A Simplified Colorimetric Procedure for Determination of Amylose in Maize Starches

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ABSTRACT

When iodine is dissolved in mixtures of water and dimethylsulfoxide (Me_2SO), triiodide ion is formed; the extent of triiodide formation is dependent upon the proportion of Me_2SO in the mixture. A method has been developed to utilize this reaction to form the blue amylose-iodine complex, providing a simple procedure for quantitative measurement of amylose in starches. Starch is dissolved in a mixture of 90% Me_2SO and 10% water containing iodine. Upon dissolution of the starch, the mixture is diluted with water to give a final Me_2SO concentration of 10%, whereupon the blue amylose-iodine complex forms immediately. Amylose is then determined by measurement of the absorbance of the complex at 600 nm. Use of Me_2SO to generate triiodide ion eliminates the necessity for preparing buffered solutions of iodine in potassium iodide. Accuracy and sensitivity of the procedure is equivalent to that of the conventional methods of preparing the amylose-iodine complex.

Determination of the amylose content of starches is commonly accomplished by measurement of the blue amylose-iodine complex. This measurement can be done quite precisely by potentiometric titration (Banks et al 1971, Knutson et al 1982) but is more conveniently done colorimetrically. The first colorimetric procedure for quantitative estimation of amylose was reported by McCready and Hassid (1943). Because of the diversity of starches from different sources, many modifications of the original procedure have been reported. Commonly used procedures for analysis of cereal starches are described by Williams et al (1970) and Wolf et al (1970). A recent report by Morrison and Laignelet (1983) describes an improved method that is rapid and accurate, and which provides a means for determination of both apparent amylose (measured in the presence of lipids) and total amylose (measured on lipid-free starch).

Formation of the amylose-iodine complex requires the presence of triiodide (I_3^-) ions in the reaction mixture to initiate the reaction (the actual nature of the iodine species in the complex has been extensively studied but not yet definitively determined). In all of the colorimetric procedures currently in use, triiodide is formed by dissolving iodine and potassium iodide in buffered solutions at neutral or slightly basic pH. However, it is possible to form the triiodide ion in a variety of basic solvents (e.g., pyridine) without addition of potassium iodide. The basic solvent, B, causes partial solvolysis and disproportionation of the I_2 molecule to give I^- and BI^+, whereupon the I^- reacts with the remaining I_2 to form the triiodide ion.

Dimethylsulfoxide Me_2SO, which is a moderately strong Lewis base, is known to form triiodide ion (Klaeboe 1964, Courtot-Coupez and Madec 1971) and is also an excellent solvent for starches (Wolf et al 1970). Thus, if both starch and iodine are dissolved in Me_2SO, an opportunity is presented to form the amylose-iodine complex in a single step, without the necessity of preparing buffer solutions. In addition, by avoiding the use of buffers, the risk of aggregation of the amylose-iodine complex is reduced. This paper describes the stoichiometry of the formation of triiodide ion in Me_2SO-water mixtures and the conditions necessary for formation of the amylose-iodine complex in such mixtures, and presents a rapid single-step method for determination of the amylose content of starches of widely varying composition. Some speculation regarding the nature of the iodine species involved in the complex formation is also included.

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MATERIALS AND METHODS

Iodine, potassium iodide, and Me_2SO were reagent-grade chemicals from commercial sources, used without further purification. The standard amylose sample had been extracted at 70° C from defatted corn starch and crystallized from an aqueous solution saturated with 1-butanol; molecular weight (by intrinsic viscosity) was 1.76×10^5, equivalent to a degree of polymerization (DP) of 1,100. Samples of dent corn, waxy maize, amylomaize V (50% amylose), and amylomaize VII (70% amylose) starches were obtained from American Maize-Products Co., Hammond, IN. Defatted starches were prepared by dissolving starch in 90% Me_2SO, precipitating with absolute ethanol, and redissolving in 90% Me_2SO.

Spectrophotometric measurements were done with a Cary model 14 recording spectrophotometer in 0.5-cm cells. Routine colorimetric measurements were done with a Beckman model DU spectrophotometer in 1-cm cells or with a Beckman model B spectrophotometer equipped to hold 19-mm matched test tubes.

Determination of total carbohydrate content of native and defatted samples in solution was done by the phenol-sulfuric acid method of Dubois et al (1956).

