[CONTRIBUTION FROM THE IOWA AGRICULTURAL EXPERIMENT STATION]

Amylose and Amylopectin Content of Starches Determined by their Iodine Complex Formation[1]

BY F. LESLIE BATES, DEXTER FRENCH AND R. E. RUNDLE

For many years the chemical literature has contained references to starch fractionation. The methods employed have been numerous and the products obtained by different methods have generally been at variance in their chemical and physical properties.[2]

Recently, however, three methods of fractionation have been developed which appear to give nearly identical results. The methods are (a) hot water extraction of one component,[3] (b) selective adsorption of this same component on cellulose,[4] and (c) the selective precipitation of this component by butanol and other alcohols.[5] In each case one fraction stains blue with iodine, is converted almost completely to maltose by β-amylase, retrogrades easily, and furnishes good quality X-ray diffraction patterns in "A," "B" and "V" modifications. The other fraction stains purple to red with iodine, is only partially converted to maltose by β-amylase, retrogrades with difficulty or not at all, and yields poor or even amorphous diffraction patterns regardless of treatment.

Meyer has made a thorough study of the two components of his fractionation,[6] and has concluded that the hot-water extracted fraction is straight-chain starch, *i. e.*, glucose residues united exclusively by α-1,4-glucosidic linkages, the other fraction is similar but highly branched, probably through α-1,6-glucosidic linkages.[7] He has used the term, "amylose" to designate the straight-chain fraction, and "amylopectin," the branched-chain fraction.[8]

At the present time the proof of the structure of the two starch components is based almost entirely upon quantitative methylation studies. These are executed only with difficulty and can easily lead to erroneous interpretation, so that Meyer's results have not been accorded universal acceptance.[8]

Indeed, far from accepting the proposed structures of amylose and amylopectin, the existence of two well-defined components in starch is still questioned.[9] Probably the best evidence to date in favor of the two component theory of starch is the selective precipitation of Schoch,[5] giving nearly quantitative separation.

In this Laboratory the properties of starch fractions have been examined recently by physical and chemical methods. The property that has provided the greatest distinction between the fractions has been the reaction with iodine to form complexes. The study of this phenomenon has given overwhelming evidence for the two component theory, and has provided a method for the rapid analytical determination of the two components present in starch.

Since Meyer[8] has clearly defined "amylose" and "amylopectin" in terms of structure, and since for independent reasons[8] the authors are convinced that Meyer's interpretation of the structural differences in amylose and amylopectin is correct, the authors will use the terms "amylose" and "amylopectin" to designate the two components of starch. In so doing the authors wish to point out that the newer definitions of the terms are not always in accord with previous ambiguous definitions in the literature.

Reaction of Amylose and Amylopectin with Iodine

Since the color of iodine-stained amylose is blue and that of amylopectin is purple to red, it

(1) Journal Paper No. J-1045 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 660. Supported in part by a grant from the Corn Industries Research Foundation.

(2) For a review see K. H. Meyer, "Advances in Colloid Science," Interscience Publishers, Inc., New York, N. Y., 1942, pp. 142–162.

(3) This method has been revived and modified by K. H. Meyer, W. Brentano and P. Bernfeld, *Helv. Chim. Acta*, **23**, 845 (1940).

(4) C. Tanret, *Compt. rend.*, **158**, 1353 (1914). This method was rediscovered by E. Pacsu and J. W. Mullen, THIS JOURNAL, **63**, 1168 (1941).

(5) T. J. Schoch, *Cereal Chem.*, **18**, 121 (1941).

(6) K. H. Meyer, *et al.*, a series of papers in *Helv. Chim. Acta*. For a summary of the results and complete references, see ref. 2.

(7) K. Freudenberg and H. Boppel, *Naturw.*, **28**, 264 (1940).

(8) As Meyer has pointed out,[2] many of the physical properties of the two components can be explained on the basis of the above structures. An independent check on the general structure is given by R. E. Rundle and R. R. Baldwin, THIS JOURNAL, in press. They find that amylose is easily oriented by flow, while amylopectin is not. Since amylopectin has the larger molecular weight,[2] the difference in orientation during flow can be explained only on the basis of molecular shape. These results suggest a branched structure for amylopectin.

