THE EFFECT OF SOME EXTRACTION SOLVENTS ON THE CHEMICAL STRUCTURE OF THE STARCHES FROM TOBACCO LEAF AND POTATO TUBERS

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Summary

Tobacco leaf starch has been isolated as granules by mechanical extraction, and the viscosity, amylose content, β-amylolysis limit, starch–iodine complex absorption spectrum, and apparent chain length compared with samples extracted by perchloric acid, sodium hydroxide, and chloral solution and purified as the starch–iodine complex. The efficiency of extraction of these methods was also compared. The same comparisons were made with potato granules and potato granules treated with these extracting reagents.

Evidence is presented that the most efficient method of extraction of tobacco leaf starch is with perchloric acid but all solvent extraction methods give a degraded starch with low viscosity. The β-amylolysis limits are similar, while the starch–iodine absorption spectra and apparent amylose contents of the extracted starches and granules may differ slightly, but solvent-extracted starches show a quite different pattern of acid release on oxidation with periodate ion which makes estimation of apparent chain length difficult.

I. INTRODUCTION

Starch is extracted from tubers and grains by mechanical disruption of the tissue followed by sedimentation of the starch grains, and protein associated with the grains is removed by shaking with toluene or n-butanol (Cowie and Greenwood 1957*a*). This method is suitable for storage organs where the main component is starch and where the cells are easily disrupted. In leaf tissue, in some fruits, and in parenchymous tissue of sapwoods the starch is a minor component and often all the cells are not readily disrupted, so the quantitative isolation of a sample mechanically may not always be possible. In some instances this has been done, e.g. from elm sapwood as described by Campbell *et al.* (1951) and from tobacco leaf by Porter and Martin (1952). The starch-like polysaccharides of simpler organisms have mostly been isolated by chemical extraction methods (Eddy, Fleming, and Manners 1958), although Meeuse, Andries, and Wood (1960) have mechanically isolated floridean starch from a number of algal species.

When starch is chemically extracted some structural breakdown can be expected to take place. If the extraction is not complete there may be preferential removal of one of the components (Cowie and Greenwood 1957*b*). Although the efficiency of different extraction methods has been compared, e.g. for barley grains by MacWilliam, Hall, and Harris (1956), and the effect of acidic and alkaline reagents on starches is known, a comparison of the structural properties of granules and

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starches isolated by chemical methods has not been reported. Starch can be brought into solution, after gelatinization by boiling, by a number of agents that disperse the swollen grains. The most common extraction solutions used have been chloral at 80°C (Meyer and Bernfeld 1940), perchloric acid at or below room temperature (Pucher, Leavenworth, and Vickery 1948), and sodium hydroxide at room temperature (MacWilliam, Hall, and Harris 1956). It is well known that degradation of starch by alkali and hot water (Greenwood 1956; Machell and Richards 1958) and by acids (Greenwood 1956; Cowie and Greenwood 1957*a*) occurs. These solvents are not selective but remove other polysaccharides, e.g. cell wall polysaccharides, proteins, and inorganic salts, so that further purification is necessary. This is usually carried out by precipitation of the starch as the iodine complex in the presence of iodine–potassium iodide solution. Starch content can be estimated by hydrolysing this complex and estimating the glucose produced (Pucher, Leavenworth, and Vickery 1948). To make structural studies, e.g. apparent chain length of amylopectin fraction by estimation of formic acid produced on periodate oxidation of the whole starch (Anderson, Greenwood, and Hirst 1955) or isopotential absorption of iodine by potentiometric titration (Bates, French, and Rundle 1943) it is necessary to remove inorganic ions. This is usually carried out by decomposition of the starch–iodine complex by alkali followed by dialysis, a process that involves the possibility of further degradation.

Starch offers an example of a polysaccharide that can be isolated in a form that involves minimum degradation (as granules) (Banks, Greenwood, and Thomson 1959*a*) for comparison with chemically extracted samples. Most polysaccharides cannot be isolated without taking them into solution, and even neutral aqueous solutions can cause degradation of some, e.g. pectin (Alberscheim, Neukom, and Deuel 1960). Eddy, Fleming, and Manners (1958) found that an α-1,4-glucosan could be extracted from the alga *Dunaliella bioculata* by perchloric acid without degradation.