The stoichiometry of triiodide ion formation in Me_2SO-water mixtures was evaluated by measuring absorbance spectra of iodine solutions, from 0 to $6 \times 10^{-4} M$, in 0–100% Me_2SO, both in the presence and absence of potassium iodide (KI). The molar absorptivity of I_3^- in Me_2SO-water mixtures of varying composition was determined by extraction of a $1.25 \times 10^{-3} M$ iodine solution in hexane with an equal volume of Me_2SO-water mixture containing $0.01 M$ KI. Hexane is almost totally immiscible with Me_2SO-water mixtures. Any I_3^- formed was extracted into the Me_2SO-water layer, and unreacted I_2 remained in the hexane layer. The layers were separated and absorbance of each measured immediately. The amount of unreacted I_2 was determined by measuring the absorbance at 522 nm (molar absorptivity 900 in hexane). Absorbance of I_3^- in the Me_2SO-water layer was measured at maximum, 350–365 nm, and the molar absorptivity was calculated from the known amount of iodine that had been lost from the hexane layer. The same extraction procedure, using Me_2SO-water mixtures containing no KI, was used to evaluate the equilibrium of the I_3^- formation reaction.

The amylose-iodine complex was formed by dissolving 400 μg amylose in 10 ml of Me_2SO-water mixtures that contained varying iodine concentrations. The absorbance spectra were measured, and optimum conditions for complex formation were identified. These conditions were then incorporated into the following standard procedure, which was used to analyze starch samples of varying amylose content. Starch sample (1–5 mg) is dissolved in 10 ml 90% Me_2SO containing $6 \times 10^{-3} M$ iodine. Purified starch will dissolve overnight at room temperature; ground whole corn or endosperm requires heating to 50° C overnight. One milliliter of this solution is diluted with 8 ml of water and mixed thoroughly. The complex forms immediately and stabilizes to maximum absorbance within

30 min. After 30 min, absorbance is measured at 600 nm. At this wavelength, absorbance is 99% of maximum (at 620 nm), and it is not necessary to use such a wide slit width on the spectrophotometer. Apparent amylose, uncorrected for amylopectin, is calculated from a standard curve obtained with pure amylose; this value is corrected for amylopectin by the equation:

$$\% \text{ Amylose} = \frac{\% \text{ Apparent amylose} - 6.2}{93.8}.$$

RESULTS

Absorbance of the triiodide ion in varying concentrations of iodine and Me_2SO is shown in Figure 1. In 100% Me_2SO the absorbance changes linearly with iodine concentration. At lower Me_2SO content, there is an initial lag in I_3^- formation until iodine concentration reaches $2 \times 10^{-4} M$, beyond which absorbance again varies linearly with iodine concentration, with the extent of variation dependent upon the Me_2SO content of the solution. Maximum absorbance in 100% Me_2SO occurs at 365 nm and shifts downward as Me_2SO content is decreased, to 350 nm at the lowest detectable absorbance.

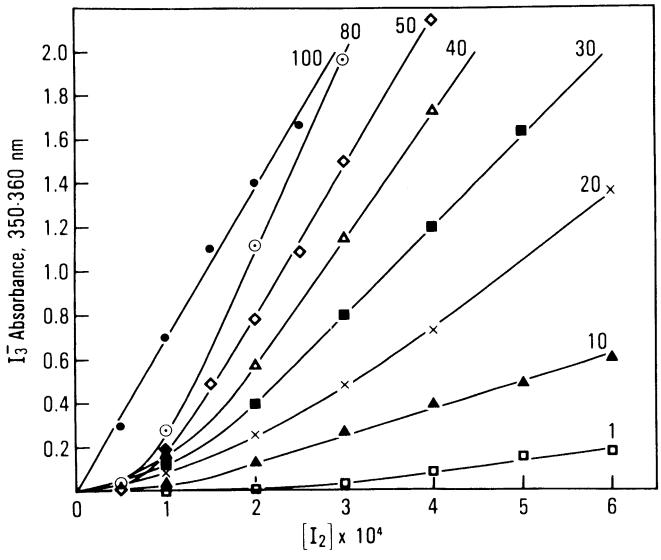

Fig. 1. Variation of absorbance with iodine concentration in Me_2SO-water mixtures: ●——●, 100% Me_2SO; ⊙——⊙, 80%; ◇——◇, 50%; △——△, 40%; □——□, 30%; x——x, 20%; ▲——▲, 10%; □——□, 1%.

TABLE I
Effect of Potassium Iodide on the I_3^- Absorbance in Me_2SO-Water Mixtures[a]

		KI Concentration, Molar			
% Me_2SO	λ max	0	10^{-3}	10^{-2}	10^{-1}
0	353	0	0.540	0.942	1.070
10	355	0.054	···	0.845	1.005
50	360	0.248	0.680	0.885	0.998
100	365	0.702	1.068	1.126	1.226

[a] Initial iodine concentration $5.1 \times 10^{-5} M$; path length 1 cm.

