating in a three-hole reflux condenser at 90°C for 45 min. with constant stirring using a Bayer stirring apparatus. The resulting slurry was cooled, and sodium trimetaphosphate, STMP (0.4 g) with sodium sulphate (4 g) added. The

pH was adjusted to 11 with of 0.1 M sodium hydroxide and the slurry stirred at 40°C for 3 h using a magnetic stirring set up (Corinho, Brasil). The pH was then re-adjusted to 6.5 with 1 M HCl. The slurry was washed with de-ionised water (4 x 300 ml) to remove all traces of unreacted salts. The cross-linked starch was then precipitated with ethanol (1 : 5), dried for 24 h in an oven (Gallenkamp, England) at 60°C, and pulverized. The process was repeated for 0.8 and 1.2 g concentrations of STMP.

Moisture content of cross-linked starch

The moisture content of the cross-linked starch was determined in triplicates as earlier described.

IR spectroscopy

A 2 % dispersion of the cross-linked starch was prepared by heating in de-ionised water at 80°C for 30 min. These were subjected to IR spectroscopic studies using the Perkin Elmer, IR equipment (England) using water/AgBr system. All samples were scanned in the IR range from 400 - 4000 cm⁻¹.

¹³CNMR spectroscopy

¹³CNMR spectrum was obtained in D₂O solution at an Amidex starch concentration of 30 g/L with a Varian spectrometer, Model Mercury 200 (Palo Alto, CA) operating at a ¹³C frequency of 75.4 MHz. The experiment was performed in the Fourier-transform mode. Free-induction decays were accumulated with a 90° pulse and a repetition time of 1 s. Spectra were recorded at probe ambient temperature under conditions of high power hydrogen decoupling, given by a field frequency lock of 7.4 KHz. The ¹³CNMR spectra were referenced to the chemical shift of the methyl carbons of sodium 3-trimethyl silyl proportionate (TMSP) (Cambridge Inc, USA) (0). Peak areas were measured after amplifications of the C₁ (104 - 101), C₄ (81 - 78.5), and C₆ (70.5 - 68.5) regions. The spectra were carried out for the dispersions containing 0 and 1.2 g of STMP.

Thermogravimetric analysis

Thermographic analysis (TGA) was carried out on the native starch as well as the cross-linked starch.. A 6 mg quantity of each sample was used for the analysis in a Perkin Elmer TGA 7 Analyzer (Perkin Elmer, USA). The samples were heated in the temperature range of 30 - 700°C.

Results and discussion

Crosslinking by pregelatinisation

The cross-linking processes yielded the following percentages of the native starch: 61.75, 75.94, 72.28 and 68.75 for the starches cross-linked with 0, 0.4, 0.8 and 1.2 g of STMP respectively. The determined moisture contents of the cross-linked starch was: 9.99, 8.49, 10.11 and 7.85 for the native starch and starches cross-linked with 0.4, 0.8 and 1.2 g of STMP respectively. The percentage concentration of the pregelatinized non cross-linked starch was found to be 6.58.

STMP is reported to be an effective cross-linking agent at high temperature with semidry

starch and at warm temperature with hydrated starch in an aqueous slurry (15). Cross-linking with STMP after gelatinisation would yield a greater cross-linking efficiency since the starch molecules would be sufficiently opened up for easier attachment of the cross-linking molecules. The pH of 11 used also favours the cross-linking reaction, as Kasemsuwan and Jane (20) have shown that cross-linking of starch molecules to give a distarch monophosphate is favoured by alkalinity above 10. Increasing the alkalinity in the cross-linking medium increases starch anion concentration. Kitamura et al (21) have also indicated that ionization of amylose in alkali at pH 11 has been detected by changes in the polarization of fluorescein-substituted amylose, intrinsic viscosity, and optical rotation. Wu and Seib (14) have equally shown that cross-linking of starch molecules especially to give distarch monophosphate is also favoured by the presence of a neutral sodium salt. Otherwise, monostarch monophosphate esters are formed. Increasing sodium ion concentration increases both the ionic strength of the reaction medium and uptake of alkali by starch.

The presence of sodium sulphate in the reaction mixture, according to Rutenberg and Solarek (13) enhances cross-linking as it builds water structure and allows deeper penetration of the cross-linking agent into the granules. Another possible explanation is that ionic strength promotes reaction between a starch alkoxide ion and an ionic phosphoryl reactant (22). Reactions between two negatively charged species are expected to increase with ionic strength (23, 24). Moreover, ionization of weak acids (starch hydroxyls) is also promoted by ionic strength. Finally, sodium ions increase the alkali adsorbed by starch granules (25), which has been attributed to the ion-exchange properties of starch (26).

