

Determination of the maximum water solubility of eight native starches and the solubility of their acidic-methanol and -ethanol modified analogues

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Abstract—The maximum water solubilities of eight native starches from potato, shoti, tapioca, maize, waxy maize, amylo maize-7, wheat, and rice and their acid-methanol and acid-ethanol modified analogues have been determined. Maximum solubilities of 18.7 and 17.4 mg/mL were obtained for waxy maize and tapioca and 12.4 mg/mL for potato and maize starches by autoclaving 220 mg/10 mL at 121 °C; 8.7 mg/mL was obtained for shoti starch by stirring in 85:15 (v/v) Me₂SO–H₂O at 20 °C; and 7.0 and 5.2 mg/mL for rice and amylo maize-7 starches by stirring in 1 M NaOH at 20 °C. The acid-alcohol treated starches were 4–9 times more soluble than their native starches. The compositions of the solubilized starches had, in general, much higher ratios of amylose to amylopectin than the ratios in their native granules. A major exception to this was the acid-methanol treated potato, shoti, and rice starches that had much lower ratios of amylose to amylopectin than the ratios in their granules.

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1. Introduction

Starch is the reserve substance of the energy of sunlight. It is widely distributed in higher plants, appearing in green leaves, stems, tubers, cereal grains, seeds, nuts, and fruits. Starches provide the major caloric intake in the diets of non-photosynthesizing organisms and are increasingly being used industrially as a biorenewable material to be used for the production of various products, such as ethanol, cyclomaltodextrins, maltodextrins, fructose syrups, and so forth.

Starch is composed of two glucans, a linear α -(1→4) linked glucan, amylose, and an α -(1→4) linked glucan with 5% α -(1→6) branch linkages, amylopectin. The mixture can have varying amounts of amylose (15–

30%) and amylopectin (85–70%) with the exception of high amylose starches that have 53–70% amylose and 47–30% amylopectin, respectively, and the so-called waxy starches that have 100% amylopectin.¹ Amylopectin is the larger of the two molecules with 10⁴–10⁵ D-glucose units per molecule and amylose with 250–5000 D-glucose units.¹ Amylopectin can also vary in the distribution and clustering of the branch linkages and the lengths of the chains between branch linkages and the lengths of the branch chains themselves.² The two types of α -glucans are combined together into a water-insoluble granule that varies in size and shape, depending on their biological source.³

Starch granules from various sources also vary in the amounts of secondary (helical and double helical chains) and tertiary structures (inter- and intra-molecular chain associations), which hold the molecules together and give the insoluble character to the starch granules. These differences are manifested by different kinds of X-ray

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diffraction patterns, A, B, and C for the various starches;⁴ different susceptibilities to hydrolysis by α -amylases⁵ and glucoamylase;⁶ different swelling properties and gelatinization temperatures.⁷

Because of the presence of secondary and tertiary structures in the starch granules, the preparation of water-soluble solutions presents special problems. Three general methods are most often used to solubilize starch granules: (1) by pouring a water slurry of the starch granules into boiling water or by autoclaving a suspension of the granules at 121 °C (high temperature gelatinization); (2) by stirring a suspension of the granules in 1 M NaOH, followed by dilution and neutralization (a chemical or cold gelatinization method); and (3) by stirring or shaking a starch suspension in aqueous dimethylsulfoxide (Me₂SO)–water solutions of 90:10 or 85:15, followed by dilution with water to give 10–15% Me₂SO (a second chemical or cold gelatinization method).

Because the water solubility of starches has not been systematically studied and reported previously, we have determined and compared in this study the water solubilities of eight relatively common kinds of starches, solubilized by the three methods mentioned above. It was found that the maximum degree of solubility varied for the different kinds of starches. Solubility is defined here, as the maximum amount of starch that can be obtained in a specific volume of water. The eight kinds of starches were chosen for (a) their being a major food starch (e.g., potato, rice, wheat, maize and tapioca starches), (b) their ease of gelatinization (e.g., potato, shoti, and tapioca starches), (c) their unusual composition of amylose and amylopectin (e.g., amylo maize-7 starch with 70% amylose and waxy maize starch with 100% amylopectin), (d) their differences in their type of X-ray patterns (e.g., A-type for maize, wheat, and waxy maize starches; B-type for potato, amylo maize-7, and shoti starches; and C-type for tapioca starch), and (e) the somewhat exotic character of shoti starch with its unusual flat-plate granule morphology and its relatively wide use in Asia as a therapeutic agent for stomach and intestinal problems.