To find the extent of degradation of starch during chemical extraction, tobacco leaves and purified potato granules were extracted by a variety of methods and a number of characteristic structural properties of the different samples compared.

II. Experimental

(*a*) *Extraction of Tobacco Leaf Starch*

(i) *Source of Material.*—About 25 leaves were collected from glasshouse plants of *Nicotiana tabacum* cv. Hicks. They were fully expanded but had not yet begun to senesce as indicated by a lack of chlorophyll breakdown and they were removed from the lower half of the plants at leaf numbers 15–18 as counted from the cotyledons. The plants had begun to flower. The lamina were separated from the midribs and the latter discarded. The lamina were chopped into smaller pieces and five similar portions of about 100 g wet weight taken. Four portions were immediately macerated in hot ethanol, filtered, and washed several times with ethanol and then extracted in Soxhlet extractors for 18 hr with ethanol, dried, and kept for further extraction. The fifth sample was immediately extracted mechanically.

(ii) *Mechanical Separation of Starch Granules.*—The method of Porter and Martin (1952) was used and the granules purified by the method of Greenwood and Robertson (1954).

(iii) *Extraction by Chloral Hydrate.*—The leaf, previously extracted by ethanol, was extracted three times at 80°C for 1 hr with 33% chloral solution and the starch precipitated into acetone and washed with ethanol and ether by the method of Meyer and Bernfeld (1940). A portion (2·0 g) of the precipitate was extracted twice with 0·25N sodium hydroxide (2×200 ml) and acidified with hydrochloric acid; sodium chloride was then added, followed by 3% iodine in 3% potassium iodide solution. The precipitate was washed with 2% ethanolic sodium chloride, dissolved in 0·25N sodium hydroxide, and deionized by electrodialysis as described for perchloric acid extraction and then freeze-dried.

(iv) *Extraction by Perchloric Acid followed by Electrodialysis.*—The macerated leaf after extraction with hot ethanol was dried, macerated again in cold water, the solution boiled for 15 min, cooled in an ice–salt-bath, and an equal volume of cold 60% perchloric acid added slowly with vigorous stirring. The solution was stirred for 30 min. During the addition and later stirring the temperature was not allowed to rise above 10°C. The mixture was centrifuged and the supernatant immediately treated with sodium chloride (15 g/100 ml) and 3% iodine in 3% potassium iodide solution. The residue was again extracted with cold 30% perchloric acid for 30 min, centrifuged, and the residue extracted with cold water. The starch–iodine precipitates were combined, washed twice with 0·25M ethanolic sodium chloride, and stored in a refrigerator overnight. The starch–iodine precipitate was dissolved in 0·25M sodium hydroxide (100 ml) and centrifuged to remove any insoluble material. The residue was washed with alkali (50 ml) and centrifuged again. The residue after this washing was free of starch and gave tests for protein. The sodium hydroxide solution of the starch was deionized by electrodialysis in a "Perspex" tank (5·5 by 6·5 by 7·5 cm) fitted with "Permaplex" C-10 and A-10 resin-coated membranes. The apparatus was cooled in an ice-bath and the current did not exceed 0·4 A. This cooling was sufficient until the final stages when it was found necessary to place ice directly into the cathode and anode compartments. The temperature was kept below 30°C at all times and mostly below 25°C. After 4–6 hr the current passing was not detectable on a one-ampere ammeter, but it was found desirable to pass current for about 1 hr more on full line voltage to remove all traces of iodide ion as it interfered with later measurements. The contents of the chamber were removed, the chamber washed, the volume reduced to 150 ml by distillation under diminished pressure below 40°C, and the solution freeze-dried.

(v) *Extraction by Perchloric Acid followed by Dialysis.*—The extraction was carried out in the same way as the previous method. The starch–iodine complex was washed with 2% ethanolic sodium chloride and decomposed by treatment with ethanolic sodium hydroxide (16 g NaOH in 80 ml water made up to 480 ml with ethanol), washed again with 2% ethanolic sodium chloride and dissolved in 0·25N sodium hydroxide solution, neutralized with dilute hydrochloric acid, and dialysed against running tap water for 48–60 hr. The volume was reduced by distillation under diminished pressure below 40°C and the solution freeze-dried.