(9a) R. W. Kerr and O. R. Trubell, *Cereal Chem.*, **18**, 530 (1941); (b) R. W. Kerr, O. R. Trubell and G. Severson, *ibid.*, **19**, 64 (1942); (c) Alsberg, *Plant Physiol.*, **13**, 295 (1938); (d) Badenhuizen, *Rec. trav. botan. neerland.*, **35**, 559 (1938); (e) Badenhuizen, *Protoplasma*, **33**, 440 (1939).

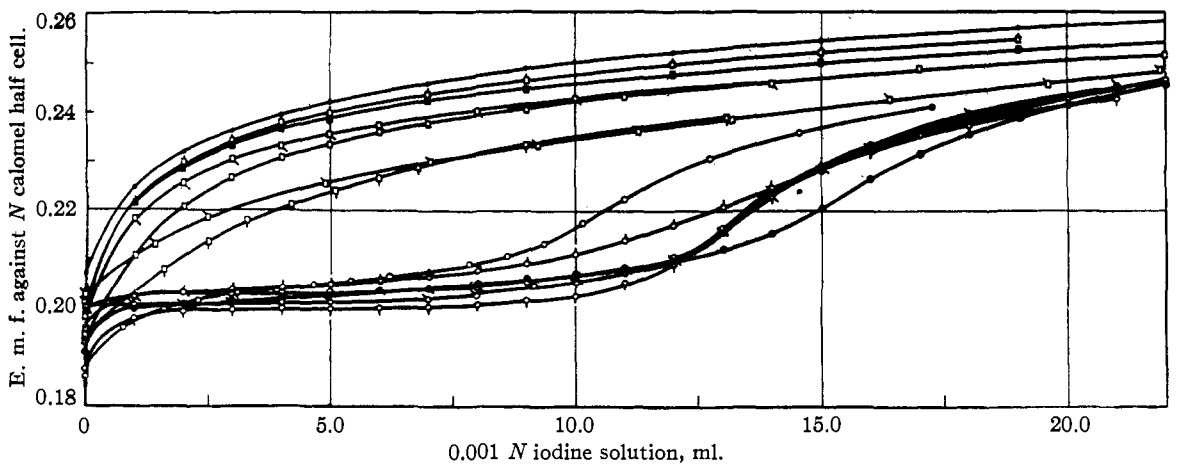

Fig. 1.—The uppermost curve is a titration of 0.05 *N* potassium iodide.

Titration of amylose materials 100 ml. of 0.01% solution		Titration of amylopectin materials 100 ml. of 0.04% solution	
○	gamma amylose	□	waxy barley
○	butanol ppt. from potato	■	waxy rice
○	butanol ppt. from corn	□	waxy maize
○	butanol ppt. from lily bulb	□	glycogen
○	synthetic starch	□	corn amylopectin by Schoch (after treatment with cotton)
●	Kerr's "crystalline amylose"	□	potato amylopectin by Schoch

seemed likely that a difference in the ability of amylose and amylopectin to bind iodine in complex formation might exist. Accordingly, solutions of amylose and of amylopectin were titrated potentiometrically with iodine, and the iodine bound by complex formation was determined. The results were very striking, and can best be compared by reference to Fig. 1. It is seen that the activity of iodine in an amylose solution remains essentially constant upon addition of iodine until complex formation is complete, while there is a continual rise in the iodine activity when iodine is added to an amylopectin solution. In Fig. 2 it can be seen that starches containing both components show a break in the curve, potential *vs.* iodine added. The point of inflection might then be expected to give a measure of the amylose fraction present in the starch.

Analytical Procedure

Completely dispersed starch is a prime requisite in this method, and for this reason solutions containing only a few hundredths of a per cent. of starch are used. The sample may be dispersed in several ways, but the use of alkali is recommended.

In our procedure 0.01 to 0.04 g. of starch is dispersed in 10 ml. of 0.5 *N* potassium hydroxide. Although the ease with which complete dispersal is attained varies greatly with the type of starch, alkali works much more effectively on dry material. When dispersal is complete, the mixture is diluted with distilled water, made neutral to methyl orange by the addition of hydriodic acid, and then diluted to 100 ml. The solution thus prepared is 0.05 *N* in potassium iodide.