We also report on the comparison of the solubility of the autoclaved starch analogues that were obtained by treating the eight starches each with 0.36% (w/v) HCl in anhydrous methanol and anhydrous ethanol. These treatments give modified starches that are more soluble than their native starches.

2. Experimental

2.1. Materials

2.1.1. Starches. Potato starch was obtained from Penford Products Co. (Cedar Rapids, IA); mature maize

was obtained from Archer Daniel Midland Co. (Clinton, IA); mature waxy maize, amylo maize-7, rice, and tapioca starches were obtained from National Starch and Chemical Co. (Bridgewater, NJ); and mature wheat starch was isolated from wheat berries in our laboratory, using a standard procedure.⁸ Shoti starch was isolated in our laboratory from mature *Curcuma zedoaria* tubers obtained from Dr. M. Kitaoka, National Food Research Institute, Tsukuba, Japan, using a standard procedure for isolating potato starch from potato tubers.⁹

2.2. Methods

2.2.1. Acid–alcohol modifications of the starches. The eight starches were modified in methanol and ethanol as previously described.¹⁰ Starches (25 g) with 10–15% (w/w) water were suspended in 100 mL of the anhydrous alcohol (methanol or ethanol); 1.0 mL of concentrated HCl (36%, w/v) was added with mixing and the modification was allowed to go at 20 °C for 72 h. The starches were filtered and washed with 70% (v/v) ethanol until neutral to pH paper and then air-dried at 20 °C for 48 h.

2.2.2. Solubilization and analysis of the native starches. The native starch granules were solubilized by three methods: (1) The starch granules (110 mg) were suspended in 8 mL of water and autoclaved at 121 °C for 30 min, cooled, diluted to 10 mL and centrifuged at 10,000g for 20 min at 20 °C. The supernatants were removed and diluted to 10.0 mL and 4-vol of ethanol were added to precipitate the solubilized starch, which was centrifuged. The supernatants were removed and the two precipitates, the starch that did not go into solution and the solubilized starch that was precipitated with the 4-vol of ethanol, were dried by treating them 4 times with 1 mL of acetone and 1-time with 1 mL of ethanol, followed by drying in a vacuum oven at 40 °C for 10–15 h. (2) The starch granules were suspended in 1 mL of 1 M NaOH, which was stirred for 15 h at 20 °C, diluted to 7 mL with water, neutralized with 1 M HCl, diluted to 10.0 mL, and centrifuged at 10,000g for 20 min. The solubilized starches were precipitated and treated as in (1) above. (3) The starch granules were stirred or shaken in 1 mL of 85:15 (v/v) Me₂SO–H₂O at 20 °C for 8 h and then carefully diluted to 10.0 mL with H₂O, mixed, and centrifuged. The solubilized starches were precipitated and treated as in (1) above. The fractions of starches that were not solubilized by the three methods were also dehydrated with acetone and ethanol, dried, and weighed, as described in (1) above.

2.2.3. Solubilization and analysis of the acid–alcohol modified starches. The acid–alcohol modified starch granules (110, 220, 330, and 550 mg) were solubilized

by autoclaving as described for the native starches in (1) above and the precipitated solubilized starches were treated in the same way, but with 1, 2, 3, and 5 mL of acetone and ethanol, for the different amounts of the starches, respectively. The supernatants from the precipitated starches were concentrated to dryness in a Savant Speed Vac Concentrator (SVC100H) and the resulting solid materials were treated 4 times with 1 mL of acetone and once with 1 mL of ethanol, dried, and weighed. The total amount of solubilized starch was the amounts precipitated and found in the dried supernatants.