(vi) *Extraction by Sodium Hydroxide.*—The macerated leaf, after extraction by ethanol and drying, was boiled with water, cooled to room temperature, and an equal volume of 10% sodium hydroxide added and the mixture stirred for 1 hr. No precautions were taken to exclude oxygen. The solution was centrifuged and the residue extracted twice with 5% sodium hydroxide for ½-hr periods. The supernatants after centrifuging were immediately acidified with dilute hydrochloric acid and the starch–iodine complex precipitated and deionized by electrodialysis or dialysis as in the perchloric extraction method.

(*b*) *Extraction of Potato Granules*

Potato granules were prepared by maceration of commercial potatoes of unknown variety and history by the method of Cowie and Greenwood (1957*a*) and purified by shaking in a salt solution with toluene and n-butanol (Greenwood and Robertson 1954) and the granules defatted with hot methanol. These granules were extracted with perchloric acid and sodium hydroxide by methods similar to those described for tobacco leaf starch.

(*c*) *Estimation of Glucan Content of Starch Samples*

The starch content of the samples was estimated by the method of Pirt and Whelan (1951) with slight modifications for freeze-dried samples which quickly dissolved in 1·5N sulphuric acid so that it was not necessary to dissolve in sodium hydroxide. Granules were dissolved in sodium hydroxide and neutralized. Starch (12–15 mg) was heated in a boiling water-bath with 1·5N sulphuric acid (10 ml) for 2 hr, cooled, transferred to a 250 ml volumetric flask, neutralized with 1N NaOH and 0·1N oxalic acid, and made up to volume. Samples (5 ml) were taken for glucose estimation by the Nelson (1944) modification of the Somogyi method. The error was ±0·5%.

(*d*) *Estimation of Sulphated Ash*

Ash was determined as sulphated ash after ignition of a sample (*c.* 100 mg) in a platinum crucible.

(*e*) *Protein Content*

Protein contents were estimated by the colorimetric microKjeldahl method of Lang (1958). Starch (*c.* 5 mg) was digested with sulphuric acid, selenium oxychloride, and potassium sulphate and the ammonia estimated by nesslerization. The light absorption was measured at 420 mμ and compared with standard samples.

(*f*) *Estimation of Arabinose and Galactose Content*

Starch (*c.* 50 mg) was hydrolysed in 1N sulphuric acid, neutralized with barium carbonate, and the volume made up to 50 ml. The sugars were separated by paper chromatography in n-butanol (5 parts), water (3 parts), pyridine (3 parts), benzene (1 part), sprayed with anisidine hydrochloride, and the developed spot eluted. They were estimated colorimetrically by comparison with standard samples from the same chromatogram, using the method of Pridham (1956).

(*g*) *Limiting Viscosity Number*

The limiting viscosity number, η, was determined graphically from the relationship

$$\eta = \lim_{c \to 0}[\eta_{sp.}/c].$$

The specific viscosities were measured in 1M potassium hydroxide in an Ubbelohde viscometer in a bath at $25 \pm 0{\cdot}01$°C. The solvent flow time was 178·3 sec. Solutions were made by shaking the starch sample (*c.* 80 mg) vigorously at room temperature for 30 min in potassium hydroxide solution and the solution from this filtered through a grade 3 followed by a grade 4 sintered-glass filter. This solution was diluted to allow four different concentration measurements. The results are expressed as g/100ml. No kinetic energy or shear correction factors were applied.

(*h*) *Isopotential Iodine Absorption of Starch*

The method of Bates, French, and Rundle (1943) was used. Starch (*c.* 120 mg) was dissolved in 0·2M potassium hydroxide (25 ml) by heating in a boiling water-bath for 3 min. The solution was immediately cooled and neutralized with 0·2M hydrochloric acid using methyl orange as an indicator. Potassium iodide (0·1M, 50 ml) was added and the solution titrated at $30 \pm 0{\cdot}05$°C with a 0·005M solution of iodine in 0·05M potassium chloride and 0·05M potassium iodide. The potential changes were measured with calomel and platinum electrodes connected to a potentiometer reading to 0·1 mV. The isopotential iodine absorption was found by plotting free iodine in solution (found from a standard curve) against bound iodine (found by difference of total iodine less free iodine) and extrapolating the plateau portion of the curve to the "bound iodine" axis. The values of individual samples were reproducible to $\pm 0{\cdot}06$. The percentage amylose was calculated by assuming that amylose absorbs 19·2 g of iodine per 100 g. For a discussion of iodine binding power see Greenwood and Robertson (1954) and Anderson and Greenwood (1955).