Though a wide range of concentrations of iodide may be used in the titration, the authors find that the amount of iodine taken up by amylose decreases with increasing iodide concentration, so that a standard iodide concentration must be adopted.[10] The choice of 0.05 *N* iodide allows the use of a convenient amount of alkali of the strength required for dispersing the starch. It is also sufficient to prevent any appreciable change in the iodide concentration due to triiodide formation during the course of the titration.

The solution, which should be slightly acid, is titrated with 0.001 *N* iodine solution containing potassium iodide of the same normality as that of the solution being titrated. The exact value of the *p*H is not important, since the potential of the iodine–iodide half cell is nearly independent of *p*H.

In carrying out the titration, it should be borne in mind that a slow, non-ionic reaction is involved. If the starch is well dispersed, however, equilibrium is nearly always reached two to five minutes after the addition of 1 ml. of 0.001 *N* iodine solution, the shorter interval being sufficient in most cases. If the change of potential near the end of the interval takes place at a rate exceeding a few tenths of a millivolt per minute, more time should be allowed between successive additions of titrating solution.

A Leeds and Northrup type K potentiometer was used in conjunction with a normal calomel cell and a bright platinum electrode. Any galvanometer permitting adjustment of the potentiometer within 0.1 mv. may be used.

Starches.—The whole starches that were used in this investigation were, for the most part, milled in this Labora-

(10) The amount of iodide in the complex apparently increases with the increasing iodide concentration. A complete study of the iodide concentration will be reported in a subsequent paper.

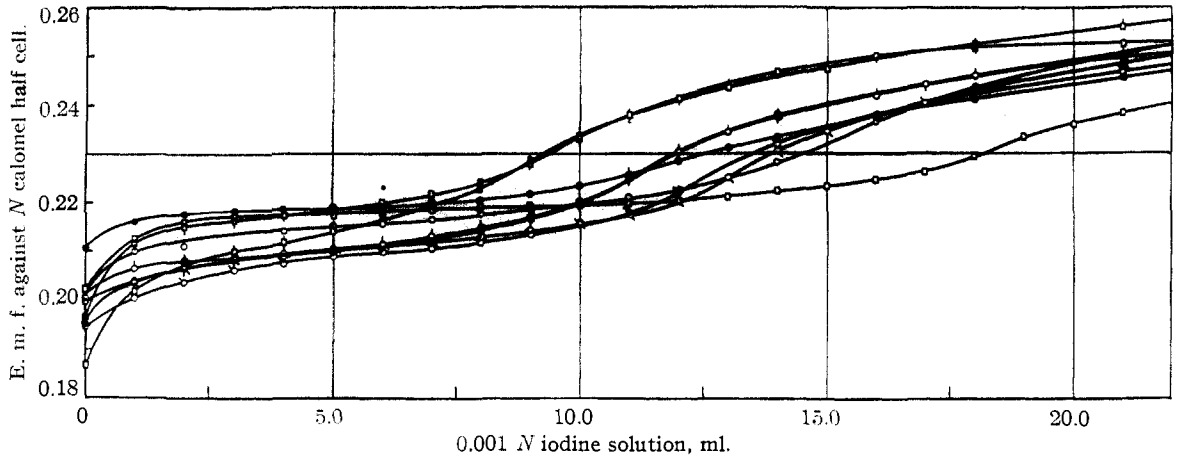

Fig. 2.—Titration curves of some whole starches: 100 ml. of 0.04% solution; O sago, ○ popcorn, □ lily bulb, ◌ banana, ◌ wheat, ◌ rice, ○ corn, ● potato, ▽ tapioca.

tory. Dr. T. J. Schoch supplied samples of the amylose and amylopectin components prepared by his butanol fractionation.[5] He also provided details of his fractionation procedure, which enabled the authors to separate the components of lily bulb starch. The "crystalline amylose" was prepared by Dr. R. W. Kerr, who also supplied a sample of his "gamma-amylose."[11] Dr. W. Z. Hassid provided a sample of the synthetic starch that he has prepared in quantities sufficient for methylation studies.[12] Lily bulb starch was provided by Dr. S. L. Emsweller.[13]

