Polarimetric Determination of Starch in Corn with Dimethyl Sulfoxide as a Solvent[1]

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ABSTRACT

Polarimetric starch analyses in which starch is extracted from the corn kernel with 90% dimethyl sulfoxide (DMSO) at room temperature gave results in good agreement with those of the hot calcium chloride extraction customarily used. Recovery of different starches from corn varied in the following order of increasing difficulty: High-amylose, waxy, and ordinary. High-amylose starch was readily extractable; however, length of grinding and agitation time with DMSO governed the completeness of extraction of waxy and ordinary corn starches. More than 98% of the starch was recovered from amylomaize, waxy, and ordinary corn after 3, 6, and 9 min., respectively, of grinding in 90% DMSO. Heating (at 55°C.) without adequate shaking and grinding did not ensure quantitative starch recovery from ordinary and waxy corn. Such optically-active substances in corn as hemicelluloses, zein, and sugars did not interfere with starch determination by the new method. Rapid and accurate polarimetric readings were made by consecutively introducing sample solutions to a flow-through cell assembly.

The use of dimethyl sulfoxide (DMSO) as a solvent for starch, combined with accurate and rapid polarimetric readings obtainable with an electronic polarimeter, led to the development of a simplified method for the determination of amylose in corn (1). An essential step of the amylose analysis was determining starch content of the DMSO corn extract. In this method (1), complete starch extraction from the kernel was not required to determine the amylose content of the starch. However, this amylose method was potentially applicable for measuring the total starch in corn. The main problem encountered was that quantitative starch recovery from the kernel was difficult to attain in certain corn varieties, particularly in ordinary and waxy corns. Consequently, work was undertaken to establish optimal conditions for complete extraction and solubilization of starch from the kernels from different varieties of corn.

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Advantages of DMSO as a starch solvent were demonstrated by Leach and Schoch (2), who showed that there was no degradation of corn starch on prolonged standing in DMSO. High apparent solubility of a variety of starches in DMSO was also reported by Adkins et al. (3) with no insolubility effects on aging of DMSO. Other workers (4,5,6) isolated starch from corn using DMSO as a solvent. Under prescribed conditions hemicelluloses are not extracted from native cell walls with DMSO (1). Consequently, these optically-active constituents do not interfere with starch determination.

The high sensitivity of the electronic polarimeter permits accurate measurement of optical rotation of starch solutions in cells of short path length (0.2 to 0.5 dm.). Optical-rotation measurements of turbid solutions are facilitated by the use of cells of short path length. Slightly turbid solutions are sometimes encountered in the extraction of ground corn.

MATERIALS AND METHODS

All polarimetric measurements were made with a Bendix automatic polarimeter Model 143A as previously described (1). The flow-through cell assembly (0.5-dm. cell) (Fig. 1) was constructed with 1/8-in. polyethylene-tubing connectors at entrance and exit ports of the glass cell. Most tubing materials other than polyethylene or Teflon are soluble or partially soluble in DMSO. When this system is operated carefully, no air bubbles are introduced. Strict control of polarimetric cell temperature was not needed.

The only reagent required is 90% (v./v.) DMSO in water, prepared from industrial-grade DMSO. Periodic colorimetric and polarimetric checks of industrial-grade DMSO revealed no undesirable qualities.

Procedure

Whole-kernel samples (approximately 1 g.) are weighed on a top-loading balance to within 1 mg. After steeping in water at 7°C. for 4 hr., individual whole kernels

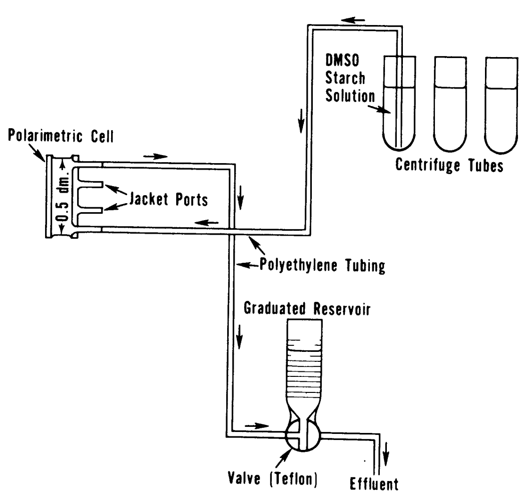


Fig. 1. Flow-through cell assembly. Sample flow rate of starch dissolved in DMSO and volume were controlled at graduated reservoir.

are cut into six to eight pieces and transferred quantitatively to a small glass screw-cap vial. To the vial is added 20.0 ml. 90% DMSO. It is advantageous, but not necessary, to store samples in this condition for at least 1 day so that the starch can swell and partially solubilize before grinding. If this step is taken, starch swells to form a turbid, viscous layer over the bottom of the vial with ordinary and waxy corns. A clear, thin solution remains with amylomaize samples and serves to differentiate between amylomaize and other types of corn. The DMSO-corn mixture, which has been ground (1) for 6 to 11 min. and shaken for 24 hr., can be allowed to settle by gravity, or the insoluble residue and dissolved starch can be immediately separated by centrifugation.

