

A Modified Starch-Iodine Method Suitable for the Study of Starch and its Hydrolysis Products

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A method of extracting excess iodine from the starch-iodine complex by carbon tetrachloride has been developed which produces a color only with the amylose present, removes turbidity due to insoluble or retrograde amylose, and is not interfered with by amylopectin or lower dextrans. By this method some new insight can be gained into the mechanism of starch degradation.

Varying quantities (1 to 5 ml. of 0.0125 to 2.0% solutions) of starch solutions were added to 25 ml. Folin-Wu tubes which had been provided with 3 ml. each of an iodine solution (0.05% iodine solution in 0.15% potassium iodide and 0.5% hydrochloric acid). Distilled water was added, 10 ml. to each tube, and the solutions were mixed by inversion. Carbon tetrachloride was then added, 5 ml. to each tube, the tubes were shaken 50 times, diluted to 25 ml., mixed again by inversion, and allowed to settle for 20 minutes. The absorption spectra of the supernatant liquids were then determined, against a standard of distilled water, by means of a Beckman DU spectrophotometer.

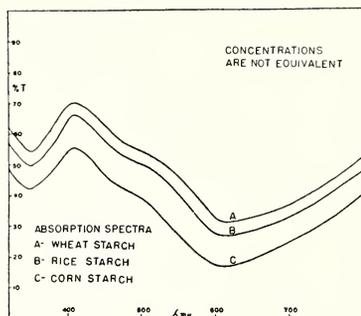
The carbon tetrachloride layer emulsifies when shaken with starch. Apparently this is due to the adsorption of a kind of retrograde amylose, since the adsorbed material will not dissolve after being shaken in repeated changes of distilled water, forms a blue color on the surface of the carbon tetrachloride droplets when iodine is added, and will dissolve in 0.1 N sodium hydroxide to form a solution which, when neutralized, produces a color the spectrum of which is identical with that of amylose. The ability to form this emulsion disappears during the early stages of hydrolysis.

The method has been found to be quite reproducible and the quantities of iodine and carbon tetrachloride are not critical. Beer's law has been found to be followed at $610m\mu$, over the range 0.2% to 0.025% starch. The color produced is related only to the amount of amylose present and is independent of the iodine concentration. This is shown by the variation of the transmittancy at $350 m\mu$ (due to iodine alone) in direct relationship with the transmittancy at $610 m\mu$ (that due to amylose-iodine complex and which depends upon the amount of amylose present). This permits a comparison of the color developed with excess iodine to the color produced with that amount of iodine utilized in the actual amylose-iodine absorption complex, and thus additional information can be obtained concerning the qualitative and quantitative changes occurring during the early stages of starch degradation.

Curves for three types of whole starches were prepared, corn starch, rice starch, and wheat starch (Fig. 1). The shapes of these curves were

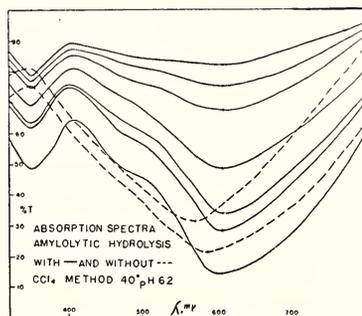
alike, including the position of maximum absorption at $610\text{ m}\mu$, and they correspond closely to the curves prepared by Simerl and Browning from crystalline corn amylose (3). The slight difference is probably due to the difference in standards used.

The hydrolysis experiments were performed with a sample of amylose prepared from rice starch by the method of Schoch (2).



Aliquots were removed from the hydrolysates at suitable time intervals and absorption spectra were run both with and without shaking with carbon tetrachloride.

Hydrolysis of amylose by beta-amyase produced substances, the spectra of which were similar throughout the range which could be followed, and there was no apparent shift in the position of the maximum absorption (Fig. 2). However, curves produced from these

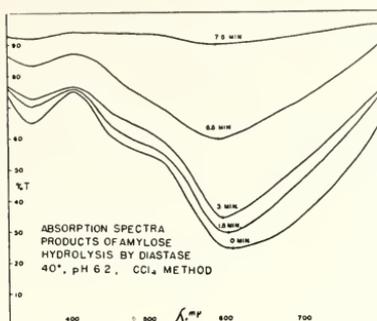


iodine complexes without extraction by carbon tetrachloride showed a definite shift in the location of the maximum toward the blue end of the spectrum.

The spectra of the hydrolysis products resulting from the action of malt diastase showed a continually changing picture and a progressive shift of the position of the maximum both before and after extraction with carbon tetrachloride (Fig. 3). The transmittancy increased steadily in both cases. Such a shift has been previously reported by Hanes and Cattle (1) and by Swanson (4). This latter paper in addition to

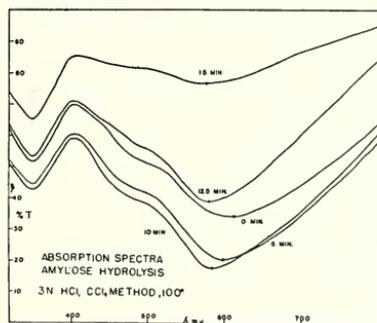
numerous others, indicates that this phenomenon is due to the appearance of increasingly shorter dextrans.

Acid hydrolysis (100° C, 0.3 N HCl) appeared to be much the same as hydrolysis by diastase except that there was a marked increase in the



apparent concentration of higher dextrans and a simultaneous shift in the position of the maximum immediately after bringing the mixture to boiling temperature (Fig. 4).

The fact that the position of maximum absorption did not change during hydrolysis by beta-amylase and after extraction with carbon



tetrachloride indicates the presence of unhydrolyzed amylose. At the same time very short chain dextrans are undoubtedly present as indicated by the shift in the location of the maximum when determined without extraction. The co-existence of these two types of molecules, at any given time after hydrolysis has started, infers the formation of a tightly bound enzyme-substrate complex in which the enzyme remains tightly bound until the macromolecule is reduced to a very short dextrin. Such a complex has been previously postulated by Swanson (4) for this enzyme.

The sudden increase in apparent concentration at the start of acid hydrolysis is believed to be due to the splitting of very long molecules near their center to produce dextrans of a sufficient length to increase the apparent color intensity. This phenomenon does not occur in

enzymatic hydrolysis, nor is it reported to occur during acid hydrolysis at 30° C. (4).

Although no quantitative studies have been presented here, it is felt that this method might well be adapted to the study of the kinetics of starch degradation, the determination of bound iodine, and the estimation of the amylose content of whole starches.

Summary

A method for studying the absorption spectrum of the amylose iodine complex in the presence of amylopectin has been described.

The method has been applied to study the hydrolysis of amylose. The results suggest that beta-amylase progressively hydrolyzes each amylose molecule until a very short dextrin molecule results, whereas acid hydrolysis initially breaks the molecules of amylose into large fragments.

Literature Cited

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