Standardization of the Analysis

All attempts to find a stoichiometric formula for the starch–iodine complex have failed.[14] Consequently, the end-point of the reaction between amylose and iodine had to be determined experimentally. To do this it was necessary to accept as pure amylose the amylose fraction which takes up the most iodine. On this basis, Kerr's "crystalline amylose" is the best of the amylose fractions yet prepared (Fig. 1). It is satisfying to note that it is also the amylose fraction which has been subject to the most careful refractionation, and the one showing the best crystalline properties. It would then appear that all the other amylose fractions are contaminated to some extent with the amylopectin fraction.

The point of inflection in the curve, potential *vs.* milliliters of iodine added, may be looked upon as determining the end-point of the titration. Such an assumption may not be perfectly valid in the case of the titration of amylose–amylopectin mixtures, since amylopectin takes up some iodine, as may be seen in Fig. 1. A more accurate idea of what is occurring at the inflection point may be obtained by plotting the amount of iodine bound per gram of starch against the concentration of free iodine in the solution, as shown in Fig. 3. The curve in Fig. 1 for 0.05 *N* potassium

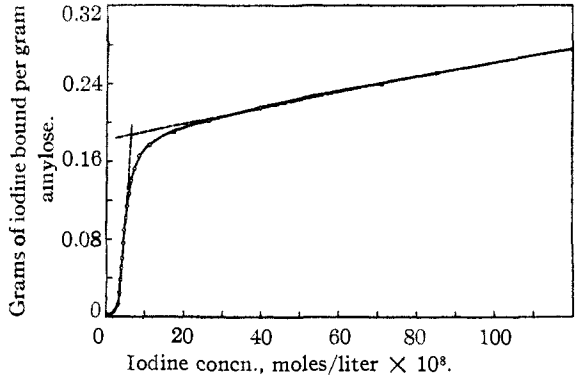

Fig. 3.—Amount of iodine bound by "crystalline amylose" as a function of iodine concentration.

iodide may be used to calculate the concentration of free iodine corresponding to any potential. This is subtracted from the total iodine to get the bound iodine. Nearly all the added iodine is bound by complex formation, until the vicinity of the inflection point is reached, after which the increase in bound iodine appears to depend upon a proportional increase in the concentration of free iodine. This latter binding of iodine appears to be adsorption, and is distinctly different from complex formation.[15] It would appear that

(11) R. W. Kerr and O. R. Trubell, *Cereal Chem.*, **18**, 530 (1941).
(12) W. Z. Hassid and R. M. McCready, THIS JOURNAL, **63**, 2171 (1941).
(13) U. S. D. A. Experiment Station, Beltsville, Maryland.
(14) G. Barger, "Some Applications of Organic Chemistry to Biology and Medicine," McGraw-Hill Book Co., Inc., New York, N. Y., 1930, pp. 127–176.
(15) For the mechanism involved in the formation of the starch-iodine complex, see R. E. Rundle and R. R. Baldwin, THIS JOURNAL, in press, and R. E. Rundle and Dexter French, THIS JOURNAL, in press.

adsorption accounts for practically all the iodine bound by amylopectin. If this assumption is correct, the amount of iodine involved in complex formation only may be determined by finding the intersection of the adsorption curve with the vertical line representing the complex formation.

By this method amylose is found to take up 18.7% iodine at the end-point in 0.05 *N* potassium iodide solution. Mixtures of amylose and amylopectin may be analyzed on this basis. Figure 4 contains curves illustrating the titration of mixtures of amylose and amylopectin in varying ratios. The quantitative aspect of these curves is evident in the equal spacing of the inflection points.

Analysis of Starch and Starch Fractions

Analyses of various whole starches are summarized in Figs. 1 and 2, and in Tables I and II. It can be seen that the amylose content runs from 0 to 34% in the whole starches. In general the amylose content is very low in the red-staining waxy or glutenous starches, and is generally about 22% in the other starches. It is to be noted that the amylose content may be quite high in both cereal and tuber starches, and that differences in the properties of these starches are not to be attributed to the ratio of the two components.