Polarimetric Measurement Techniques

Two techniques for measurement of optical rotation of a DMSO-starch solution are available:

A)The recommended technique includes a starch-precipitation step, as described earlier (1). Optically-active constituents other than starch are removed in this step and do not interfere with polarimetric measurement. The precipitated starch is redissolved in 90% DMSO (usually 50 ml.). The redissolved starch has a volume-additive effect of approximately 0.8 ml.; therefore, the volume of DMSO-starch solution is approximately 50.8 ml. if 50.0 ml. has been used to redissolve the precipitate.

B)In the second technique, the crude DMSO-starch extract is diluted before measuring the optical rotation. To make the dilution, 10 ml. of the supernatant extract is mixed with 45 ml. of 90% DMSO in an 80-ml. polypropylene screw-cap vial.

Technique A is always used if amylose content of the starch is to be determined. Precipitation of the starch ensures noninterference of fatty acids with amylose color development (1). It must also be used when optically-active substances other than starch, such as hemicelluloses, zein, or sugars, are present in sufficient amount to interfere with polarimetric measurement of the starch.

Benefits derived from technique B are: 1) It is more rapid than A, 2) minute, suspended material is diluted to an insignificant quantity, resulting in a stable polarimetric reading, 3) no starch-separation procedures are needed, and 4) results serve as a check that no starch losses have occurred during the precipitation step of technique A. Technique B could fnd applications in corn-selection operations because of its relative speed and simplicity.

Starch Computation

The following equation, which incorporates the basic polarimetric equation, is used to compute starch content in corn on a dry basis.

$$\%\ \text{Starch} = \frac{(\alpha)(100)(V_1)(V_3/V_2)}{[\alpha]^{25}_{546}\ (P)(W)(100-\%\ \text{moist.})} \tag{1}$$

where: α = observed optical rotation, in degrees; $[\alpha]^{25}_{546} = 220^\circ$ = specific rotation of starch in 90% DMSO; P = path length, in dm.; W = weight of kernels, in g.; V_1 = volume of ground crude DMSO-corn-extract mixture, in ml.; and V_3/V_2 = dilution factor, where V_2 is 10.0 ml. of crude DMSO-starch extract which is precipitated (technique A) or diluted (technique B) directly with 90% DMSO. V_3 is the volume of redissolved precipitated starch (approximately 50.8 ml., technique A) or the final diluted crude-extract volume (55.0 ml., technique B).

To correct results to a moisture-free basis, we use a 10% moisture correction based on our corn storage conditions in this laboratory.

RESULTS AND DISCUSSION

A flow-through cell assembly permits rapid measurement of optical rotation in up to 30 consecutively introduced samples per hr. Preliminary grinding of large quantities of a corn sample is not required. Digestion, as well as lengthy preparation of reagents, is eliminated. Volumetric requirements and dilutions can usually be met with the use of automatic reagent dispensers. Only inexpensive glassware and polypropylene centrifuge tubes are needed.

Besides calibration with aqueous solutions (1), it is also possible to calibrate the polarimeter with a sucrose-90% DMSO solution. The specific rotation of sucrose, $[\alpha] = 75.9°$, in 90% DMSO, is slightly lower than in water. The sucrose-90% DMSO solution is stable for almost 2 months at room temperature. With ten separate polarimetric values observed between 1 and 56 days after preparation of a sucrose-90% DMSO solution, a mean of 304.6 ±1.2 millidegrees optical rotation was obtained in a 0.5000-dm. cell at 25°C. The concentration of the solution was 8.033 g. sucrose per liter 90% DMSO.

Specific Rotation

For comparative purposes, specific-rotation data were acquired at 546 nm. for starches and starch fractions representing waxy, ordinary, and amylomaize varieties with solutions of both 90% DMSO and calcium chloride ($CaCl_2$) (7) as solvents. The weight of starch samples taken was chosen so that, in combination with the appropriate cell, polarimetric readings would range between 0.240° and 0.300°. Moisture- and protein-content corrections were made for the starches.