2.2.4. Fractionation and analysis of the solubilized starches. The starches that were solubilized and then precipitated with 4-vol of ethanol (~100 mg) were dissolved in 10 mL of water and 0.35 mL of 10% thymol in ethanol was added to precipitate the amylose component,¹¹ which was removed by centrifugation. The amylopectin component was precipitated by adding 4-vol of ethanol to the supernatant. The precipitated amylose and amylopectin were treated with 1 mL of acetone 4 times and 1 mL of ethanol and then they were dried in a vacuum oven at 40 °C for ~10–15 h and weighed.

2.2.5. Analysis of covalently linked phosphate to starches. Covalently linked phosphate was removed from the starches by hydrolyzing 100 mg with 1 M HCl in a boiling water bath for 1 h, neutralized with 1 M NaOH, 100 mg of activated charcoal added to remove the colored materials, and the inorganic phosphate was determined by a modified Fiske–Subbrow method.¹²

3. Results and discussion

3.1. Solubilities of the eight native starches

The water solubilities of native starches, that is, the maximum amount that can be dissolved in a unit volume, do not appear to have been previously reported. In this study, the maximum water solubility of eight

starches was determined by using three methods: (1) autoclaving starch suspensions at 121 °C for 20 min; (2) stirring in 1 M NaOH at 20 °C for 15 h; and (3) stirring in 85:15 (v/v) Me₂SO–H₂O at 20 °C for 8 h. The results are given in Table 1. The autoclaving method gave the highest amount of solubility for potato, tapioca, waxy maize, and maize starches in that order from 92 mg/10 mL to 75.1 mg/10 mL. The autoclaving method gave much lower amounts of solubility for amylo-maize-7, shoti, rice, and wheat starches in decreasing order from 37.1 mg/10 mL to 27.5 mg/10 mL. Shoti starch was unusual in that autoclaving gave a gelatinized mass that could not be dispersed, hence giving low water solubility. In contrast, when a slurry of shoti starch was poured into boiling water, it dissolved to the extent of 65.1 mg/10 mL.

Increasing the amounts of the starches to 220 mg, and autoclaving them gave increased solubilities from 1.34 to 2.86 times the solubilized material obtained from 110 mg of the starches (Table 2). The experiments were performed, at a minimum of 2–4 times for each starch, and gave very similar results within 1–2 mg.

Although all of the starches gave increased amounts of solubilized starch when the amount was doubled, most gave less than double the amounts obtained with

Table 2. Solubility of 220 mg of seven of the native starches solubilized by autoclaving at 121 °C for 20 min

| Starches ^a | Autoclaving at 121 °C mg/10 mL | Factor increase ^b |
|-----------------------|--------------------------------|------------------------------|
| Potato | 123.7 | 1.34 |
| Tapioca | 173.8 | 1.93 |
| Waxy maize | 186.5 | 2.23 |
| Maize | 124.4 | 1.66 |
| Wheat | 51.9 | 1.88 |
| Amylomaize-7 | 54.5 | 1.47 |
| Rice | 79.4 | 2.86 |

^a 220 mg of the starches was suspended in 7 mL of water and autoclaved and then diluted to 10.0 mL and centrifuged; the starches were precipitated from the supernatants with 4 vol of ethanol, dried, and weighed.

^b The factor increase is the amount the solubility was increased on doubling the starting amount of starch from 110 to 220 mg.

Table 1. Solubilities of the eight native starches by (A) autoclaving at 121 °C for 30 min, (B) stirring in 1 M NaOH at 20 °C for 15 h, and (C) stirring or shaking in 85:15 Me₂SO–H₂O at 20 °C for 8 h

| Starches ^a | Autoclaving at 121 °C mg/10 mL | Stirring in 1 M NaOH at 20 °C for 15 h mg/10 mL | Stirring in 85:15 (v/v) Me ₂ SO at 20 °C mg/10 mL |
|-----------------------|--------------------------------|---|--|
| Potato | 92.0 | 77.6 | 82.4 |
| Shoti | 29.5 | 74.9 | 86.8 |
| Tapioca | 90.0 | 81.5 | 90.5 |
| Waxy maize | 83.8 | 84.8 | 72.6 |
| Maize | 75.1 | 82.2 | 62.1 |
| Wheat | 27.5 | 71.6 | 47.7 |
| Amylomaize-7 | 37.1 | 52.3 | 37.9 |
| Rice | 27.8 | 70.1 | 22.1 |

^a 110 mg of the starches was suspended in 7 mL of water and autoclaved or suspended in the reagent and stirred, and finally diluted to 10.0 mL and centrifuged; the starches were precipitated from the supernatants with 4 vol of ethanol, dried, and weighed.