In Table I the percentages of amylose in various starches, as determined by butanol fractionation and iodine titration, are compared. It is to be noted that in every case where comparisons have been made the results are in good agreement.

TABLE I

COMPARISON OF METHODS FOR THE DETERMINATION OF AMYLOSE IN STARCH

	Amylose, %		
	Iodine titration	Butanol fractionation	
Starch		By Schoch[a]	By authors
Corn	21	22	...
Potato	22	22	22.5
Lily bulb	34	..	31
Waxy corn	None	None	Negligible

[a] T. J. Schoch, THIS JOURNAL, 64, 2957 (1942).

TABLE II

AMYLOSE CONTENTS OF STARCHES DETERMINED BY IODINE TITRATION

Starch	Amylose, %	Starch	Amylose, %
Waxy rice	0	Corn	21
Waxy sorghum	0	Potato	22
Waxy corn	0	Popcorn	23
Waxy barley	0	Wheat	24
Tapioca	17	Sago	27
Rice	17	Lily bulb	34
Banana	20.5		

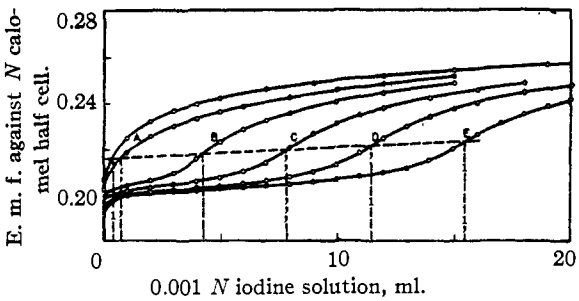

Fig. 4.—Titration of mixtures of amylose and amylopectin: A, 0.01 g. of amylopectin from potato starch; B, 0.0075 g. of amylopectin from potato starch plus 0.0025 g. of "cryst. amyl"; C, 0.0050 g. of amylopectin from potato starch plus 0.0050 g. of "cryst. amyl"; D, 0.0025 g. of amylopectin from potato starch plus 0.0075 g. of "cryst. amyl"; E, 0.01 g. of "cryst. amyl." The uppermost curve is a titration of 0.05 *N* KI for comparison.

Discussion

Though the heterogeneity of starch is no longer seriously questioned, the nature and degree of this heterogeneity has not been well established. The physical and chemical evidence for branched starch chains is, in the opinion of the authors, unquestionable. It appears then that the molecular species present in starch may differ both in size and in degree of branching. Both the chemical and physical properties of starch fractions indicate that present methods of fractionation separate starch into components differing primarily in the degree of branching. The heterogeneity that may result from this factor will be considered first.

Heterogeneity in degree of branching of the molecules present in a starch could be either continuous or discontinuous, *i. e.*, all degrees of branching between completely straight chains and highly branched chains might be represented, or a limited number of components might be present, each with a fair uniformity in degree of branching and separated from other components by a significant difference in this property. Early fractionation procedures gave such a variety of products that the idea of a continuous variation seemed most acceptable. Schoch's nearly quantitative separation[5] of starch into two distinctly different components by a single precipitation would seem to require a considerable discontinuity in the degree of branching. Such a concept is also required by the work reported here.

In the opinion of the authors, the results of this investigation, in conjunction with previous work on starch fractionation, are sufficient to prove

that most starches have two, and only two, components differing significantly in the degree of branching. The sharp breaks in the iodine titration curves certainly indicate that the molecular species that may possibly be present in starch possess a marked discontinuity in their ability to react with iodine. Components with intermediate degrees of branching would most certainly have intermediate properties. These do not appear to be present in the starches studied.