For the DMSO procedure, samples of approximately 0.3 g. were dissolved and made up to a final volume of 100 ml. in 90% DMSO. After the sample solutions were shaken thoroughly, they were left untouched for 24 hr. before polarimetric measurements were made in a 0.3982-dm. cell.

For the $CaCl_2$ procedure (7), 0.7-g. starch samples were taken to simulate polarimetric starch values obtained with about 1 g. of whole-kernel corn. The standard $CaCl_2$ method (7) was modified by heating for 15 min. and making up the final-solution volume to 100 ml. The observed optical rotation was approximately 0.300° with a 0.1967-dm. cell.

At 546 nm., the respective specific-rotation values for the DMSO and $CaCl_2$ procedures were 220 and 234. Only slight differences were detectable in specific-rotation values between amylose and amylopectin (Table I).

Solubilization of Pure Starches

Starch can be solubilized during 24 hr. of shaking at room temperature. Heating a DMSO-starch mixture for 30 min. at 50°C. also solubilizes starch.

For testing various shaking and heat treatments, 0.802-g. samples of an ordinary corn starch were ground in a Udy grinding chamber as described for the corn samples (1). The ground mixture was transferred to 4-oz. bottles and, after either appropriate shaking or heating, the optical rotation of the resultant solution was measured. Table II shows the effects on starch of shaking and heating. The observed optical-rotation data indicate that 90% DMSO will dissolve granular starch quantitatively with a 24-hr. shaking. Even in conjunction with a 24-hr. shaking, heat treatments dissolved slightly less starch. Starch was dissolved better where the volume of 90% DMSO was increased to 120 ml. and a 24-hr. shaking, because of a more-favorable ratio of DMSO-volume to weight of starch.

The recommended starch polarimetric technique A requires that starch be precipitated and that the precipitated starch in 90% DMSO be completely redissolved. To study the effects of shaking and heating on redissolving precipitated starch, duplicate 10-ml. aliquots of an ordinary corn starch-DMSO solution (1.00 g. per 80 ml. 90% DMSO) were precipitated with ethanol. To the precipitated starch was added 50.0 ml. 90% DMSO, and the starch was redissolved by shaking (1 hr.) and heating (50°C. for 30 min.). One hour of shaking is sufficient to redissolve the precipitated starch because of a high ratio of DMSO-volume to weight of starch (Table III). Solution was also readily achieved because the DMSO-starch precipitate is more soluble than the original granular starch. As it turned out, shaking and heat treatments are identical in their effects in dissolving the starch. Combined shaking and heating showed no additional improvement (Table III).

TABLE I. SPECIFIC-ROTATION VALUES BY DMSO AND Ca Cl_2 METHODS AT 546 nm.

Starch Sample	No. of Samples	Amylose Range %	DMSO Method, Mean Specific Rotation	Ca Cl_2 Method, Mean Specific Rotation
Amylopectin	4	2.5–9.5	220.8	235.8
Ordinary	3	26–28	220.1	233.9
High-amylose	5	54–80	220.1	233.3
Overall specific rotation			220.4 ±0.7	234.3 ±1.7

TABLE II. EFFECTS OF SHAKING AND HEATING ON SOLUBILIZATION OF ORDINARY CORN STARCH

Sample and Treatment with 0.802 g. Starch	90% DMSO ml.	Optical Rotation[a] (0.1967-dm. cell) degrees
Nos. 1 through 3 (three identical samples), 24-hr. shake	80	0.427 ±0.002
No. 4, no shaking, heated 30 min. at 50° C.	80	0.419
No. 5, 24-hr. shaking[b], heated 30 min. at 50° C.	80	0.424
No. 6, 24-hr. shaking	120	0.432[c]

[a]Calculated expected reading, 0.433 degrees.

[b]The heat treatment was applied before shaking whenever both treatments were combined.

[c]Corrected to 80-ml. volume.

TABLE III. EFFECTS OF SHAKING AND HEATING IN REDISSOLVING PRECIPITATED ORDINARY CORN STARCH[a]

Heat and Shake Treatments	Optical Rotation (0.1967-dm. cell) degrees[b,c]
1-hr. shaking	0.1077
No shaking; heated 30 min. at 50° C.	0.1076
1-hr. shaking[d]; heated 30 min. at 50° C.	0.1074

[a]Solution: 1.000 g. per 80 ml. 90% DMSO. Duplicate 10-ml. aliquots were precipitated, then redissolved in 50.0 ml. 90% DMSO.

[b]Average of duplicates.

[c]Expected reading, 0.1082 degrees.

[d]Heat treatment preceded shaking.