110 mg, with the exception of waxy maize, rice, and tapioca starches. The lowest increase (1.34 times) was for potato starch, which had the highest solubility using 110 mg; and the largest increase (2.86 times) was for rice starch, which had the lowest solubility at 110 mg (see Table 2). The significantly greater amounts of starch that was solubilized when the amount was doubled from 110 to 220 mg were for waxy maize, rice, and tapioca starches. These increased amounts of solubilized starch were correlated with the higher percentages of amylopectin in these starches, 100%, 82%, and 83%, respectively. Increasing the amounts of starch further to 330 mg, however, was not practical, due to the high viscosities that were obtained.

The 1 M NaOH method, however, gave higher amounts of solubilized starch for maize, shoti, wheat, rice, and amylo maize-7 starches in decreasing order from 82.2 mg/10 mL to 52.3 mg/10 mL, all considerably higher than their solubilities obtained by the autoclaving method. Both the 1 M NaOH and the autoclave methods gave identical solubilities for waxy maize, starch, potato and tapioca starches, which were 14.4 mg/10 mL to 8.5 mg/10 mL lower, respectively, than that obtained by the autoclaving method. One of the reasons for the lower amounts for these two starches is that they both started to retrograde, as soon as they were neutralized.

Both the Me₂SO–H₂O and the autoclaving methods gave about the same amounts of solubility for potato, tapioca, and amylo maize-7 starches. The solubility of shoti starch was higher in the Me₂SO–H₂O method than it was in either the boiling in water method or the 1 M NaOH method. The solubilities for waxy maize and maize starches were lower in the Me₂SO–H₂O method than they were in either the autoclave or the 1 M NaOH methods. The solubility of wheat starch in the Me₂SO–H₂O method was higher than in the autoclave method but lower than in the 1 M NaOH method. The solubility of amylo maize-7 starch was identical to the solubility by autoclaving but lower than the solubility by the 1 M NaOH method. The solubility of rice starch was the lowest by the Me₂SO–H₂O method than it was by either the autoclaving or by the 1 M NaOH methods (see Fig. 1 for comparisons of the different solubilities of the native starches by the three methods).

Leach⁷ has given the water solubility of several native starches at 95 °C, as 82% for potato starch, 48% for tapioca starch, 41% for wheat starch, 25% for maize starch, 23% for waxy maize starch, 18% for rice starch, and 12% for amylo maize-7 starch. These values obviously are too high and incorrect for the solubility of these starches in water and they most probably represent the percentages of the individual starches that were solubilized. This, however, has no meaning in that 100% of each of the starches are soluble, if the concentration is made dilute enough, for example 1 mg/mL (see below). A meaning-

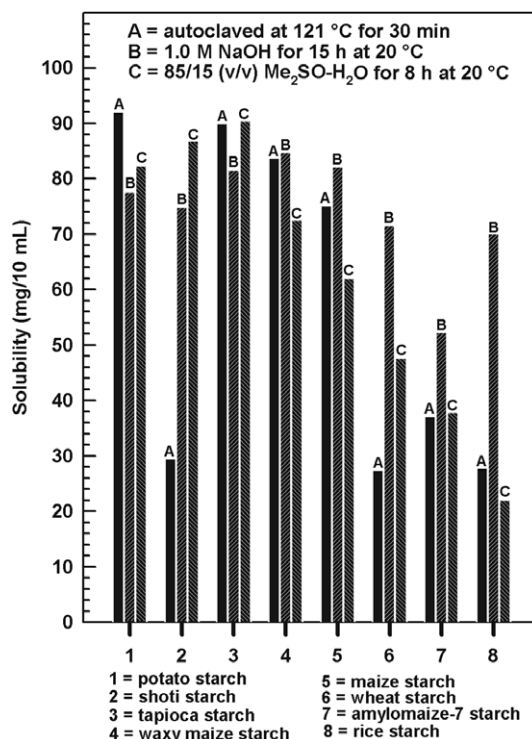


Figure 1. Comparison of the solubilities of 110 mg of eight native starches solubilized by three methods: (A) autoclaving at 121 °C for 30 min; (B) 1.0 M NaOH for 15 h at 121 °C; and (C) 85/15 (v/v) Me₂SO–H₂O for 8 h at 20 °C.