For example, the single precipitation of amylose with butanol, as carried out by Schoch, is not absolutely sharp when applied to cornstarch. The amylopectin fraction takes up an appreciable amount of iodine (Fig. 5), a fact that could be construed as evidence for the presence of material possessing the power of taking up iodine to an intermediate degree and, therefore, possessing a branched structure of intermediate character. However, the amount of iodine bound may also be accounted for by assuming that 8% of the crude amylopectin material is actually amylose impurity. Upon treating the material with cellulose, after the method used by both Tanret and Pacsu,[4] about 6% of the material is removed, the break in the curve disappears (Fig. 5), and the iodine color of the solution changes from blue to reddish-purple. All the properties thus disclosed are those of amylose and not of a component of intermediate character.

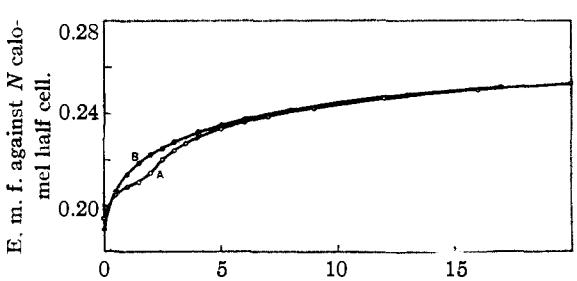

Fig. 5.—Titration of amylose fraction from cornstarch (Schoch's fractionation): A, before treatment with cotton; B, after treatment with cotton.

This work is sufficient, too, to prove that the degree of branching does not vary radically from region to region in any one molecule. It would be expected that a long unbranched portion of a molecule would behave like an unbranched molecule. It is found, however, that all the material which forms an amylose-like complex with iodine can be removed from the material which will not form such a complex. It can be safely assumed, then, that the amylopectin contains no long, unbranched portions.

These arguments provide no real proof that the amylose is free of branched portions. Since, however, Meyer and co-workers[16] have found in the case of amylose that the number of reducing ends is, within the experimental error, equal to the number of non-reducing ends, and, furthermore, that the molecular weight based on the number of non-reducing end-groups checks very well with that found by physical methods, the branching in the amylose fraction must be quite negligible. Meyer[17] has also shown that the degradation of amylose by β-amylase proceeds practically to completion. This point has been confirmed by Kerr and Severson,[18] and others working with other amylose preparations. If β-amylase does not degrade starch chains beyond the first branch-point it encounters, then amylose molecules possess a negligible number of branch points. By the same reasoning, amylose cannot consist of long straight chains linked together by branch-points, since a structure of this kind would allow a single branch to protect a long chain from β-amylase degradation.

It appears, then, that amylose may be regarded as an essentially unbranched chain of glucose residues, while amylopectin is branched, and with no large unbranched portion in the molecule. In this sense the starches examined contain two components, one unbranched and one branched, with no material of an intermediate degree of branching present; or, more specifically, variations in the degree of branching in the amylopectin are very small as compared with the variation between amylose and amylopectin.

This does not imply, of course, that amylose or amylopectin represent homogeneous molecular species; certainly each will have some heterogeneity as to molecular size. In addition there may be some variation in the degree of branching in amylopectin. The latter certainly is true for amylopectins from various starch fractions, since amylopectins from different starches do not behave alike in the iodine titration (Fig. 1). The curves of Fig. 1 are quite reproducible, and any one of them is characteristic of the amylopectin

(16) K. H. Meyer, M. Wertheim and P. Bernfeld, *Helv. Chim. Acta.*, **23**, 865-875 (1940).

(17) K. H. Meyer, P. Bernfeld and J. Press, *ibid.*, **23**, 1465 (1940).

(18) Paper presented before the Memphis meeting of the American Chemical Society, April, 1942 (joint meeting of the Division of Sugar Chemistry and Technology and the Division of Agricultural and Food Chemistry), THIS JOURNAL, **65**, 193 (1943).

from a given starch source, *i. e.*, all the corn examined produced an amylopectin fraction which, upon titration, gave a curve characteristic of corn amylopectin, and quite distinct from that of potato amylopectin, etc. It is noteworthy that glycogen, which is most highly branched according to methylation studies,[19] has the least affinity for iodine. Meyer[20] finds that the amylopectin from potato starch is more highly branched than that from corn. It also has less affinity for iodine (Fig. 1). As far as it has been tested it would appear that the more highly branched the amylopectin, the less the affinity for iodine.

