A PHYSICO-CHEMICAL STUDY

OF THE

SOLUBLE POLYSACCHARIDES OF SWEET CORN

By

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INTRODUCTION

The purpose of this study was to determine some of the physical and chemical properties of the soluble polysaccharides occurring in sweet corn kernels at two stages of ripening and to compare these properties with those of beta-amylose of starch which was obtained from the same corn.

The literature yields very little as to the physical and chemical properties of these polysaccharides. Most of the investigators have referred to them as dextrins or dextrin-like compounds. However, if we accept the definition of a dextrin as given by Abderhalden[1] we should have to conclude that these soluble polysaccharides have resulted from the partial hydrolysis of starch. From the data available we do not know whether these soluble polysaccharides have resulted from starch hydrolysis or whether they are units for the natural synthesis of starch. It is hoped by these studies to ascertain the true nature of the soluble polysaccharides in sweet corn.

RESEARCH MATERIAL

All of the polysaccharides used in these investigations were obtained from Hopeland Sweet Corn. This sweet corn is the result of a cross between Stowell's Evergreen Sweet Corn and Johnson County White Corn. This cross was made by the Agronomy Department of the University of Maryland Agricultural Experiment Station in 1919, and the resulting hybrid was known as Number 301-19. Since the cross selections have been made for sugar segregates; the corn was tentatively named Hopeland Sweet Corn in 1928. Samples of the corn were taken in

the milk stage (3) and in the air dry mature stage. The corn in the milk stage was prepared for extraction by splitting the kernels and scraping out the contents. The mature kernels where first soaked for 48 hours in 20 per cent alcohol containing $\frac{1}{10,000}$ normal iodine. The presence of this concentration of iodine inhibits the activity of starch and dextrin enzymes according to Olsson (14a). The softened kernels were then passed through a nixtamal mill.

PREPARATION OF SOLUBLE POLYSACCHARIDES

Extraction.—Preliminary work showed that the soluble polysaccharides under investigation are soluble in 20 per cent alcohol and insoluble in alcohol above 40 per cent. On the basis of this solubility the following procedure was used for extracting these polysaccharides. Samples of 1000 to 1500 grams of sweet corn which had been either pulped or passed through a nixtamal mill, depending on the stage of maturity of the corn, were twice extracted with 20 per cent alcohol containing $\frac{1}{10,000}$ normal iodine. After thorough mixing, the extracts were separated from the pulp by means of cheese cloth. The extracts thus obtained contained the soluble polysaccharides and suspended starch besides proteins and various other substances. The starch was separated by centrifuging and the soluble polysaccharides were separated from the extract by increasing the alcohol concentration of the solution to 60 per cent.

Purification: The soluble polysaccharides could be readily purified by dissolving in 20 percent alcohol, centrifuging, filtering through a mat of paper pulp and reprecipitating

in 60 per cent alcohol. The supernatant liquid was then removed and the process continued until the material was pure. This point was determined when the solution in 20 per cent alcohol deposited no residue on centrifuging and qualitative tests for proteins were negative. The entire procedure is shown in Figure 1. In purifying the soluble polysaccharides from the milk stage the precipitated material was washed with 77.5 per cent alchol. However, the values received did not justify this treatment and it was discontinued in subsequent preparations. The procedure as outlined was repeated three times on the material from the milk stage and six times on the material from the mature stage.