TABLE IV. ACCURACY OF POLARIMETRIC DATA OVER A RANGE OF STARCH CONCENTRATION (ordinary corn starch)

Starch/80 ml. 90% DMSO[a] g.	Optical Rotation millidegrees[b] 0.1967-dm. cell	Millidegrees/g. Starch (dry basis)
0.100	50.0 ±0.5	536.4
0.300	151.3 ±0.3	541.0
0.500	255.0 ±0.2	547.1
0.700	351.0 ±0.5	537.9
0.900	456.7 ±0.5	544.3
1.000	502.3 ±0.4	538.8
	Mean	540.9 ±4.3[c]

[a]Weight of starch shaken 24 hr. before rotation readings.

[b]Mean value for three separate aliquots.

[c]Calculated expected reading, 541.0 millidegrees.

Accuracy of polarimetric readings in the weight range of 0.1 to 1.0 g. starch is demonstrated in Table IV. All the foregoing data on pure starches show that quantitative starch recoveries with DMSO solubilization are possible. Furthermore, they demonstrate the accuracy and reproducibility of polarimetric measurements.

Starch Extraction from Corn

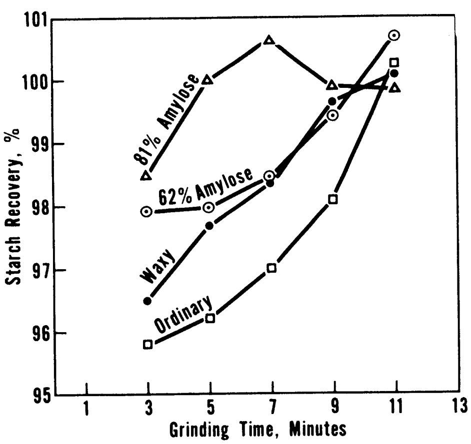
Although pure corn starches may be solubilized without too much difficulty, quantitative extraction of starch from the kernel presents a special problem because starchy tissues and cells must be completely broken down before the starch trapped inside the cell walls and protein network can be fully solubilized. Different corn varieties exhibit different starch-solubility rates. Starch from amylomaize is more easily solubilized than starch from waxy and ordinary corn varieties. Figure 2 shows grinding times required for quantitative starch recovery for four corn varieties. With 6 min. of grinding and a subsequent 24-hr. shake, more than 98% of the starch is recovered from either amylomaize. To reach this same percentage, both waxy and ordinary varieties require a 9-min. grind before the 24 hr. of shaking. DMSO-starch recoveries are based on starch data by the hot Ca Cl_2 procedure. The most effective means of extracting starch quantitatively from the kernel was to allow sufficient grinding time.

To determine how much shaking was required for starch solubilization after an 11-min. grind, two corn samples representing the most difficult to solubilize (ordinary) and a more easily solubilized (amylomaize, 62%) were ground. For each shake-time interval studied, 1 g. of the respective sample was used. Upon completion of the grind operation, the shaking procedure was started immediately for the appropriate interval (1 to 24 hr.). As shaking was completed, the samples were centrifuged immediately to separate solubilized starch in the 90% DMSO solution for subsequent polarimetric measurement. From polarimetric measurements, the solubilized starch was determined (Fig. 3). As shown in Fig. 3, after 11 min. of grinding, ordinary corn and amylomaize responded similarly to the shaking procedure.

Additionally, two corn samples (ordinary and 62% amylose) were treated like those shown on Fig. 3, except that no shaking was involved. Twenty-four hours after grinding, polarimetric measurement of the dissolved starch indicated a starch recovery of approximately 93% for both samples. This recovery demonstrated the effect of time only on starch solubilization.

The effect of heating from 2 to 5 hr. at 55°C. was also investigated. Although extraction with DMSO at 55°C. with simultaneous agitation enhanced starch recovery, subsequent shaking was still required for quantitative starch yield. Heat treatment alone without shaking gave low results (Table V, column 2), and cannot replace shaking treatments. Starch could be detected microscopically in corn residues where quantitative recovery had not been attained.

Grinding in addition to heating (2 or 5 hr.) yields about 90% starch solubilization with waxy and ordinary corn. The remaining 10% starch, being the



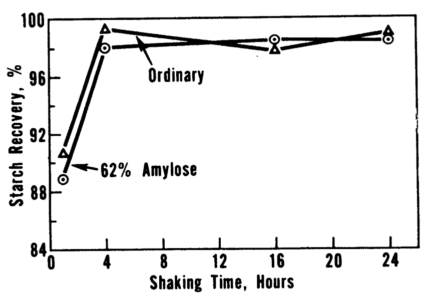


Fig. 2 (left). Starch recovery vs. grinding time for four corn varieties. Ground suspension shaken for 24 hr.