The degree of the homogeneity of the branching in the amylopectin fraction from any one starch source is not as yet known. That the variation cannot be a great one has already been seen. The fact that an amylopectin from a particular source has a characteristic degree of branching which distinguishes it from amylopectins from other sources would suggest that the degree of branching is a fairly specific property of any one amylopectin. Meyer, on the basis of the enzyme digestibility of various amylopectins, was led to the same conclusion, and he has even attempted to find a pattern for the branching.[17]

Further light on the nature of the heterogeneity is obtained by the following calculation. Whole starch from potato or corn has been found by many investigators[21] to contain about 4% end-groups, corresponding to about one branch point in every 25 glucose residues, while amylose contains, on the average, not more than one end-group in 200 glucose residues. In the whole starch, then, the chance that any one glucosidic link is not a branch link is 24/25. If one assumes that branch points occur at random in the starch chains, the chance that a chain be straight for n linkages is $(24/25)^n$. Since $(24/25)^{200}$ is 0.0003, we should expect, on this basis, only 0.03% of the material to be as straight chain as amylose. If we allow a factor of 2 for the inaccuracies of the methylation results, we find $(24/25)^{100}$ is 0.017, or 1.7% of the starch should possess chains straight for 100 or more glucose residues. Over 20% amylose is found in both corn and potato starches. Clearly anything resembling a random distribution of branch links in the starch chains will not account for the high percentage of amylose in many starches. It would again appear that the position of the branch link is determined by something other than chance in the synthesis of starch.

It is interesting, in this regard, that the synthetic starch of Hassid appears to be essentially straight chain, both according to his methylation studies[12] and according to the iodine titration. Thus far, all the starches produced *in vivo* have been found to contain much less amylose than amylopectin. It seems possible that starch is synthesized by an enzyme system and that the enzyme or enzymes responsible for branching are absent from the system usually used *in vitro*.[22] The difference in degree of branching of starch produced *in vitro* and starch produced *in vivo* may, of course, be due to external conditions. This appears less likely when one considers that the waxy characteristic (the ability to synthesize starch free of the amylose component) is a genetic factor in plants. It seems unlikely that conditions can be greatly different in waxy maize and ordinary maize kernels, for example. It also seems unlikely that some molecules should remain unbranched throughout while some molecules should be very branched within the same starch granule if external conditions played the determining role in branching.

The inhomogeneity in chain length of amylose fractions is also probably less than would ordinarily be suspected. In Fig. 1 it is seen that amyloses from different starch sources have slightly different potentials for complex formation. These differences are beyond the experimental error and quite reproducible. These differences the authors attribute to different chain lengths of amylose from different sources.

In Fig. 6 are shown results of titrations of mixtures of corn and potato amylose prepared by Schoch's fractionation. The break in the curve, corresponding to the difference in iodine potential necessary for corn and potato amylose–iodine complex formation, is clearly visible in each titration. These results can only mean that the inhomogeneities of corn and potato amylose are not so great that they overlap each other. Interpreting the results in terms of chain lengths,

(19) K. H. Meyer and M. Field, *Helv. Chim. Acta*, **24**, 375 (1941).

(20) K. H. Meyer, M. Wertheim and P. Bernfeld, *ibid.*, **24**, 382 (1941).

(21) E. L. Hirst, *J. Chem. Soc.*, 2375 (1932).

(22) Synthetic glycogen has been prepared using enzymes from both liver and muscle. See C. F. Cori, *Endocrinology*, **26**, 285–296 (1940). Although the products do not appear uniform, and have not received the same study that has been applied to synthetic starch, the iodine colors reported for these synthetic glycogens suggest that they possess far straighter chains than does glycogen produced *in vivo*.