Generally, cross-linking increased the overall hardness of the starch granules. This was evidenced in the difficulty encountered in trying to pulverise the cross-linked starches as opposed to the non-cross-linked forms. The degree of difficulty increased with increasing concentration of the cross linking agent - sodium trimetaphosphate. The increased strength of granules is attributable to the reinforcement of the granule integrity due to the introduced cross-linking bonds.

¹³CNMR spectroscopy

Figures 1 and 2 show the ¹³CNMR spectra of the non cross-linked starch as well as the starch cross-linked with 1.2 g of STMP. The assignments of the lines are those reported by Kalinowski et al (27) and Rosenthal et al (28). The ¹³CNMR spectra of the non- cross linked corn starch and the probe cross-linked starch agreed well with those of Kalinowski et al and Rosenthal et al. Almost all the different carbon lines are resolved and their chemical shifts are recorded in Table 1. The starches showed distinct similar absorption peaks at 58 - 64, 70 - 73, 74 - 75, 76 - 78 and 98 - 103 bands. There was however a slight general decrease in the ppm of the respective chemical shifts in the spectra of the starch cross-linked with 1.2 g of STMP. This is most probably as a result of the minor substitutions arising from the incorporated phosphoryl groups that formed the cross-links.

Kalinowski et al had posited that because of the complexity of biopolymers ¹³CNMR spectra, complete interpretation is possible only in a few cases. Low solubility of biopolymers is also a limiting factor in recording ¹³CNMR spectra of polymeric substances. NMR studies of biopolymers are not in principle different from those of low molecular weight substances. Nonetheless, owing to the limited molecular motion in high molecular weight molecules, a dipolar broadening of the resonances is observed, which can amount to

more than 10 KHz in proton spectra, but is not observable in ^{13}C spectra because of its smaller gyromagnetic ratio. This fact, combined with the larger chemical shift range of the in ^{13}C nuclide, and the possibility of recording spectra with ^1H broad band decoupling, makes ^{13}C NMR spectroscopy especially suitable for investigating polymer structure and microstructure, which determines the physical properties of the molecules.

IR spectroscopy

Figures 3 - 6 show the IR spectra of the cross-linked and non-cross-linked starches. The figures show that there was an increase in the number of peaks from 18 in the non cross-

Table 1: Assignment of peaks for the ^{13}C NMR spectra of Amidex starch

Sample	Chemical shifts ^a					
	C-1	C-2	C-3	C-4	C-5	C-6
Non cross-linked Amidex starch	102.24	76.04	74.23	79.28	73.77	63.10
Cross-linked Amidex starch (Cl-1.2)	99.56	73.38	71.55	76.62	71.10	60.40

linked starch to 37, and 42 for the starches cross-linked with 0.8 g and 1.2 g of STMP respectively. The additional peaks are presumably due to the added phosphoryl groups during the cross-linking process, the number of which increased as the amount of STMP added, increased.

Thermogravimetric analysis

The results of the thermogravimetric analysis of the non crosslinked starch and starch cross-linked with 0.4 g of STMP are presented in Table 2. The loss of matter, mostly water, from the samples was continuous in the cross-linked starches up till the onset of degradation. The moisture content of the starches as determined from the TGA decreased with cross-linking. The temperature for the onset of degradation was slightly higher in the non-modified starch. This difference was however not significant enough and can be attributed to instrumental error. Cross-linking increased the peak degradation temperature i.e. the temperature of maximal velocity of degradation from 327.81°C in the non-modified starch to 332.10 in the starch cross-linked with 0.4 g of STMP. Cross-linking also increased the inorganic matter content of the starch while decreasing the total organic matter. The percentage organic matter content of the starch cross-linked with 0.4 g of STMP was 84.50 as against 87.38 for the non-modified starch. The presence of incorporated phosphorous as a result of the process of cross-linking could account for this increased inorganic matter content.