ful percentage solubility should be expressed as the maximum weight per volume that can be obtained. None of the values for the native starches in the present study were as high as 10 mg/mL if one used 110 mg of starch (see Table 1). A significant amount of the starches did not dissolve, but it should be noted here that all 110 mg and 220 mg of the eight starches could be completely solubilized by using 100 or 200 mL of water, respectively, giving solutions of ≤ 1 mg/mL.

3.2. Solubilities of the acid–alcohol modified starches

The treatment of starch granules by dilute acid in anhydrous alcohols produces an increase in the water solubility and a decrease in the molecular size of the starch. These changes are dependent on the kind of alcohol used, the amount of starch per unit volume, the temperature, and the concentration of the acid.^{10,13–15} To date, only three kinds of starches have been reported to be modified: potato, waxy maize, and amylo maize-7 starches in four alcohols, methanol, ethanol, 2-propanol, and 1-butanol.^{10,13–15} Modifications of these three starches have also been obtained by using mixtures of different ratios of methanol and 2-propanol in 10% v/v differences ranging from 90:10 to 10:90 to give a family of modified starches with decreasing degrees of polymerization.¹⁵

In the present study, we modified the same three starches along with the five others: maize, wheat, tapioca, shoti, and rice starches in methanol and ethanol at 20 °C with 0.36% (w/v) HCl and their water solubilities were determined. The modifications were limited to these two alcohols to give the higher molecular sizes. The starches were solubilized by taking different amounts, 110, 220, 330, and 550 mg of starch, and autoclaving them in 10 mL of water.

Tables 3 and 4 show that the solubilities for the eight acid–alcohol treated starches are greatly increased over the solubilities of their native granules. Also increasing the amount of starting material gives significant increases from ~9 mg/mL, using 110 mg, to ~40 mg/mL, using 550 mg.

An unusual phenomenon was observed for the acid–methanol treated potato and shoti starches. At all amounts (110–550 mg) of these two modified starches, the amount of starch precipitated by the 4 vol of ethanol left a substantial amount (35% for 220 mg potato starch and 31.3% for 550 mg of shoti starch) that was not precipitated (see Table 3). Only these two starches showed this phenomenon and only for the acid–methanol treated starches. The two starches are known to be high in covalently linked phosphate.^{16,17} Analysis for phosphate showed that it was primarily found in the starch that was not precipitated, 70.58% for potato starch and 77.93% for the shoti starch. Analysis of the supernatants from all of the other starches, both native starches and acid–alcohol treated starches, found only very low