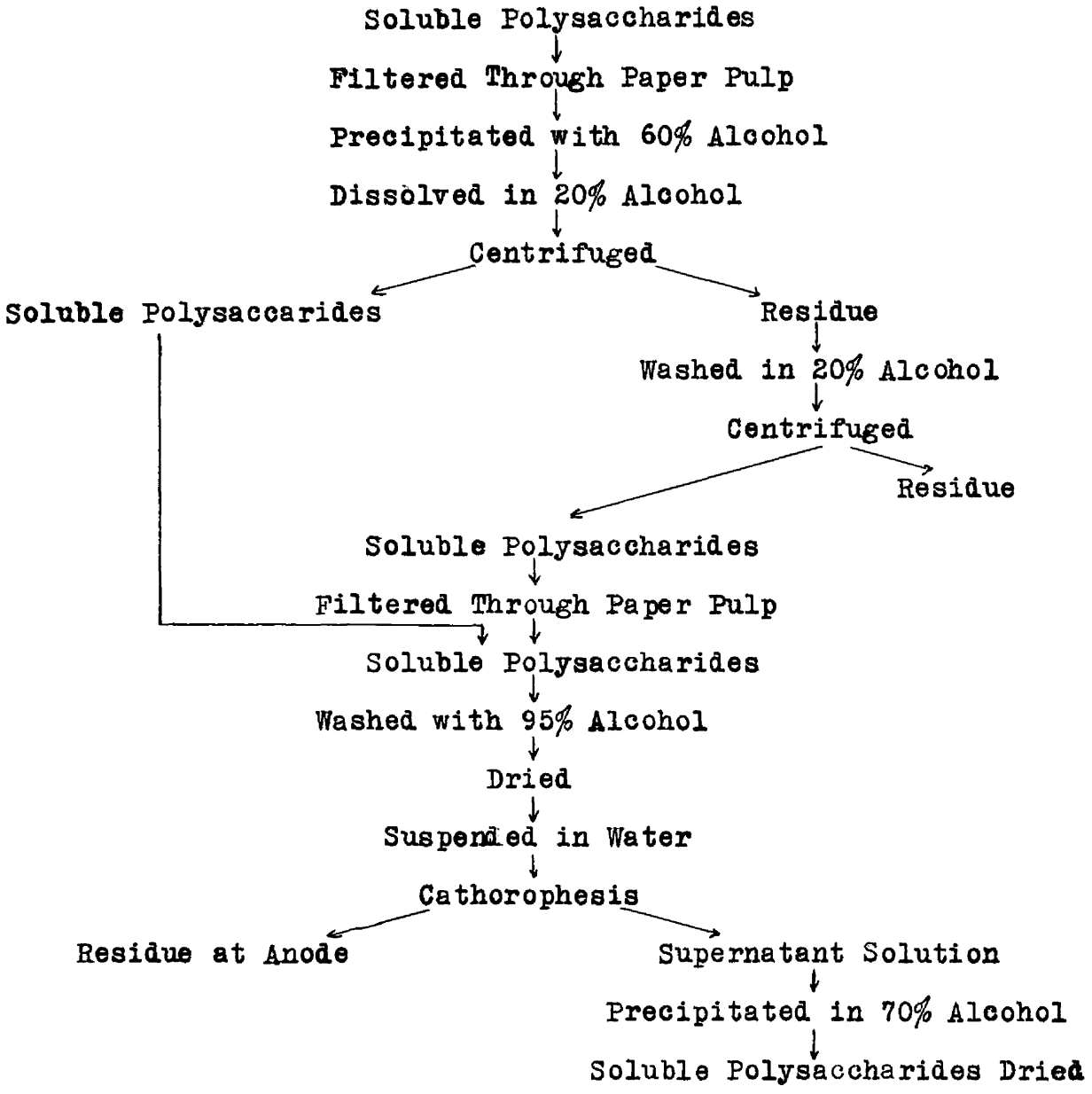

Figure 1. Diagram showing the method of purifying the soluble polysaccharides

Cathorophesis.-- After completing the purification as outlined the material was subjected to cathorophesis. The purpose of this was to remove the inorganic impurities which remained after the final alcohol treatment, and to determine the effect of an electric current on the soluble polysaccharides.

The apparatus used in this investigation was a modified form of the apparatus devised by Taylor and Iddles (20). This apparatus is shown in Figure 2. The arrangement allows for the convenient change of water in the anode and cathode compartments. The membranes that separated the cathode from the inter-chamber and the inter-chamber from the anode were made of collodion supported on cheese cloth. These membranes did not allow the passage of the soluble polysaccharides but allowed the passage of inorganic ions. A direct current of 250 volts was supplied through two platinum electrodes.

Many investigators have mentioned the migration of alpha-amylose when subjected to the action of the electric current. Samec (17), in particular, has studied the migration of alpha-amylose. However, the action of the electric current had not been determined on the soluble polysaccaharides under investigation.

The purified soluble polysaccharides were made up in sufficient water to give a four per cent suspension. The suspension was then placed in the cathorophesis chamber under a direct current of 250 volts. During the first day the water in the electrode chambers was changed frequently;after this period the water was changed twice a day.In a short time a slimy,yellowish white material began to collect around the lower membrane,and

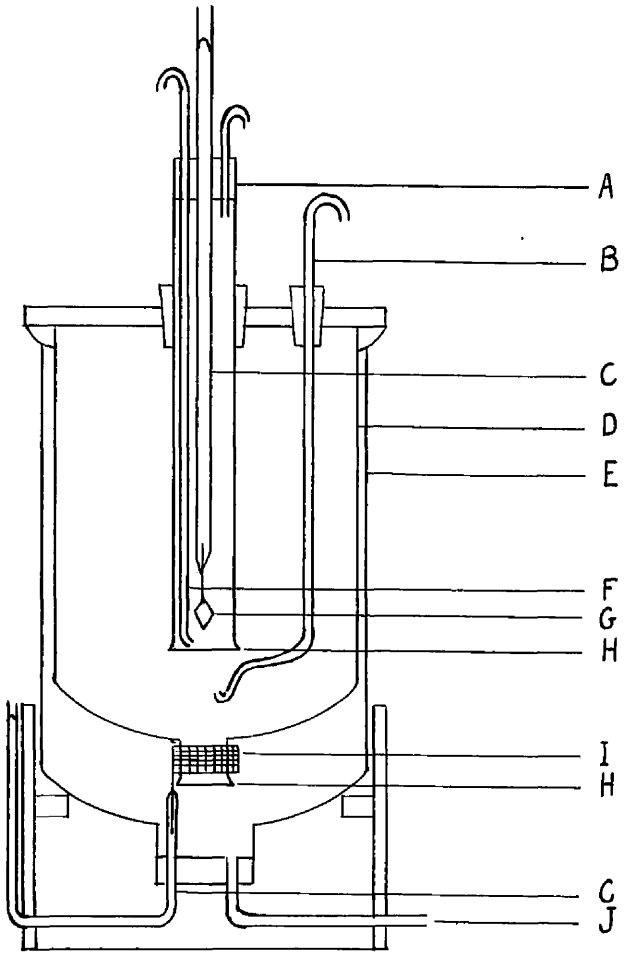

Figure 2. Cathorophesis Apparatus

A	Negative Electrode Chamber	F	Siphon for Negative Electrode Chamber
B	Siphon for Inter Chamber	G	Platinum Foil Electrode
C	Mercury Connection	H	Collodion Membrane
D	Inter-Chamber	I	Platinum Gauze Electrode
E	Positive Electrode Chamber	J	Outlet for Positive Electrode Chamber

at the end of 40 hours a definite separation had occurred. The slimy material was around the lower membrane and the milky solution of the polysaccharides remained in suspension. This supernatant liquid was siphoned off and poured into sufficient 95 per cent alcohol to give a final concentration of 70 per cent alcohol. Precipitation of the pure white polysaccharides occurred immediately. The residue around the lower membrane was again suspended in water and cathorophesis continued. This process was repeated until the supernatant liquid above the residue gave no precipitate in 70 per cent alcohol.

The soluble polysaccharides were washed with absolute alcohol and dried in a vacuum oven at room temperature under 3-4 cm. pressure.

The slimy residue was washed into 70 per cent alcohol and readily precipitated in the presence of a trace of electrolyte. Considerable more residue was present in the soluble polysaccharides from the mature stage than from the milk stage. On drying these residues in a vacuum desiccator over sulfuric acid they gave a horny translucent material.