TABLE II
Molar Absorptivity of I_3^- in Varied Concentrations of Me_2SO-Water

% Me_2SO	Molar Absorptivity
0	22,306
10	23,172
20	26,417
30	24,919
40	26,493
50	26,118
60	25,663
80	26,811
100	26,411
Mean (20–100%)	26,119
SD	637.8

The addition of KI to iodine-Me_2SO-water mixtures increases the absorbance, as shown in Table I. When I^- is provided in the form of KI, more I_2 can react directly to form I_3^-; at I^- concentrations higher than $10^{-2} M$, essentially all I_2 present can go into I_3^- formation. That the absorbance is not constant at the higher KI concentrations indicates a change in the equilibrium of the reaction with varying Me_2SO content, or a variation in the molar absorptivity of I_3^-.

To determine the molar absorptivity of I_3^- at varying concentrations of Me_2SO, the hexane extraction procedure described in the Methods section was used. Results are given in Table II. The molar absorptivity of I_3^- in aqueous solutions is 26,400 (Awtrey and Connick 1951). The values in the table agree well with this value, except at the lowest concentrations of Me_2SO. However, under the conditions of this experiment, little I_3^- is formed and extracted into the Me_2SO-water layer at these Me_2SO concentrations (0 and 10%), so the absorbance measurement is subject to correspondingly larger errors. If the 0 and 10% Me_2SO values are disregarded, the average of the remaining values is 26,119, which is in good agreement with the reported value.

The same extraction experiment without KI in the mixtures was then conducted to study the equilibrium of the I_2-I_3^- formation at varying concentrations of Me_2SO. Theoretical maximum I_3^- concentration was calculated assuming two I_2 molecules consumed for every I_3^- ion formed. Results are shown in Table III. Above 80% Me_2SO, I_3^- formation is essentially 100%, whereas below 10% Me_2SO there is essentially no I_3^- formed. In the intermediate concentrations I_3^- variability is dependent upon Me_2SO concentration, and there is a significant amount of iodine not accounted for either as I_2 or I_3^-. This iodine presumably has been disproportionated to form I^- but has not reacted with molecular iodine to form I_3^-.

Various compositions of I_2-Me_2SO-water were then evaluated for their capacity to form the amylose-iodine complex. Absorbance values for solutions of 400 μg amylose in 10 ml of Me_2SO-water mixtures containing 1 to $6 \times 10^{-4} M$ iodine are shown in Figure 2. Amylose-iodine complex could be formed in Me_2SO concentrations up to 40%. Maximum binding occurred with Me_2SO concentrations of 10% or less and at iodine concentrations of 4×10^{-4} or higher. In 40% Me_2SO maximum absorbance was at 570 nm, and at 10% or less the maximum was at 620 nm. In 40% concentrations the complex formed very slowly, requiring 60–90 min to reach maximum absorbance.

A sample of waxy maize starch, previously identified as amylose-free, was analyzed under the conditions of optimum amylose-iodine complex formation, i.e., $6 \times 10^{-4} M$ iodine in 10% Me_2SO, to determine the extent of interference from amylopectin. The

TABLE III
Concentration of I_2 and I_3^- at Equilibrium in Varied Concentrations of Me_2SO-Water[a]

	Concentration, $M \times 10^{-3}$			
% Me_2SO	$[I_2]$	$[I_3^-]$	Total $[I_2] + 2 \times [I_3^-]$	% of Theory
0	1.26	0	1.26	100.9
10	1.18	0.003	1.19	95.0
20	1.12	0.006	1.13	90.6
30	1.05	0.015	1.08	86.5
40	0.888	0.044	0.976	78.2
50	0.531	0.171	0.872	69.9
60	0.347	0.287	0.921	73.8
70	0.154	0.500	1.15	92.5
80	0	0.617	1.23	99.0
100	0	0.617	1.23	99.0

[a] Original iodine concentration, $1.25 \times 10^{-3} M$.

absorbances of amylose and amylopectin under these conditions are shown in Figure 3. Amylopectin has a small absorption band with a maximum at 520 nm and an extinction coefficient at 600 nm that is 6.2% that of amylose. On the basis of this evaluation, the standard procedure was established for the routine analysis of starch samples. The standard procedure was used to analyze both native and defatted samples of maize starches of varying amylose content. These results were compared to values obtained using conventional I_2-KI reagents as described by Wolf et al (1971) and Knutson et al (1982). Comparative results are shown in Table IV. The values obtained with iodine-Me_2SO on defatted starches are equivalent to the other procedures; native samples of high-amylose starches do not agree as well and have significantly higher standard deviations.