Table 3. Solubilities of eight acid–methanol treated starches

| Starches ^a | Ppted ^b (mg/10 mL) | Supern ^c (mg/10 mL) | Totals ^d (mg/10 mL) | Insoluble ^e (mg) |
|---|-------------------------------|--------------------------------|--------------------------------|-----------------------------|
| <i>110 mg of starch in 10 mL solubilized by autoclaving</i> | | | | |
| Potato | 70.2 | 22.9 | 93.1 | 16.9 |
| Shoti | 65.0 | 27.1 | 92.1 | 17.9 |
| Tapioca | 87.5 | 1.5 | 89.0 | 21.0 |
| Waxy maize | 88.5 | 0.9 | 89.4 | 20.6 |
| Maize | 87.8 | 1.4 | 89.2 | 20.8 |
| Wheat | 80.8 | 0.6 | 81.4 | 28.6 |
| Amylomaize-7 | 41.8 | 1.1 | 42.9 | 67.1 |
| Rice | 92.3 | 1.0 | 93.3 | 16.7 |
| <i>220 mg of starch in 10 mL solubilized by autoclaving</i> | | | | |
| Potato | 117.4 | 62.0 | 179.4 | 40.6 |
| Shoti | 132.9 | 53.6 | 186.5 | 33.5 |
| Tapioca | 176.3 | 1.7 | 178.0 | 42.0 |
| Waxy maize | 190.5 | 1.7 | 192.2 | 27.8 |
| Maize | 149.6 | 1.0 | 150.6 | 69.4 |
| Wheat | 175.4 | 1.0 | 176.4 | 43.6 |
| Amylomaize-7 | 95.8 | 1.2 | 97.0 | 123.0 |
| Rice | 144.4 | 0.9 | 145.3 | 74.4 |
| <i>330 mg of starch in 10 mL solubilized by autoclaving</i> | | | | |
| Potato | 238.3 | 43.7 | 282.0 | 48.0 |
| Shoti | 206.1 | 72.1 | 278.2 | 51.8 |
| Tapioca | 272.0 | 1.0 | 273.0 | 57.0 |
| Waxy maize | 280.0 | 1.9 | 281.9 | 48.1 |
| Maize | 217.2 | 2.6 | 219.8 | 110.2 |
| Wheat | 216.3 | 1.3 | 219.6 | 112.4 |
| Amylomaize-7 | 130.0 | 0.9 | 130.9 | 199.1 |
| Rice | 257.8 | 2.3 | 260.1 | 69.9 |
| <i>550 mg of starch in 10 mL solubilized by autoclaving</i> | | | | |
| Potato | 401.2 | 74.4 | 475.6 | 74.4 |
| Shoti | 309.5 | 141.0 | 450.5 | 99.5 |
| Tapioca | 424.2 | 2.8 | 427.0 | 123.0 |
| Waxy maize | 472.6 | 2.1 | 474.7 | 75.3 |
| Maize | 376.0 | 2.3 | 478.3 | 171.7 |
| Wheat | 357.3 | 7.3 | 364.6 | 185.4 |
| Amylomaize-7 | 171.6 | 1.0 | 172.6 | 377.4 |
| Rice | 419.7 | 5.4 | 425.1 | 124.9 |

^a Starches were suspended in 7 mL of water, autoclaved, diluted to 10 mL and centrifuged.

^b After removing the starch by centrifugation that did not go into solution, the solubilized starch was precipitated with 4 vol of ethanol.

^c The supernatants were concentrated to dryness and the solids were dried and weighed.

^d mg/10 mL of solubilized starch.

^e Amount of starch that did not dissolve after autoclaving.

Table 4. Solubilities of eight acid–ethanol modified starches

| Starches ^a | Ppted ^b (mg/10 mL) | Supern ^c (mg/10 mL) | Totals ^d (mg/10 mL) | Insoluble ^e (mg) |
|---|-------------------------------|--------------------------------|--------------------------------|-----------------------------|
| <i>110 mg of starch in 10 mL solubilized by autoclaving</i> | | | | |
| Potato | 85.7 | 4.6 | 90.3 | 19.7 |
| Shoti | 76.5 | 0.4 | 76.9 | 33.1 |
| Tapioca | 79.4 | 1.1 | 80.5 | 82.4 |
| Waxy maize | 81.5 | 0.9 | 82.4 | 27.6 |
| Maize | 90.7 | 2.6 | 93.3 | 16.7 |
| Wheat | 78.6 | 1.4 | 80.0 | 30.0 |
| Amylomaize-7 | 64.6 | 1.5 | 66.1 | 43.9 |
| Rice | 92.5 | 1.2 | 93.7 | 16.3 |
| <i>220 mg of starch in 10 mL solubilized by autoclaving</i> | | | | |
| Potato | 173.4 | 6.0 | 179.4 | 40.6 |
| Shoti | 154.0 | 0.5 | 154.5 | 65.5 |
| Tapioca | 129.9 | 1.0 | 130.9 | 89.1 |
| Waxy maize | 175.8 | 1.4 | 177.2 | 42.8 |
| Maize | 153.6 | 1.6 | 155.2 | 64.8 |
| Wheat | 144.2 | 2.2 | 146.4 | 73.6 |
| Amylomaize-7 | 122.0 | 3.1 | 125.1 | 94.4 |
| Rice | 177.4 | 7.1 | 184.5 | 35.5 |
| <i>330 mg of starch in 10 mL solubilized by autoclaving</i> | | | | |
| Potato | 256.9 | 4.4 | 261.3 | 68.7 |
| Shoti | 207.5 | 1.3 | 208.8 | 121.2 |
| Tapioca | 246.6 | 2.6 | 248.6 | 80.8 |
| Waxy maize | 262.7 | 1.7 | 264.4 | 65.6 |
| Maize | 217.1 | 2.7 | 219.8 | 110.2 |
| Wheat | 218.4 | 4.0 | 222.4 | 107.6 |
| Amylomaize-7 | 122.0 | 3.1 | 125.1 | 94.9 |
| Rice | 177.4 | 7.1 | 184.5 | 35.5 |
| <i>550 mg of starch in 10 mL solubilized by autoclaving</i> | | | | |
| Potato | 434.6 | 6.3 | 440.9 | 109.1 |
| Shoti | 413.0 | 3.4 | 416.4 | 133.6 |
| Tapioca | 471.8 | 2.1 | 473.9 | 76.1 |
| Waxy maize | 440.0 | 2.0 | 442.0 | 108.0 |
| Maize | 380.8 | 3.1 | 383.9 | 166.1 |
| Wheat | 358.4 | 7.7 | 366.1 | 183.9 |
| Amylomaize-7 | 255.0 | 6.2 | 261.2 | 288.8 |
| Rice | 409.4 | 2.2 | 411.6 | 138.4 |