PREPARATION OF BETA-AMYLOSE FROM STARCH

Purification of Starch.--The impurities in the extraction residue which contained the starch consisted chiefly of proteins and soluble polysaccharides. Zein was probably the most abundant and this protein is the most soluble in 85 to 95 per cent alcohol, according to Osborne (13). The proteins could also be separated from the starch mechanically due to the fact that on centrifuging the starch was thrown down rapidly and the impurities formed a distinct layer on the surface of the starch. The soluble polysaccharides could be washed out with 20 per cent alcohol. By taking advantage of the above properties the starch was purified by washing and centrifuging eleven times in 20 per cent alcohol and five times in 80 per cent alcohol. The procedure for this purification is shown in Figure 3. The starch was finally washed with 95 per cent alcohol and ether, and dried in a vacuum oven at 40° C. under 3-4 cm. pressure.

Separation of Starch Components.--It is well known that the starch grains must be completely ruptured before a separation of alpha- and beta-amylose can be made. Many methods have been proposed for this process, including both chemical and mechanical rupture. The method of mechanical rupture as reported by Alsberg and Perry (2) and by Taylor and Beckmann (19) was adopted in order to eliminate suspicion of any possible change in the beta-amylose, arising from the use of chemical agents.

The method employed was as follows: Twenty grams of dry starch were placed in a quart ball mill which was rotated at 70 to 75 r.p.m. for one week. This period of time gave com-

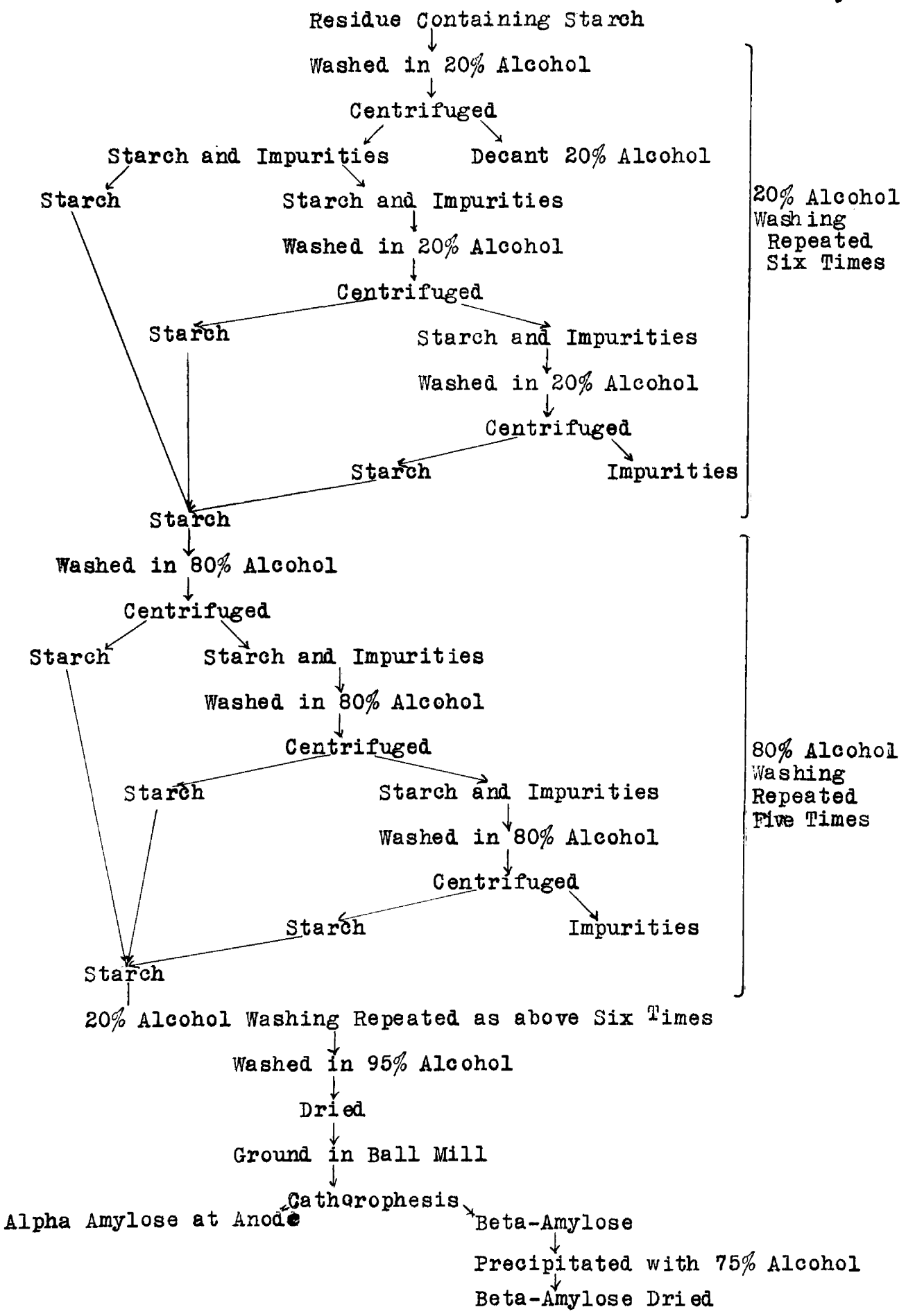

Figure 3. Diagram showing the method of purifying the Starch and Beta-Amylose

plete rupture of the grains as evidenced by the disappearance of the typical black cross on the starch grains under a polarizing microscope. Five lots of starch were prepared in this manner. The completely ruptured starch was then made up into an eight per cent suspension with water and placed in the cathorophesis cell previously described. The alpha-amylose collected around the lower membrane and the clear beta-amylose solution was siphoned off. Water was again added to the residue and the cathorophesis continued until another clear solution of beta-amylose resulted. The dry beta-amylose was obtained by precipitating in 75 per cent alcohol, washing with absolute alcohol, and drying in a vacuum oven at room temperature under 3-4 cm. pressure.