(*i*) *Estimation of Apparent Chain Length by Formic Acid Produced on Periodate Oxidation*

The method of Anderson, Greenwood, and Hirst (1955) was followed with the modification that a few glass beads were included in the reaction mixture. Starch granules (*c.* 300 mg) were suspended in 0·56M potassium chloride solution (60 ml) and 0·2M sodium metaperiodate (20 ml) and some glass beads added and the flask shaken in the dark at 10°C. Samples (10 ml) were withdrawn at intervals up to 400 hr. Ethylene glycol (1 ml) was added to the aliquot and after shaking in the dark for 10 min, nitrogen gas, free of carbon dioxide, was bubbled for 10 min, and the solution titrated potentiometrically under nitrogen with 0·01N sodium hydroxide solution using a Cambridge pH-meter. The amount of alkali used at pH 6·25 was determined graphically. The external chain length of the amylopectin fraction was calculated from the percentage of amylopectin in granules found by iodine titration. The small contribution of formic acid from amylose was neglected. It was at first found to be difficult to carry out oxidations on the freeze-dried samples as they tended to

aggregate, so that when withdrawing samples for titration, the solution left had a different starch concentration. Glass beads were added to break up the aggregates. The addition of glass beads to granules had no effect on their formic acid production curves. The extracted freeze-dried samples formed a colloidal solution after shaking for 100–125 hr.

(*j*) *Estimation of Periodate Uptake*

This was determined by the method of Fleury and Lange (1933). Separate aliquots containing starch (*c.* 30 mg), 0·2M sodium metaperiodate (3 ml), and 0·56M potassium chloride (9 ml) were shaken under similar conditions as for the formic acid determinations. At the required time, saturated sodium bicarbonate solution (10 ml), 0·05M sodium arsenite solution (12 ml), and 20% potassium iodide in saturated sodium bicarbonate solution (1 ml) were added and the flask shaken for 15 min before being titrated with 0·05M iodine solution.

(*k*) *Estimation of Percentage β-Amylolysis and Iodine Complex by Absorption Spectrophotometry*

Soybean β-amylase free from α-amylase was prepared by the method of Peat, Pirt, and Whelan (1952*b*) and the β-amylolysis measurements carried out by a similar method to their described method (Peat, Pirt, and Whelan 1952*a*). Starch (*c.* 25 mg) was dissolved in 0·25N sodium hydroxide (5 ml) by heating for 3 min in a boiling water-bath, cooled, and the solution neutralized with 1N sulphuric acid. Acetate buffer (0·2M, pH 4·8, 25 ml) was added and the solution made up to 50 ml with water. A portion (2 ml) was transferred by pipette to a 25-ml volumetric flask and 0·01% iodine in 0·1% potassium iodide solution added and the volume made up to 25 ml with water. The absorption was measured against water in a Beckmann DU spectrophotometer. To the original solution, β-amylase (0·5 ml) was added and the solution incubated at 37°C for 1½ hr. A further 2 ml was taken for iodine complex absorption readings and then 20 ml of the solution was diluted to 50 ml with water and 5-ml samples taken for glucose determinations by the Nelson (1944) modification of the Somogyi method and the maltose produced by β-amylase action estimated from a standard maltose calibration curve.