DISCUSSION

Stoichiometry of I_3^- Formation

The formation of I_3^- in Me_2SO solutions has been recognized for some time. Klaeboe (1964) mistakenly assumed that it was caused by impurities in the Me_2SO. Courtot-Coupez and Madec (1971) evaluated the formation of triiodide in Me_2SO-water mixtures in the presence of 0.1 *M* lithium perchlorate and speculated on the nature of the ionic species involved. The reaction is most probably analogous to the disproportionation of Cl_2 in Me_2SO described by Gavreau et al (1980). Such a reaction would proceed thusly:

$$A)\ (CH_3)_2SO + I_2 \rightleftarrows (CH_3)_2SO\text{-}I^+ + I^-.$$

The iodide thus formed would then combine with available molecular iodine in the usual manner for triiodide formation:

$$B)\ I^- + I_2 \rightleftarrows I_3^-$$

and the overall reaction would be

$$C)\ (CH_3)_2SO + 2I_2 \rightleftarrows (CH_3)_2SO\text{-}I^+ + I_3^-.$$

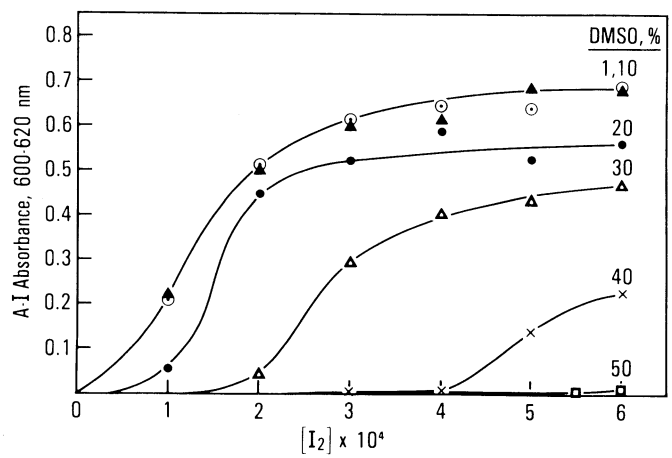

Fig. 2. Absorbance of amylose-iodine complex in varying concentrations of iodine and Me_2SO: ⊙——⊙, 1% Me_2SO; ▲——▲, 10%; ●——●, 20%; △——△, 30%; x——x, 40%; □——□, 50%.

The fact that the formation of I_3^- must occur in two steps serves to explain the lag in I_3^- formation observed in the data in Figure 1 and Table III. There must be an increase in either Me_2SO or I_2 to drive reaction A toward I^- formation; only after sufficient I^- is available, as well as sufficient unreacted I_2, can reaction B proceed to form I_3^-. As shown in Table III, all the I_2 in the system remains unreacted in the absence of Me_2SO, and is all converted to I_3^- in Me_2SO concentrations of 80% or higher.

It must be noted that the equilibrium established by extraction from hexane is quite different from that established in Me_2SO-

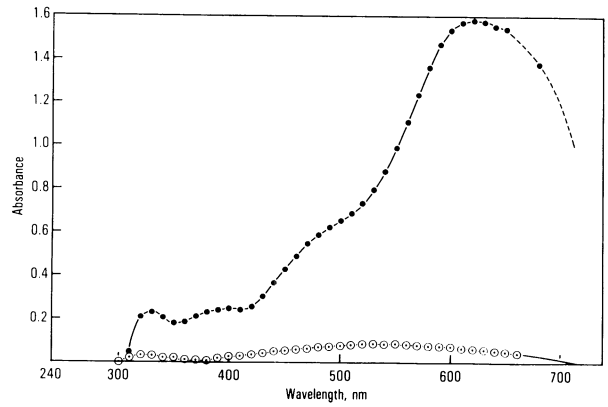

Fig. 3. Absorbance spectra of amylose-iodine complex (●——●) and amylopectin-iodine (⊙——⊙). Carbohydrate 40 mg/ml, 10% Me_2SO, 6 $\times 10^{-4}$ *M* I_2.