COMPARISON OF SOLUBLE POLYSACCHARIDES WITH BETA-AMYLOSE

Physical Properties

Color with Iodine.--For this work one per cent solutions of the three materials under investigation were prepared. To one ml. of each solution was added ten drops of $\frac{N}{100}$ iodine potassium iodide. The beta-amylose gave a deep dark blue on the addition of one drop of iodine solution. The soluble polysaccharides from the mature stage developed a purplish violet, increasing in intensity on the addition of each drop of the iodine solution. The soluble polysaccharides from the milk stage also developed a purplish violet color. However, the color always remained lighter than that of polysaccharides from the mature stage, regardless of the amount of iodine added. These results are shown in Table 1. The colors that developed were extremely hard to judge, due to the opalescence of the polysaccharide solutions before the addition of any iodine.

Table 1. Color of Beta-amylose and soluble polysaccharides from the milk and mature stages with N/100 iodine potassium iodide.

Material	Color of one per cent solution on adding ten drops of N/100 IKI
Beta-Amylose	Dark Blue
Polysaccharides from mature stage	Purplish Violet with bluish tint
Polysaccharides from milk stage	Purplish Violet

Viscosity.--An Ostwald viscosimeter was employed to determine the relative viscosity of beta-amylose and the soluble polysaccharides from the sweet corn in the mature and the milk stages. The measurements were made in a thermostat at 25°C ±.03°.

The water value of the viscosimeter was determined with five ml. of water. After allowing the system to reach the constant temperature of the thermostat the time of flow between the two given points on the viscosimeter was recorded. The results are shown in Table 2.

Table 2. Time of flow for water at 25°C±.03°.

Time of Flow	
Sample 1	Sample 2
Seconds	Seconds
161.2	161.4
161.0	161.0
161.1	161.3
161.1	161.6
161.3	161.4
161.1	161.2
161.0	161.4
161.1	161.2
160.9	161.4
	161.6
Avg. 161.1	Avg. 161.3
Avg. 161.2	

Solutions of the three materials were prepared with a concentration of one per cent. It was necessary to determine the density of each solution so that the relative viscosity might be calculated after the time of flow had been determined. These densities were determined by the pycnometer method and the results are shown in Table 3. The time of flow for the three solutions was then determined under the conditions previously described. The results are shown in Tables 4, 5 and 6.

Table 3. Density of one per cent solutions of Beta-amylose and Soluble Polysaccharides from milk and mature stages of Sweet Corn

Material	Density of 1% solution at 25°C ±.03°
Beta-amylose	1.0016
Soluble Polysaccharides from milk stage	1.0019
Soluble Polysaccharides from mature stage	1.0020

Table 4. Time of flow for a one per cent solution of Beta-amylose at 25°C ±.03°

Time of flow	
Sample 1	Sample 2
Seconds	Seconds
188.9	188.0
188.0	188.0
188.8	187.8
187.4	187.6
187.7	187.7
Avg. 188.1	Avg. 187.8

Avg. 187.9

Table 5. Time of flow for 1% Solution of Soluble Polysaccharides from the mature stage at 25°C ±.03°.

Time of flow	
Sample 1	Sample 2
Seconds	Seconds
174.3	174.9
174.3	174.9
174.7	174.4
173.9	174.2
173.9	174.4
Avg. 174.22	Avg. 174.56
Avg. 174.39	

Table 6. Time of flow for 1% Solution of Soluble Polysaccharides from the milk stage at 25°C ±.03°.

Time of Flow	
Sample 1	Sample 2
Seconds	Seconds
170.4	170.4
170.3	170.5
170.9	170.2
170.3	170.2
170.4	170.3
Avg. 170.46	Avg. 170.32
Avg. 170.39	

From this data the relative viscosity of the three solutions was calculated by means of the following formula:

$$\frac{N_1}{1} = \frac{d_1 t_1}{d_w t_w}$$

where N_1, d_1, and t_1 denote the viscosity, density and time of flow, respectively, of the solution under investigation, and d_w and t_w denote the density and time of flow of water. The relative viscosities of the three solutions is shown in Table 7.

Table 7. Relative Viscosity of Beta-amylose and Soluble Polysaccharides from Sweet Corn in the milk and mature stages at 25°C±.03°.

Material	Relative Viscosity of 1% solutions at 25° ± .03°
Beta-amylose	1.1710
Soluble Polysaccharides from milk stage	1.0622
Soluble Polysaccharides from mature stage	1.0872

It is evident from these results that the relative viscosity of the polysaccharides from the mature stage is slightly less than that of the polysaccharides from the milk stage. However, the viscosity of the beta-amylose is much higher than either of the polysaccharides.

Optical Rotation.--A Schmidt and Haensch, half shadow saccharimeter was employed for the determination of the optical rotation.

It was necessary to use very dilute solutions of the polysaccharides due to their extreme opalescence. By using five-tenths per cent solutions and a three centimeter polariscope tube satisfactory reading could be obtained. The polariscope tube was filled with the solution to be examined and immersed in a thermostat at $25^{\circ}C \pm .03^{\circ}$. After the tube containing the solution had reached the temperature of the thermostat it was removed and the water was rapidly blotted from its surface. Two readings were then made and the tube returned to the thermostat. This procedure was continued until five readings had been obtained.

The beta-amylose solution was perfectly transparent and satisfactory readings could be obtained when a one per cent solution was used in a ten centimeter tube. The procedure for the determination of the optical rotation was the same as that previously described.

The results for these determinations are in Table 8.

Table 8. Optical Rotation of Beta-amylose and the Soluble Polysaccharides from the milk and mature stages of ripening, in degrees Ventzke at 25°C.