Fig. 3 (right). Starch recovery vs. hours of shaking time after an 11-min. grind. Circles = 62% amylose corn; triangles = ordinary corn.

TABLE V. EFFECTS OF DIFFERENT TREATMENTS ON STARCH RECOVERY FROM WHOLE-KERNEL CORN

Corn Variety	% Starch Recovery by DMSO Procedure[a]				
	6-min. grind[b] 2-hr. heat No shake	6-min. grind[c] 5-hr. heat No shake	3-min. grind[b] 5-hr. heat 16-hr. shake	6-min. grind[b] 4-hr. heat 24-hr. shake	11-min. grind[d] No heat 24-hr. shake
High-amylose (81%)	101.3	101.7	99.2	100.4	100.2
High-amylose (62%)	98.2	96.3	97.6	98.9	100.7
Waxy	90.7	93.6	98.2	99.3	99.8
Ordinary	88.5	94.3	98.0	94.5	100.2

[a]Average of duplicate aliquots. Same samples analyzed by $Ca\ Cl_2$-starch method. $Ca\ Cl_2$ results taken as 100%.

[b]Heated with agitation at 55° C.

[c]No agitation during heat treatment at 55° C.

[d]Microscopic examination of corn residues revealed that all starch had been removed.

most difficult to remove, requires prolonged shaking for complete solubilization. Agitation has a shearing action which promotes dispersion of swollen starch granules. Quantitative recovery of amylomaize starch was accomplished easily with only 3 to 6 min. of grinding under the conditions studied. From data in Table V it is apparent that prolonged shaking (24-hr.) of the corn-DMSO mixture after extended grinding (11 min.) was the most effective means of ensuring quantitative starch recovery.

Extraction with DMSO vs. Hot $Ca\ Cl_2$

Comparative starch values for the DMSO and the $Ca\ Cl_2$ methods are in good agreement when the appropriate grinding time is used for the different corn varieties. Table VI records comparative starch recoveries for different varieties of corn with starches that solubilize with difficulty. Although not shown in Table VI, starch recoveries with a 6-min. grind have been excellent in a 60-sample-per-day amylomaize analytical program.

Because starch is the main optically-active constituent in whole-kernel corn with a relatively high specific rotation, starch results are essentially identical if either of the two polarimetric techniques described in the DMSO procedure is followed. The two optically-active constituents in corn that are most likely to interfere with polarimetric determination of starch are probably zein (levorotatory) and sucrose (dextrorotatory). They would be present if technique B were selected, but would be absent with technique A. Their effect, if any, on the observed optical rotation of diluted crude-DMSO extract was insignificant (Table VI). Finely ground (60-mesh) sorghum and millet flour responded well to starch solubilization with only a 3-min. grind, but with the sample size reduced to 0.5 g. (Table VI).

An extension of this direct DMSO method to include other products is desirable. Because of the accuracy available with the electronic polarimeter, the determination of starch where it exists as a minor constituent is also possible. Recently the determination of starch in commercial defatted corn germ, which contained 12% sugar, has been accomplished satisfactorily according to polarimetric technique A. The ground corn germ (60-mesh) was treated with only a 6-min. grind.

TABLE VI. COMPARATIVE STARCH RESULTS BETWEEN DMSO AND $CaCl_2$ POLARIMETRIC METHODS

Sample	DMSO Method % Starch (average of duplicate aliquots)		$CaCl_2$ Method % Starch (average of duplicate samples)
	Redissolved precipitated starch[a]	Diluted crude DMSO-starch extract[b]	
Corn grain[c]			
Ordinary dent			
Pioneer 3306	70.8	70.7	70.8
PAG SX 29	74.5	74.2	73.9
High oil			
Alexho 1	67.3	65.8	67.3
Alexho 2	69.1	68.1	69.6
Alexho 3	67.2	67.2	69.0
Sorghum flour[d]			
OK612	87.8	86.5	87.5
RS626	87.4	88.1	86.5
TE77	86.6	87.8	87.1
Millet flour[d]			
White Proso	76.8	78.1	80.3

[a]Technique A: Crude DMSO-starch extract was precipitated and redissolved in 90% DMSO.

[b]Technique B: Crude DMSO-starch extract diluted with 90% DMSO.

[c]1-g. corn kernels with 15-min. grind in 90% DMSO, followed by 24-hr. shaking.

[d]0.5-g. ground sample (−60 mesh) with wet grind in 90% DMSO for 3 min., followed by 24-hr. shaking.

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