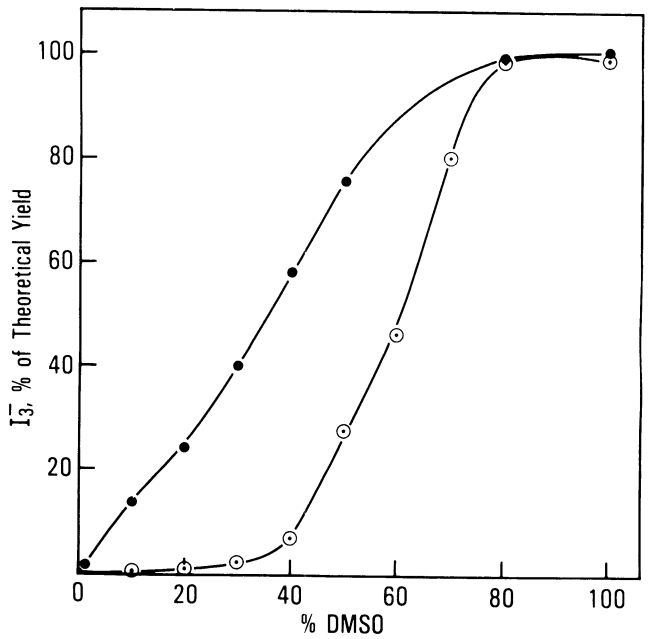

Fig. 4. Formation of I_3^- with increasing Me_2SO concentration: ●——●, single phase reaction; ⊙——⊙, hexane extraction reaction.

TABLE IV
Comparison of Amylose Values Obtained by Measurement of Amylose-Iodine Complex Formed in I_2-Me_2SO and in I_2-KI

	Amylose Content, %											
	I_2-Me_2SO						I_2-KI (defatted samples only)					
	Native			Defatted			Method A[a]			Method B[b]		
Sample	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Dent starch	8	23.6	1.7	5	22.9	1.2	10	21.9	1.5	8	23.0	0.9
Amylomaize V	8	47.9	4.5	5	48.9	1.3	8	48.8	2.1	5	51.4	2.0
Amylomaize VII	8	66.3	7.1	5	70.1	2.0	8	65.1	2.7	5	68.2	3.0

[a] Method A, Wolf et al, 1970.
[b] Method B, Knutson et al, 1982.

water mixtures alone at low Me_2SO concentrations. This is illustrated in Figure 4, where I_3^- concentrations from Figure 1 (at $3 \times 10^{-4} M$) and Table III are expressed as percentages of the theoretical value and plotted versus Me_2SO concentration. This difference in equilibria is to be expected in view of the low solubility of molecular iodine in water; at low Me_2SO concentrations, I_2 is retained in the hexane layer, and the equilibria of reactions A and B are shifted to the left.

Nature of the Amylose-Iodine Complex Formed in Me_2SO-Water Mixtures

It must be assumed that when the amylose-iodine complex is formed in solution, the ratio of I_2 to I_3^- in the complex reflects the composition of the solution, because deviation from this composition is energetically unfavorable. We have previously relied on this assumption to estimate iodine species involved in aqueous I_2-KI systems from potentiometric and spectrophotometric data (Knutson et al 1982). In the present study, only spectrophotometric data are available. This precludes precise calculation of I_2/I_3^- ratios because of the large disparity between the absorptivity of I_3^- and I_2 (746 at 445 nm). Estimated values calculated from spectrophotometric data of Me_2SO-water mixtures between 10 and 50% Me_2SO ranged from an I_2/I_3^- ratio of 11 at 10% Me_2SO to 2 at 50%. Although these calculations may not be accurate, they do reflect the trend of the system, and the variation in absorbance of the amylose-iodine complex formed in different Me_2SO concentrations (Fig. 2) demonstrates the relative effectiveness of the different I_2/I_3^- compositions to form the amylose-iodine complex.

It is significant that in this and our previous work (Knutson et al 1982) there is a downward shift in the absorbance maximum of the amylose-iodine complex as the triiodide content of the solution is increased, indicating a change in the nature of the complex as more of the ionic species is incorporated.

Variability in Analysis of High-Amylose Starches

The data in Table IV show considerable variability between native and defatted high-amylose starches, although the difference is minimal in dent starch. This variability is undoubtedly caused by the interference of lipids, as high-amylose starches are known to contain more lipid than normal starches (Acker and Schmitz 1967, Tan and Morrison 1979, Morrison and Milligan 1982). Therefore, if accurate results are to be obtained, defatting of the sample is necessary with this method. The results on native samples do demonstrate the usefulness of the method without defatting for initial screening to assign samples to different amylomaize classes.

Applicability of the Method to Other Starches

This method was developed for analysis of maize starches and has only been tested on maize starches. However, it can be safely assumed that use of iodine-Me_2SO as a substitute for I_2-KI will be applicable to any starch, and that successful adaptation to other starches merely requires satisfactory procedures for dissolving and defatting the starch.

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