Rotation of the Polysaccharides in Ventzke Degrees at 25°C			
Soluble Polysaccharides from milk stage	Soluble Polysaccharides from mature stage	Beta-amylose A	Beta-amylose B
0.8	0.8	5.8	5.8
0.8	0.8	5.8	5.8
0.8	0.8	5.8	5.8
0.8	0.8	5.8	5.8
0.8	0.8	5.8	5.8

The optical rotation was calculated by means of the formula: $(\alpha)_D^{25} = \frac{100 \times r \times 0.34657}{L \times C}$

where r = reading in Ventzke degrees, L = tube length in decimeters; C = concentration in grams per 100 ml; and 0.34657 = conversion factor for Ventzke degrees to angular degrees.

In making the calculations the weight of the sample was reduced to the dry basis by means of moisture determinations which had been made previously. In order to check on these determinations two samples of beta-amylose were run. The calculations on Sample 1 were based on the moisture determinations previously made and the calculations on Sample 2 are based on a dry weight figure resulting from taking ten ml. of the solution in an evaporating dish containing dry sand and drying it

to a constant weight at 103°C. It is to be noted that these results agree within 0.3 of a degree.

The Angular Rotation of the three materials under investigation is shown in Table 9. Although the soluble polysaccharides showed the same optical rotation in degrees Ventzke a slight difference in the dry weight of the samples gave them a different optical rotation.

Table 9. Optical Rotation of Beta-amylose and Soluble Polysaccharides from the mature and milk stages of Sweet Corn at 25°C.

Material	Optical Rotation at 25°C	
	Sample 1	Sample 2
Beta-amylose	213.9	214.2
Soluble Polysaccharides from mature stage	196.9	
Soluble Polysaccharides from milk stage	198.1	

Electrical Conductivity.--The apparatus used to determine the electrical conductivity of material under investigation was set up according to the diagram shown in Figure 4. A Leeds and Northrup Student's Potentiometer was employed for the wheatstone bridge.

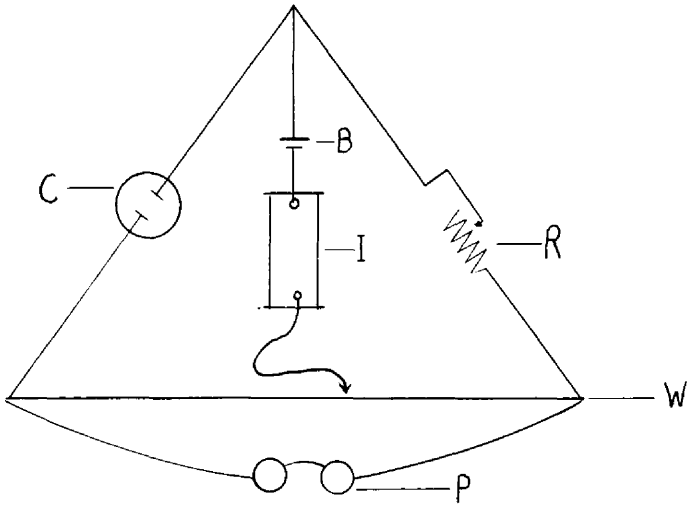

Figure 4

C -- Conductivity Cell

B -- Battery

I -- Microphone Hummer

W -- Wheatstone Bridge

P -- Phones

R -- 4-Dial Resistance Box

All of the water used in this experiment had a specific conductivity of 2.45×10^{-6}. The constant of the conductivity cell was determined by means of $\frac{N}{50}$ Potassium chloride at $25^{\circ}C \pm .02^{\circ}$. Table 10 shows this data.

Table 10. Conductivity of N/50 Potassium chloride at 25°±.02°.

Sample	Conductivity of N/50 Potassium chloride		
	Resistance Reading	*Bridge Reading	Conductivity
	Ohms	Ohms	
1	100	330	.0204
	50	495	.0203
	10	830	.0205
2	100	325	.0207
	50	490	.0208
	10	830	.0205

*Each reading average of 3 readings

AVERAGE CONDUCTIVITY of N/50 Potassium chloride = .0205

From these measurements the constant of the cell was calculated, using the value as found by Kohlraush for the specific conductances of N/50 potassium chloride:

$$K = \frac{.002768}{.0205} = .1350$$

Solutions of beta-amylose, soluble polysaccharides from mature corn and from corn in the milk stage were prepared with a concentration of two per cent. The solutions were then placed in a thermostat at 25°C ± .02°. After equilibrium had been reached, conductivity measurements were made. Before each measurement the cell was carefully rinsed with a portion of the solution to be measured. The results are shown in Tables 11,12,13,14.

The data shows the conductivity of beta-amylose and the soluble polysaccharides from the corn in the milk stage is approximately the same, but the polysaccharides from the corn in the mature stage has a conductance much lower than that of either the two other materials.

Table 11. Conductivity of Beta-Amylose at 25°C ±.02°

Resistance Reading	*Bridge Reading	Conductivity x 10^{-4}
OHMS	OHMS	
9999	280	2.57
4000	491	2.59
1000	794	2.59
		Avg. 2.58 x 10^{-4}

Table 12. Conductivity of Soluble Polysaccharides from Sweet Corn in the Milk Stage at 25°C ±.02°

Resistance Reading	*Bridge Reading	Conductivity x 10^{-4}
OHMS	OHMS	
9999	296	2.38
4100	510	2.34
2300	650	2.34
		Avg. 2.35 x 10^{-4}

*Each reading average of three readings.

Table 13. Conductivity of Soluble Polysaccharides from Sweet Corn in the mature stage at 25°C ±.02°.

Resistance Reading	*Bridge Reading	Conductivity x 10^{-5}
OHMS	OHMS	
9900	542	8.53
8000	590	8.68
4800	746	8.51
		Avg. 8.57 x 10^{-5}

*Each reading average of three readings

Table 14. Specific Conductance of the Materials under Investigation at 25°C ±.02°.