III. Results and Discussion

From Tables 1, 2, and 3 a comparison of the yields obtained by the different methods indicates that mechanical disruption did not give a quantitative isolation of the starch from fully expanded tobacco leaves, and perchloric acid treatment gave the highest yield. Porter and Martin (1952) found that six mechanical extractions left only traces of starch in the leaf residue. It is possible that the ease of extraction is dependent on the age of the leaves and that some starch is left in the toluene layer containing lipids and pigments. Extraction of the leaf macerate with ethanol to remove lipids and pigments, prior to separation of the starch grains, did not increase the yield but rather reduced it. However, leaf which had previously been extracted by ethanol tended to sediment with the starch grains and the reduction in yield may have been due to the longer purification procedure necessary with this greater

co-precipitation. Chloral hydrate solution followed by acetone precipitation gave a much higher yield than any other method. Subsequent analysis of this for starch indicated that two-thirds of the extract was non-starchy and that three extractions with chloral hydrate removed slightly less starch than perchloric acid, as did alkaline extraction. The contamination of the chloral hydrate extract by non-starchy material makes this an unsatisfactory method unless it is followed by a subsequent purification of the starch as the iodine complex. As this involves treatment with acid and alkali it is no longer a neutral extraction procedure.

TABLE 1

COMPARISON OF STARCH EXTRACTED FROM TOBACCO LEAF BY VARIOUS METHODS

Method of Extraction	Starch Extracted per 100 g Green Leaf (g)	Glucan Content of Starch (%)	Glucan Extracted per 100 g Green Leaf (g)	Sulphated Ash Content (%)*	Protein Content (%)†	Galactose and Arabinose Content on Acid Hydrolysis (%)
Mechanical	3·4	80·0	2·7	n.d.	0·03	<0·01
Perchloric acid and dialysis	5·3	84·9	4·5	0·07	1·50	<0·01
Perchloric acid and electrodialysis	5·6	82·6	4·6	n.d.	0·35	<0·01
Chloral hydrate and acetone precipitation	13·0	32·4‡	4·2	16·2	6·2	*c.* 10, 5 respectively§
Chloral hydrate and electrodialysis	5·4	77·2	4·2	n.d.	1·7	<0·01
Alkali and electro-dialysis	5·1	80·8	4·1	0·02	n.d.	<0·01

* Expressed as percentage of glucan content.

† i.e. N × 6·25. Expressed as percentage of glucan content.

‡ Determined by solution in perchloric acid, precipitation of the iodine complex, and followed by estimation of glucose by the Nelson (1944) modification of the Somogyi method.

§ Estimated by the method of Pridham (1956). Glucose content of extract *c.* 35%.

As the mechanical isolation of starch granules from leaf gave only a fraction of the yield obtained by the other methods, any difference in properties could be due to a selective removal of a majority of one type of granule. The granules isolated might not be a representative sample of the total starch in the leaf. A comparison in properties was also made between potato starch granules and the starch isolated from these granules by similar extraction methods to those used for tobacco leaf starch.

The glucan content of the granules and freeze-dried samples varied between 77 and 90%. The difference between these values and 100% could not be accounted

for as ash or protein but was probably water as indicated by the behaviour of leaf starch isolated by perchloric extraction when it was dried at 100°C and 20 mm pressure. After 1 hr there was an 8% loss in weight and after 3 hr a 9% loss. Electrodialysis reduced the ash content below the value that could be significantly measured while the dialysed samples gave a low but significant ash content.

TABLE 2

COMPARISON OF PROPERTIES OF STARCH EXTRACTED FROM TOBACCO LEAF BY VARIOUS METHODS

Method of Extraction	Limiting Viscosity (g/100 ml)	Iso-potential Iodine Absorption (g/100 g starch)	Amylose Content (%)*	Amylo-pectin Chain Length†	β-Amylolysis		
					Starch Degraded (%)	Max. Absorption of Iodine Complex‡	
						Before Amylolysis (590 mμ)	After Amylolysis (550 mμ)
Mechanical	1·79	4·18	21·8	21, 21	62	0·640	0·285
Perchloric acid and dialysis	1·24	4·00	20·8	18, 20(15)	60	0·610	0·290
Perchloric acid and electrodialysis	1·11	4·18	21·8	17, 19(15)	59	0·620	0·300
Chloral hydrate and acetone precipitation	n.d.	n.d.	n.d.	§	n.d.	n.d.	n.d.
Chloral hydrate and electrodialysis	0·92	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Alkali and electrodialysis	0·94	4·32	22·5	17, 19(15)	59	0·650	0·310

* Calculated by assuming that amylose absorbs 19·2 g iodine per 100 g (Anderson and Greenwood 1955).