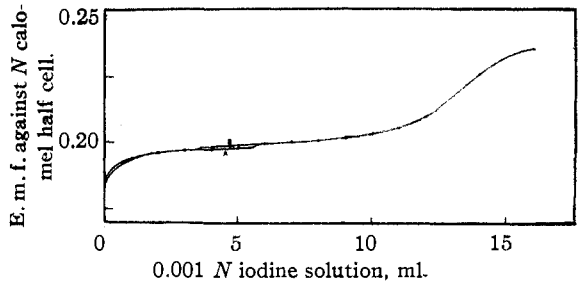

Fig. 6.—Titration of mixtures of corn and potato amyloses: A, 0.0067 g. of potato + 0.0033 g. of corn amylose; B, 0.0033 g. of potato + 0.0067 g. of corn amylose.

as the authors are inclined to do, the results would mean that amyloses from corn and potato have different chain lengths, and the inhomogeneity in the chain length of the amylose from neither source is so great that there is an appreciable overlap in the ranges of chain lengths of the two amyloses.

Preliminary studies on molecular size by viscosity techniques[23] indicate that potato amylose has a greater molecular weight than corn amylose. This result in conjunction with the data in Fig. 1 would mean that the shorter amylose chains require higher iodine potentials for complex formation. This is confirmed by the titration of very short but essentially straight chain amylodextrin (Fig. 7). This material requires a very much larger iodine activity for complex formation than potato amylose. Obviously this point deserves further study.

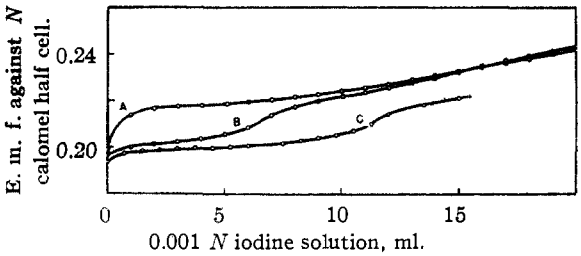

Fig. 7.—Titration of a mixture of amyloses of different chain lengths: A, 0.01 g. of amylose from amylodextrin; B, 0.005 g. of amylose from amylodextrin plus 0.005 g. of potato amylose; C, 0.01 g. of potato amylose.

(23) R. M. Hixon and J. F. Foster, THIS JOURNAL, to be published.

Summary

1. Starch has been shown to possess two components which are quite distinct in their reaction with iodine to form iodine complexes.

2. A potentiometric method has been developed for the rapid quantitative determination of the amylose components of starch.

3. The analytical method has been applied to a number of starches and starch fractions.

4. The amount of iodine bound by the amylose component of starch varies inversely with the iodide concentration.

5. Preliminary results indicate that affinity for iodine varies inversely with the degree of branching of the starch chains.

6. Preliminary results indicate that affinity for iodine varies directly with the length of the starch chain.

7. The amylose component of any one starch appears fairly homogeneous in chain length.

8. The synthetic starch of Hassid, in agreement with his methylation studies, appears to be essentially amylose.

AMES, IOWA RECEIVED SEPTEMBER 21, 1942

[CONTRIBUTION FROM THE LABORATORIES OF INSTITUTUM DIVI THOMAE]

The Ultraviolet Absorption Spectra of Nitrogenous Heterocycles. V. The Blocking Effect of Methyl Groups on the Ultraviolet Absorption Spectra of Some Hydroxypurines and Pyrimidines[1]

BY JOHN R. LOOFBOUROW, SR. MIRIAM MICHAEL STIMSON, O.P., AND SR. MARY JANE HART, O.P.

It has previously been shown that the change in the absorption of uracil may be ascribed to lactim–lactam isomerism.[2] The lactam or hydroxy form was postulated to exist in alkaline solution on the assumption that it would tend to neutralize basicity. On the other hand Fromherz and Hartmann[3] on the basis of absorptiometry of uric acid concluded that its acid nature cannot depend on the complete or partial enolization and dissociation of the hydrogen from oxygen but rather on dissociation of hydrogen from an unsaturated nitrogen. Therefore, uric acid is assumed by these workers to be in the enol form in acid solution and in the keto form in alkaline solution. In order, therefore, to obtain further information as to the *p*H effect on hydroxy com-

(1) Presented at the Buffalo meeting, September, 1942.
(2) Heyroth and Loofbourow, THIS JOURNAL, **53**, 3441 (1931).
(3) Fromherz and Hartmann, *Ber.*, **69**, 2420 (1936).