Material	Specific Conductivity
Beta-Amylose	3.48 x 10^{-5}
Soluble Polysaccharides from milk stage	3.17 x 10^{-5}
Soluble Polysaccharides from mature stage	1.15 x 10^{-6}

Flocculation by Salts.--In order to determine the effect of salts on the flocculation of the polysaccharides 10 ml. aliquots of two per cent solutions were employed. The first series of aliquots were treated with 10 ml. of saturated ammonium sulfate. Observations made immediately and at the end of 24 hours failed to detect any flocculation. To another series of samples basic

lead acetate was added drop by drop; however, no flocculation occurred in any of the polysaccharides. The final series of samples were treated with approximately five ml. of 0.1 N iodine-potassium iodide. Flocculation did not occur in any of the solutions. Baldwin (5) reports that beta-amylose is almost totally flocculated by 0.1 N. iodine-potassium iodide. This difference might be due to the fact that the beta-amylose which Baldwin prepared was not free of electrolytes, while the beta-amylose used in this investigation was practically free of electrolytes. On adding a trace of electrolyte to the 0.1 N iodine-potassium iodide, beta-amylose system, flocculation occurs immediately. However, the soluble polysaccharides plus the 0.1 N iodine-potassium iodide are not flocculated even on the addition of an electrolyte. The results are shown in Table 15.

Table 15. Flocculation of Beta-amylose and Soluble Polysaccharides from the milk and mature stages with various salts

Material	Flocculation with Various Salts			
	Half Saturated Ammonium Sulfate	Basic Lead Acetate	0.1 N Iodine-potassium iodide	0.1 N Iodine-potassium Iodide plus Electrolyte
Beta-amylose	None	None	None	Nearly complete
Soluble Polysaccharides from milk stage	None	None	None	None
Soluble Polysaccharides from mature stage	None	None	None	None

CHEMICAL PROPERTIES

Moisture.--Samples of the three materials were placed in tared moisture dishes which were clamped and weighed immediately. These samples were dried to a constant weight in a vacuum oven at 80° under a reduced pressure of 3-4 cm. of mercury. The results are shown in Table 16.

Table 16. Moisture Determinations on Beta-Amylose and Soluble Polysaccharides from Sweet Corn in the milk and mature stages

Material	Per cent Moisture		Average per cent Moisture	Per cent dry weight
	A	B		
Beta-amylose	5.35	6.79	6.07	93.93
Soluble Polysaccharides from mature stage	6.03	6.24	6.13	93.87
Soluble Polysaccharides from milk stage	6.67	6.76	6.71	93.29

Ash.--The samples which had been used for moisture determinations were transferred to tared platinum crucibles and ignited very slowly to a red heat in a muffel furnace. The samples were in duplicate and no weighable ash was found in any of the materials. These results are shown in Table 17.

Table 17. Ash Content of Beta-amylose and Soluble Polysaccharides from Sweet Corn in the mature and milk stages

Material	Per cent Ash
Beta-amylose	None
Soluble Polysaccharides from mature stage	None
Soluble Polysaccharides from milk state	None

Phosphorus Determinations.--Several methods for the determination of phosphorus were tried before any satisfactory results were obtained. The official volumetric method of the A.O.A.C. was not sensitive enough for the small amounts of material which it was necessary to use in this work. The colorimetric methods of Deniges as modified by Troug and Meyer (24) and the colorimetric method of Zinzadze (25) did not give consistent results. Finally the colorimetric method of Bell-Doisey (6) as modified by Briggs (7) and improved by Roe, Irish and Boyd (16) was found to give sufficiently accurate results for this investigation.

After trying several methods of ashing, the wet ash method was adopted for this investigation. The procedure used was recommended by Zinzadze (25) and was as follows: To the sample , (0.5--0.6 gms.) was added 5 ml. of concentrated nitric acid and 5 ml. of 30 per cent hydrogen peroxide. This was evaporated down to one to two ml. volume on an electric hot plate at 165°C, since Roe, Irish and Boyd (16) have shown that phosphoric acid is volatized off above 200° C. Then 2.5 ml. of nitric acid and 2.5 ml. of 30 per cent hydrogen peroxide were again added to the material. The mixture was evaporated to one to two ml. volume as before. To complete the ashing one ml. of concentrated sulfuric acid was added and the mixture was evaporated down to one ml. volume or until no yellow fumes were evident.

After the ash had cooled it was transferred to a 50 ml. volumetric flask. Before making to volume two ml. of ammonium molybdate solution (25 gms. of ammonium molygdate dissolved in 300 ml. water plus 200 ml. of a solution consisting of 125 ml. of water plus 75 ml. of sulfuric acid) and one ml. of 0.5 per cent hydroquione in 20 per cent sodium sulfite solution were added. The solution was made to volume and placed on a boiling water bath for fifteen minutes and cooled in running water. The solutions were then matched against standards by means of a Bausch and Lomb Duboscq colorimeter using 50 mm. cups. The standards were prepared from potassium dihydrogen phosphate simultaneously with the unknown solutions. It was necessary to add one ml. of concentrated sulfuric acid to the standards in order to make the acidity the same as that of the unknown solution. Roe, Irish and Boyd (16) have shown that the acidity has a marked

influence on the color produced, and recommend working in the region of 0.5 N. sulfuric acid.

Samples of beta amylose and soluble polysaccharides from the milk and mature stages of sweet corn were treated as described above. The results in Table 18 show that the soluble polysaccharides from the milk stage contain much more phosphorus than the soluble polysaccharides from the mature stage. The amount of phosphorus in beta-amylose was approximately the same as that from the soluble polysaccharides from the mature corn. Baldwin (5) reported a much higher phosphorus content in precipitated beta-amylose (0.19 per cent phosphorus) while her retrograded beta-amylose contained .006 per cent to .0009 per cent phosphorus. The source of her beta-amylose, however, was potato starch.