Conclusion

Amidex starch was cross-linked with varying amounts of sodium trimetaphosphate. The cross-linking affected the spectral and thermogravimetric characteristics of the starch. From the ^{13}C NMR spectroscopy, cross-linking resulted to a reduction in the chemical shifts of the starch. From the IR spectral analysis, cross-linking introduced more peaks due probably to the incorporated phosphoryl groups. The thermogravimetric analysis of the starches showed

Table 2 - Results of the thermogravimetric analysis on native Amidex starch and Amidex starch cross-linked with 0.4 g of STMP

Sample	Moisture content (%)	On-set of degradation temperature (°C)	Peak degradation temperature (°C)	Total amount of organic matter (&)	Total amount of inorganic matter (%)
Cl-0	13.22	307.70	327.81	87.38	12.62
Cl-0.4	11.24	304.80	332.10	84.50	15.50

Cl-0 Non-modified starch; Cl-0.4 Starch crosslinked with 0.4 g of STMP

that cross-linking reduced the total organic matter present, while increasing the peak degradation temperature and total inorganic matters.

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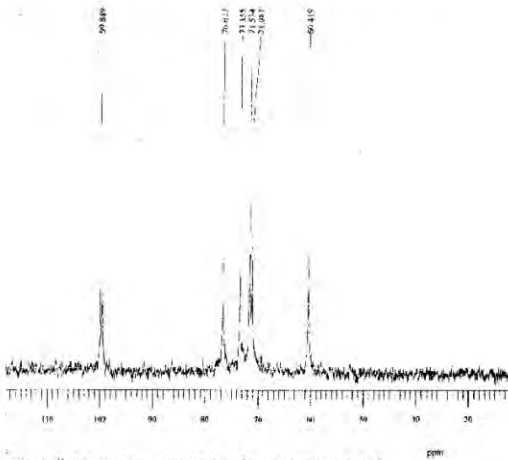


Fig. 1: ^{13}C NMR spectra (75 MHz) of a solution (60 mg/ml) in D_2O (at pK7 and 90° with k transients) of unmodified Amidex[®] starch with ^1H broad-band decoupling (in D_2O , 4 KHz region)

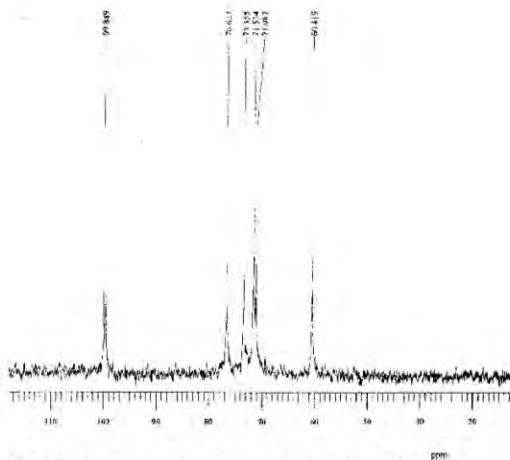


Fig. 2: ^{13}C NMR spectra (75 MHz) of a solution (60 mg/ml) in D_2O (at pK7 and 90° with k transients) of Amidex[®] starch crosslinked with 1.2 g of STMP, with ^1H broad-band decoupling (in D_2O , 4 KHz region)

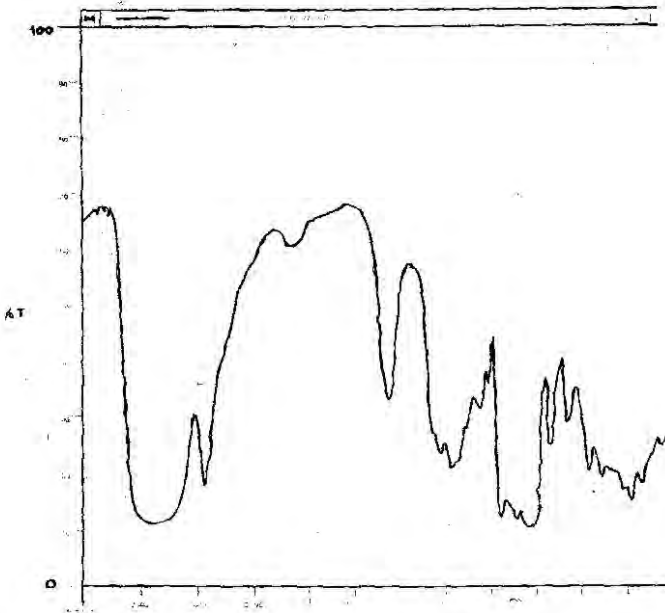


Fig. 3 Infra red spectra (H₂O/AgBr) of native Amidex starch.