† Values given represent chain lengths at 300 and 350 hr for starch granules and at 175 and 225 hr for extracted starches respectively. Values in brackets are the apparent chain lengths at 300 hr.

‡ Using a 1-cm cell.

§ See text, p. 322.

Acidic hydrolysis and examination of the samples for sugars by paper chromatography showed that the chloral extract contained significant amounts of arabinose and galactose. Chloral extraction without subsequent purification of the starch would give a product that contained pectin and water-soluble polysaccharides. The very low amounts of arabinose and galactose in the other starch samples would be unlikely to affect any measurements.

The protein contents indicate that extraction of a leaf with perchloric acid can lead to a product with a higher protein content than in granules isolated from the leaf. Perchloric acid is a protein solvent and the starch–iodine complex can

TABLE 3

COMPARISON OF STARCH EXTRACTED FROM POTATO GRANULES BY VARIOUS METHODS

Method of Extraction	Glucan Content of Starch (%)	Sulphated Ash (%)*	Protein Content (%)†	Limiting Viscosity (g/100 ml)	Isopotential Iodine Absorption	Amylose Content (%)‡	Amylo-pectin Chain Length§	β-Amylolysis		
								Starch Degraded (%)	Max. Absorption of Iodine Complex‖	
									Before Amylolysis (590 mμ)	After Amylolysis (560 mμ)
Mechanical	78·3	n.d.	0·47	2·38	4·04	21·0	25, 25	63	0·610	0·295
Perchloric acid and electrodialysis	90·3	n.d.	n.d.	0·84	4·10	21·4	20, 23 (18)	58	0·610	0·260
Alkali and electro-dialysis	85·4	n.d.	n.d.	0·96	4·32	22·5	19, 21 (18)	58	0·580	0·240
Alkali and dialysis	85·7	1·2	0·06	0·50	4·08	21·2	19, 22 (17)	59	0·570	0·240

* Expressed as percentage of glucan content.

† i.e. N × 6·25. Expressed as percentage of glucan content.

‡ Calculated by assuming that amylose absorbs 19·2 g iodine per 100 g (Anderson and Greenwood 1955).

§ See second footnote, Table 2.

‖ Using a 1-cm cell.

co-precipitate protein. Alkaline extraction gives a product with less protein contamination, but all methods except chloral extraction gave values low enough not to interfere with periodate oxidation (Anderson, Greenwood, and Hirst 1955).

The limiting viscosities of all the starches isolated by extraction were much lower than for granules, indicating that acidic and alkaline degradation accompanied isolation. The changes are comparable with those obtained by Cowie and Greenwood (1957*a*) on acidic degradation of potato starch granules and by Bottle *et al.* (1953) who followed the alkaline degradation of potato amylose in the presence of oxygen, which has been suggested (Baum and Gilbert 1954) to cause a random scission of the glycosidic linkages. The stepwise alkaline degradation from the reducing end of the polymer to produce acidic products (Machell and Richards 1958) would reduce the viscosity more slowly. Lansky, Kooi, and Schoch (1949) found that autoclaving of starch granules in water caused a drop in viscosity. The reduction in viscosity found is compatible with a random scission of the glucan chains by the acid and alkali in the presence of oxygen, which is used in extraction. α-Amylase, which randomly splits α-1,4-glucose linkages has a similar effect on the viscosity of amylose (Banks, Greenwood, and Jones 1960). There is unlikely to be any significance in the relative order of the viscosities as this was not reproducible. In another comparison of tobacco leaf starch from young leaves where the granules had a value of 1·29, perchloric extraction followed by electrodialysis gave a value of 0·78, and perchloric extraction followed by dialysis 0·67, the reverse of the order of values in the table.

In the isopotential iodine absorption of the starches, an unpredictable source of difference is the protein content. Anderson and Greenwood (1955) found that potato starch, free of protein, had an iodine affinity of 3·94 and with 2·0% added protein the value was 3·78 but that the presence of protein in oat starch increased the iodine affinity.