Table 18. Colorimetric readings and percentages of phosphorus in Beta-amylose and Soluble Polysaccharides from the milk and mature stages of Sweet Corn

Material	Weight of Sample	*Reading of Standard	**Reading of Sample	Milligrams P_2O_5	Per cent P_2O_5
	gms.	mm.	mm.	mgs.	Per cent
Beta-Amylose	.5588	10	26.7	.02621	.0046
Beta-Amylose	.6390	10	29.1	.02405	.0039
Soluble Polysaccharides, Mature stage	.5518	10	27.2	.0257	.0046
Soluble Polysaccharides, Mature stage	.8794	20	35.6	.0393	.0044
Soluble Polysaccharides, milk stage	.5915	20	32.2	.0434	.0073
Soluble Polysaccharides, milk stage	.5513	20	29.7	.0471	.0085

*Standards contained .07 mgs. P_2O_5

**Each reading is the average of four or more readings

The formula used for calculating the amount of phosphorus was as follows:

$$\frac{\text{Standard Reading}}{\text{Sample Reading}} \times \text{mg. } P_2O_5 \text{ in Standard} = \text{Amount of } P_2O_5 \text{ in Sample}$$

Determination of Fatty Acids Before and After Hydrolysis of the Polysaccharides.--Taylor and Nelson (22) have shown that fatty acids are present in the corn starch grain and that these fatty acids are not liberated until the starch is hydrolyzed. In a later investigation Taylor and Iddles (20) proved that these fatty acids present in starch are associated chiefly with the alpha-amylose fraction. Therefore in comparing the polysaccharides under investigation with beta-amylose, it is important to determine whether fatty acids are freed by hydrolyzing them.

It was necessary to determine first whether or not any fatty substances could be extracted from the materials under investigation. Samples of soluble polysaccharides from the milk and the mature stages of sweet corn and beta-amylose were dried at 100°C under 3-4 cm. pressure. These samples were then placed in Whatman's single thickness extraction thimbles which were 25 x 40 mm. These thimbles were placed in glass siphon cups which rest in the flasks of the Bailey-Walker (4) extraction apparatus employed. Ethyl ether was added and the materials were extracted for 24 hours. The ether was evaporated and the residues in the extraction flasks were dried to constant weight at 100°C under 3-4 cm. pressure. The results showed that no extraneous substances soluble in ether were present in any of the materials.

Samples of beta-amylose and the soluble polysaccharides from the milk and the mature stages were then hydrolyzed with approximately nine per cent hydrochloric acid until no iodine

color was evident in a drop of the hydrolyzing material. The solutions were then filtered through fat-free filter paper and the residues were washed with water until free of acid. The filters containing the residues were dried at 50°C. After drying the filters containing the residues were transferred to extraction cups and extracted with redistilled ethyl ether for 24 hours. The ether residues in the extraction flasks were evaporated and dried to constant weight at 100°C under 3-4 cm. pressure.

The results are shown in Table 19. From these it is quite evident that no fatty material was liberated in the hydrolysis of these materials.

Table 19. Weight of Ether Residue from Hydrolyzed Samples of Beta-amylose and Soluble Polysaccharidesfrom Milk and Mature Stages of Sweet Corn

Material	Weight of Sample	Weight of Dried Residue
	Gms.	Gms.
Beta-amylose	1.9928	.0001
Beta-amylose	2.0804	.0005
Soluble Polysaccharides from Mature Stage	2.1438	.0001
Soluble Polysaccharides from Mature Stage	1.8795	.0000
Soluble Polysaccharides from Milk Stage	2.1343	.0003
Soluble Polysaccharides from Milk Stage	1.9241	.0000

The Reduction of Fehling's Solution before and after Hydrolysis of the Polysaccharides.--The solutions of beta-amylose and the soluble polysaccharides from the milk and the mature stages had a concentration of two per cent. The reducing power of 50 ml. of these solutions was determined by means of the official gravimetric method of Munson and Walker (12). A blank determination on the Fehling's solution was made before each series. The results are shown in Table 20. Series B was run several days later than Series A.

Table 20. The Weight of Cuprous Oxide formed from Samples of Beta-amylose and Soluble Polysaccharides from the Milk and Mature Stages of Sweet Corn

Material	Weight of Cu_2O formed		Weight of Cu_2O formed Corrected for Blank	
	A	B	A	B
	gms.	gms.	gms.	gms.
Beta-Amylose	.0088	.0081	.0074	.0071
Soluble Polysaccharides from Mature Stage	.0046	.0022	.0032	.0011
Soluble Polysaccharides from Milk Stage	.0018	.0003	.0004	.0000
Blank	.0014	.0010	--	--

Samples of beta-amylose and soluble polysaccharides from the milk and the mature stages were hydrolyzed for two and a half hours with 100 ml. of two and a half per cent sulfuric acid. The samples were then made up to 250 ml. volume and after neutralizing with anhydrous sodium carbonate a Munsen and Walker (12) gravimetric determination for reducing sugars was

run on a 50 ml. aliquot. The results are shown in Table 21.

Munsen and Walker tables (12) show that one-tenth of a gram of d-glucose will form 0.2233 grams of cuprous oxide. The percentage conversion of beta-amylose and the soluble polysaccharides was calculated from this figure. These results are shown in Table 22. Link (11) reported a conversion of 92 per cent with a soluble polysaccharide which he obtained from young corn seedlings. The soluble polysaccharides from the milk stage correspond very closely to his polysaccharide.