^a Starches were suspended in 7 mL of water, autoclaved, diluted to 10 mL and centrifuged.

^b After removing the starch that did not go into solution by centrifugation, the solubilized starch was precipitated with 4 vol of alcohol.

^c The supernatants were concentrated to dryness and the solids were dried and weighed.

^d The sum of columns 1 and 2.

^e Amount of starch that did not dissolve after autoclaving.

amounts (between 0.1% and 0.2%) of starch that was not precipitated by the 4 vol of ethanol.

3.3. Composition of the starches solubilized by autoclaving

The compositions of the solubilized starches were determined by precipitating the amylose component with thymol and then taking the supernatant and precipitating the amylopectin component with 4 vol of ethanol. The results, with a comparison of the amounts of amylose and amylopectin in the eight native starches, are given in Table 5.

The amylose component exceeded the amylopectin component for all of the native starches and for the acid–methanol and acid–ethanol treated starches.

Exceptions were the solubilized components of shoti starch in which the amylose–amylopectin amounts were very nearly equal to the amounts in the native shoti starch granules. Other exceptions were the acid–methanol treated potato and shoti starches in which the ratios of amylose to amylopectin were much less than the ratios in their native starch granules. Another exception was rice starch that had been treated with acid–methanol and acid–ethanol in which the ratios of amylose to amylopectin were also less than the ratios in the native starch granules.

Wheat starch had very high ratios for the amounts of amylose to amylopectin for all three types, native, acid–methanol, and acid–ethanol modified, but especially so for the solubilized native starch and the acid–methanol modified starch. The amylose components of the solubi-

Table 5. Comparison of the percent amylose and amylopectin of eight native starches, acid–methanol modified starches, and acid–ethanol modified starches, solubilized by autoclaving 110 mg for 30 min at 121 °C

| Starches | Native starches | | Autoclaved native starches | | Autoclaved acid–methanol modified starches | | Autoclaved acid–ethanol modified starches | |
|--------------------|-------------------|-------------------|----------------------------|-------|--|-------|---|-------|
| | %Aml ^a | %Amp ^a | %Aml | %Amp | %Aml | %Amp | %Aml | %Amp |
| Tapioca | 17 | 83 | 62.3 | 37.7 | 34.4 | 65.6 | 48.2 | 51.8 |
| Potato | 25 | 75 | 52.7 | 47.3 | 3.4 | 96.6 | 23.3 | 76.7 |
| Shoti ^b | 30 | 70 | 30.5 | 69.4 | 4.1 | 95.5 | 39.2 | 60.8 |
| Wheat | 25 | 75 | 92.9 | 7.1 | 50.9 | 49.1 | 32.5 | 67.5 |
| Rice | 20 | 80 | 67.1 | 32.9 | 15.7 | 84.3 | 12.7 | 87.3 |
| Amylomaize-7 | 70 | 30 | 75.5 | 24.5 | 76.6 | 23.4 | 64.6 | 35.4 |
| Maize | 28 | 72 | 44.9 | 55.1 | 34.6 | 65.4 | 38.0 | 62.0 |
| Waxy maize | 0 | 100 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 |

^a Aml = Amylose and Amp = Amylopectin.