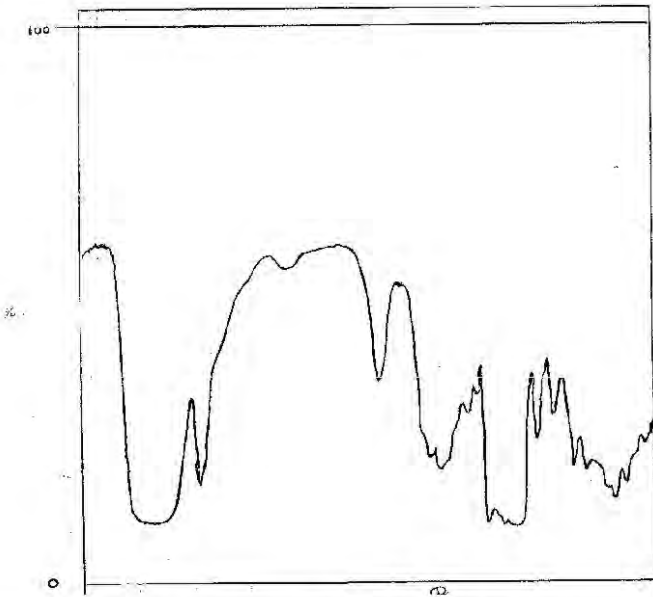


Fig. 4 Infra red spectra (H₂O/AgBr) of Amidex starch cross linked with 0.4 g of sodium trimetaphosphate

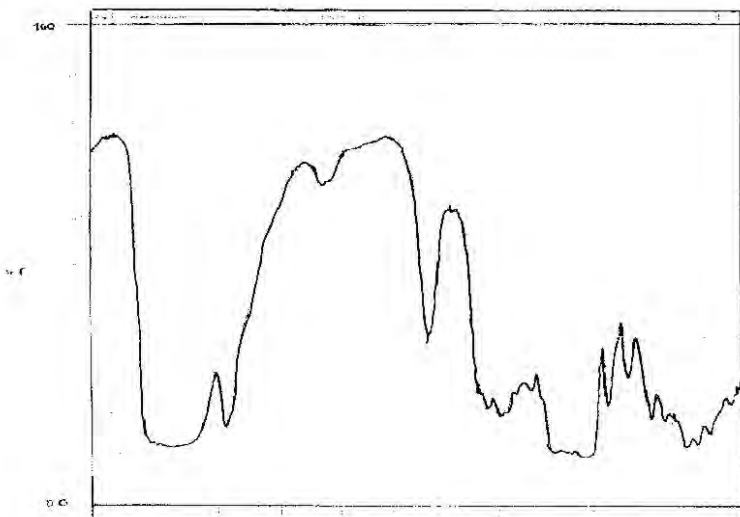


Fig. 5 Infra red spectra ($\text{H}_2\text{O}/\text{AgBr}$) of Amidex starch cross linked with 0.8 g of sodium trimetaphosphate

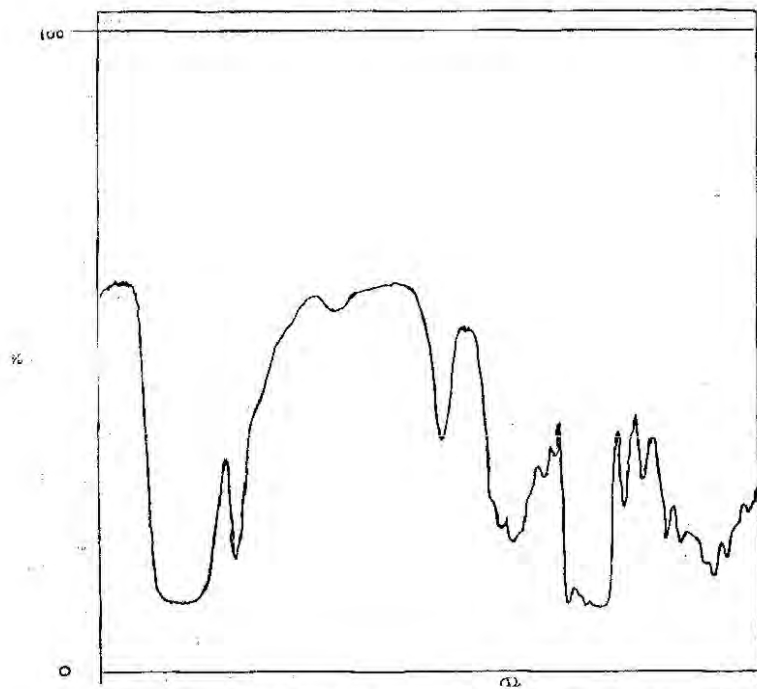


Fig. 6 Infra red spectra ($\text{H}_2\text{O}/\text{AgBr}$) of Amidex starch cross linked with 1.2 g of sodium trimetaphosphate.