Table 21. The Cuprous Oxide formed from Samples of Hydrolyzed Beta-amylose and Soluble Polysaccharides from Milk and Mature Stages

Material	Weight of Sample for reduction determination	Weight of Cu_2O formed	
		A	B
	gms.	gms.	gms.
Beta-amylose	.1000	.2159	.2132
Soluble Polysaccharides mature stage	.1000	.2185	.2184
Soluble Polysaccharides milk stage	.1000	.2046	.2039

Table 22. Percentage Conversion to d-glucose of Hydrolyzed Samples of Beta-amylose, Soluble Polysaccharides from Milk and mature stages

Material	Percentage Conversion to d-glucose
	per cent
Beta-amylose	96.05
Soluble Polysaccharides from Mature Stage	97.80
Soluble Polysaccharides from Milk Stage	91.44

Melting Points of Phenylosazone Crystals from Hydrolyzed Samples of Soluble Polysaccharides and Beta-Amylose.--Samples of soluble polysaccharides from the milk and the mature stages of sweet corn and beta-amylose were hydrolyzed for one hour with two and one half per cent sulfuric acid. After neutralizing the sample with anhydrous sodium carbonate, phenylhydrazone hydrochloride and sodium acetate were added. The samples were then placed in a boiling water bath for one hour. At the end of this period abundant osazone crystals were present.

After recrystallization from fifty per cent alcohol plus a trace of pyridine, the crystals were dried. The melting points on these osazones were carried out by the capillary tube method in a sulfuric acid bath. The data are shown in Table 23.

The osazone crystals from beta-amylose were orange-red

rosettes, while the osazones from the soluble polysaccharide were yellow needles. However, the melting points of the osazones are all within the range given for phenylglucosazone.

Table 23. Melting Points of Osazones Formed from Hydrolyzed Samples of Beta-Amylose and Soluble Polysaccharides from the Milk and Mature Stages

Material	Melting Point of Osazone Uncorrected	Melting Ppint of Osazone Corrected	Average Melting Point of Osazone Corrected
	Degrees Cent.	Degrees Cent.	Degrees Cent.
Beta-Amylose	207.0	209.2	
"	207.5	209.7	209.4
Soluble Polysaccharides from Mature Stage	208.5	210.4	
"	208.5	210.2	
"	208.5	210.6	210.6
Soluble Polysaccharides from Milk Stage	207.0	208.9	
	207.0	208.9	
	207.0	208.7	208.8

The formula of Kopp (8) was used to correct the melting points. . The formula is as follows:

$$\text{Correction} = N\,(T - t)\alpha$$

N = the portion of the mercury column above the level of the bath or not heated by the vapors, read in degrees.

T = the temperature registered by the thermometer.

t = the average temperature of the exposed column of mercury, obtained from a second thermometer hung so that its bulb is midway between the level of the bath and the top of the mercury column of the first thermometer.

α = 0.000154 the coefficient of apparent expansion of mercury in glass.

DISCUSSION

From the data obtained in this work it is evident that the soluble polysaccharides are not chemically the same as beta-amylose. However, the two products have some very similar properties. It should be noted that the solutions of the soluble polysaccharides are milky and opalescent while those of beta-amylose are perfectly clear. Link (11) has isolated a water-soluble polysaccharide, which he calls dextrin, from young corn seedlings. He reports that this polysaccharide is very similar to the trihexosan which Pictet (15) obtained by the thermal depolymerization of potato starch. The properties of Link's polysaccharide however, differ somewhat from those investigated in this work. They are similar in the respect that a glucosazone is obtained from the hydrolyzed material and that acid hydrolysis gives a 92 per cent conversion to glucose. However, they differ in respect to iodine coloration and optical rotation.

The soluble polysaccharides from the mature stage differ somewhat from the polysaccharides from the milk stage. The greatest difference seems to be in the amount of phosphorus bound in the polysaccharides. Another very marked difference is found in the material which migrated to the positive electrode during cathorophesis. Preliminary work on these residues has shown them to contain considerable phosphorus and bound fatty material. The residue obtained from the soluble polysaccharides in the milk stage was higher in fat while the mature stage residue was higher in phosphorus. The amounts of fat and phosphorus in these residues has exceeded the percentage reported by Samec (17) for corn alpha-amylose.

Taylor and Werntz (23) have shown that alpha-amylose looses its polarity when the fatty acid is removed. The removal of this is so difficult that they concluded that alpha-amylose is really a fatty acid derivative of a carbohydrate complex. Taylor and Lifschitz (21) have also shown that this alpha-amylose produces gentiobiose on hydrolysis. With these facts in mind we readily see that starch is composed of two materials, chemically different.

Lampe and Meyers (10) have studied the development of sweet corn kernels microchemically and report that globules first form and that carbohydrate grains, which give characteristic starch reactions, may or may not form within these globules. They advance the theory that these globules in sweet corn contain the water soluble polysaccharides. This idea was later given more strength by Lampe (9). Lampe and Meyers (10) also report that they found no evidence of a reversal or a hydrolysis after the carbohydrate grains were once formed.

In the light of these facts it may be possible that the soluble polysaccharides which have been investigated are elementary units which make up beta-amylose. However, further work on these residues must be completed before any definite conclusions can be made as to this hypothesis.

SUMMARY

1. A comparative study has been made of the water-soluble polysaccharides from sweet corn in the mature and milk stages and beta-amylose.

2. The soluble polysaccharides from the mature and the milk stages have practically the same optical rotation, viscosity and color with iodine. The beta-amylose is different in all three of these properties.

3. On hydrolysis the three materials yield glucose; however, the polysaccharides from the milk stage are not converted into glucose as much as the polysaccharides from the mature stage and beta-amylose. The latter two have practically the same percentage conversion.

4. The conductance of the beta-amylose is similar to that of the polysaccharides from the milk stage. The conductance of the polysaccharides from the mature stage is lower than the two other substances.

5. The phosphorus content of the polysaccharides from the mature stage and beta-amylose are the same, while the percentage of phosphorus in the polysaccharide from the milk stage is higher than any of the other materials.

6. The materials under investigation were not flocculated by ammonium sulfate, basic lead acetate or iodine-potassium iodide. On adding a trace of electrolyte the beta-amylose was flocculated by iodine-potassium iodide.

7. There was no extraneous fat or fat freed by hydrolysis in any of the materials investigated.

8. All of the three materials investigated were free of ash.

9. A residue was removed from the soluble polysaccharides by means of cathorophesis.

10. Preliminary work on these residues shows them to have a high percentage of phosphorus and fatty material.

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