^b Shoti starch was solubilized by pouring a slurry of 110 mg of starch into 8 mL of boiling water, which was stirred with boiling for 30 min and then diluted to 10.0 mL.

lized native amylomaize-7 starch and the acid–methanol treated starch only slightly exceeded the amount of amylose in the native starch.

3.4. Conclusion

A comparison of the solubilities of the eight native starches, solubilized by the three methods, is shown in Figure 1, which shows that the maximum solubilities from 110 mg of starch were obtained for potato and tapioca starches by autoclaving at 121 °C; maximum solubilities were obtained for shoti and tapioca starches by stirring in 85:15 (v/v) Me₂SO–H₂O at 20 °C; and maximum solubilities were obtained for waxy maize, maize, wheat, amylomaize-7, and rice starches by stirring in 1 M NaOH at 20 °C. Increasing the starting amount of starch to 220 mg increased the water solubility of the eight native starches from 1.34 to 2.23 times over that obtained with 110 mg of the starches (see Table 2). The amount of the starting native starches, however, could not be increased above 220 mg, using any method, because of the very high viscosities that were obtained.

Figure 1 shows that the most soluble native starches were potato, shoti, tapioca, and waxy maize in decreasing order, and the least soluble starches were maize, wheat, amylomaize-7, and rice in decreasing order. Figure 2 shows that the acid–alcohol treated starches were 4–9 times more soluble than their native starches. The compositions of the solubilized starches had, in general, much higher ratios of amylose to amylopectin than the ratios in their native granules. A major exception to this was the acid–methanol treated starches of potato, shoti, and rice starches that had much lower ratios of amylose to amylopectin than the ratios in their granules.

The differences observed for the solubilities of the starches are postulated to be due to the differences in the amounts and strengths of the non-covalent bonds

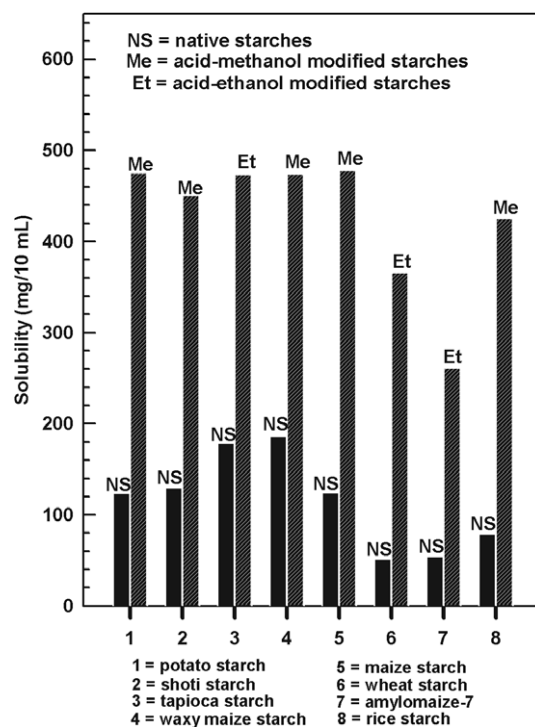


Figure 2. Comparison of the maximum solubilities obtained for 220 mg of native starches and 550 mg of the acid–alcohol modified starches; Me, acid–methanol modified starches; and Et, acid–ethanol modified starches.

that result in the secondary and tertiary structures of the starch molecules in their granules. These differences in the structures of the starches also have been postulated to be the cause of the significant differences observed in the activities of a single type of α -amylase, reacting with the eight solubilized starches. In this case, it was postulated that a certain percentage of the secondary and tertiary structures of the starch molecules remain in solution when the starches are solubilized and these structures lower the activity of the enzyme.¹⁸

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