In two alkali extractions the apparent amylose content was high and in one perchloric extraction low, but the significance of differences when the error in glucan content and the unknown effect of protein contamination is considered is difficult to determine. High apparent amylose contents could result from incomplete extraction of starch and low values from degradation of the amylose molecule. When the values are converted to amylose contents by assuming that amylose absorbs 19·2% of iodine (Anderson and Greenwood 1955), tobacco starch is found to consist of 21·8% amylose. Baker and Whelan (1950) reported an amylose content of 22% for tobacco leaf starch and Radwan and Stocking (1957) found an iodine-binding capacity of 3·4%.

The acidity produced on periodate oxidation followed a different pattern for the granules and for the extracted samples. The comparison of formic acid production is particularly interesting as the periodate oxidation does not involve the dissolution of the granules in sodium hydroxide prior to measurement, with the possibility of alkaline degradation. The granules showed the normal behaviour of constant production of acid when periodate uptake was theoretical. The oxidations of the chemically extracted samples (Fig. 1) gave a much more rapid increase in acidity with time after the initial rapid oxidation was complete. Unlike the granules, after the uptake of periodate reached the theoretical value, no point of constant release of formic

acid appeared. When the periodate uptake was estimated it was found to become as constant for the freeze-dried samples as for the granules. In Figure 1, the third point would indicate the possibility of a point of inflexion in the formic acid production from the freeze-dried sample. This inflexion was present in almost all the freeze-dried samples indicating the possibility of a real tapering off in formic acid production near 200 hr before hyperacidity appears. It is not possible to estimate how much of the acidity at 200 hr in the extracted samples is due to acidity other than formic acid, but the apparent chain lengths at this point (the time of theoretical uptake of periodate) are lower than the values found for the granules at constant acid production. As the potato starch fractions show a similar behaviour to tobacco leaf starch,

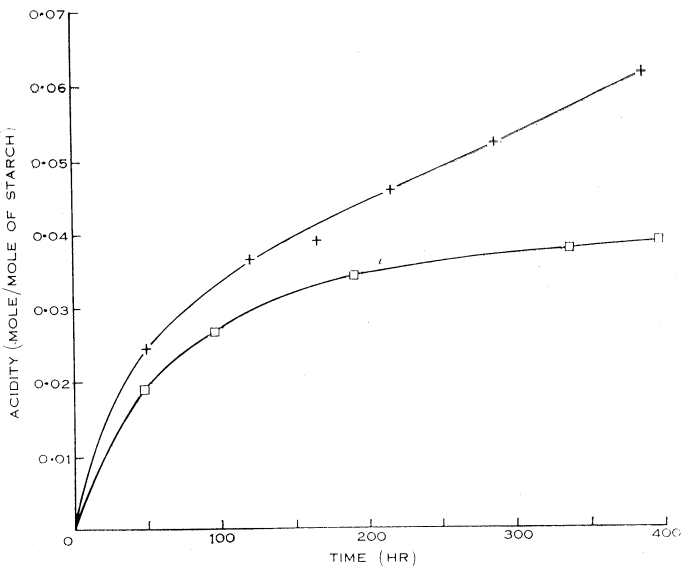

Fig. 1.—Moles of acid per mole of starch liberated by periodate oxidation of tobacco leaf starch. + Starch extracted by sodium hydroxide and deionized by electrodialysis. □ Starch granules.

the difference in tobacco granules and chemically extracted tobacco starch is unlikely to be due to a preferential separation of starch of high chain length mechanically as granules. The starch extracted by chloral and precipitated into acetone gave an abnormal titration curve. Anderson, Greenwood, and Hirst (1955) have noted that in the presence of high protein impurity chain lengths cannot be determined by formic acid production on periodate oxidation.

The β-amylolysis limit conversions showed no differences within the accuracy of measurement. Several factors could produce differences. Banks, Greenwood, and Thomson (1959*b*) found that molecular oxygen produces barriers to the action of β-amylase on amylose. If the apparent chain lengths found by periodate oxidation are real values, then the smaller chain length would produce a lower conversion to maltose. Random degradation could increase the β-amylolysis limit by bypassing the barriers to β-amylolysis in amylose. The iodine complex absorption maximum

was measured before and after β-amylolysis. In replicate samples of the same starch, the wavelength of absorption was found to be constant, but the log $E_{max.}$ value at this wavelength was no more reproducible than the differences between samples recorded in the table, so no significance can be attached to the variation in log $E_{max.}$ values.

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