Amylose Determination in Dimethyl Sulfoxide Extracts of Maize[1]

M. J. WOLF, E. H. MELVIN, W. J. GARCIA, R. J. DIMLER, and W. F. KWOLEK[2],
Northern Regional Research Laboratory, Peoria, Illinois

ABSTRACT

About 1 g. of corn is ground in 90% dimethyl sulfoxide (9:1 DMSO-water, v./v.) and then shaken for 24 hr. at room temperature to dissolve most of the starch. After undissolved solids are removed, the starch in 10 ml. of the clear extract is purified by precipitation with ethanol. The DMSO-starch precipitate is redissolved in 50 ml. of 90% DMSO. Starch in this solution is determined polarimetrically in an electronic polarimeter ($[\alpha]^{25^\circ}_{546\ m\mu} = 220^\circ$). Amylose is determined spectrophotometrically on a separate portion of the same solution by measuring the absorbance of the amylose-iodine complex at 615 mμ. Purified linear starch fractions and waxy starch serve as reference standards for calibration.

Amylomaize starches solubilize more readily in 90% DMSO than do either ordinary or waxy starches. However, the method is applicable to determination of amylose in the entire range of corn from ordinary corn to amylomaize. DMSO-extractable substances of germ and pericarp do not interfere with determination of amylose in endosperm starch. In fractional extraction of corn with DMSO, initial amylose content is high and decreases in successive extracts. Selective precipitation of starch with ethanol from crude DMSO extracts is equivalent to conventional solvent extraction as a means of minimizing fatty-acid interference with iodine sorption. Rate of amylose analysis is one and one-half to two determinations per man-hour.

---

[1]Presented at the 54th annual meeting, Chicago, April-May 1969. Contribution from the Northern Regional Research Laboratory, Peoria, Illinois 61604. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

[2]Biometrical Services, Agricultural Research Service, U.S. Department of Agriculture, stationed at Peoria.

Development of corn having starch with a high amylose content has been the objective of an intensified breeding program in which the Northern Laboratory has co-operated. Interest in high-amylose corn, which has been given the name "amylomaize," stems from properties of the linear molecular component of the starch—amylose. The potential usefulness of high-amylose starch and the properties of amylose have been discussed previously (1,2,3).

To report percent amylose in starch, it is necessary to determine first the starch content and secondly the concentration of amylose. In the procedure now used, starch is determined polarimetrically in a partially purified dimethyl sulfoxide (DMSO) extract of corn. Determination of amylose on a separate portion of the same extract is based on measuring spectrophotometrically the blue color of the amylose-iodine complex (4,5).

The procedure is similar in general approach to that of Shuman and Plunkett (6), except that these workers dissolved the starch with a hot, concentrated calcium chloride solution at a pH of 2.0. Under these conditions, starch degrades considerably.

DMSO was chosen rather than calcium chloride because it had been shown by Foster and co-workers (7,8) that DMSO was an effective starch solvent that apparently did not alter the dissolved starch chemically. Furthermore, solutions of starch are stable in the solvent for long periods (9,10).

Flexibility of the procedure for amylose determination is enhanced because starch is stable in DMSO. Corn may be extracted with the solvent, either at room temperature or more rapidly at higher temperature, without significant difference in amylose results. Samples which cannot be analyzed immediately may be steeped in DMSO until it is convenient to proceed with the analysis. The solvent penetrates readily into endosperm cells, swells the starch granules, and facilitates subsequent grinding and extraction. When kept in the dark, the amylose-iodine complex is stable for more than 24 hr. in the presence of the small volume of DMSO (0.5 to 1.0 ml. in 100 ml. of aqueous solution) introduced with the starch sample.

McGuire and Erlander (11) used DMSO as a solvent to isolate starch from corn rather than to determine amylose. Shannon (12) extracted starch primarily from immature maize by exhaustive extraction with hot (70°C.) DMSO and determined total carbohydrate in the extract.

Our procedure was developed primarily to determine amylose rather than total starch. Lipids, proteins, soluble sugars, and polysaccharides (such as hemicelluloses) which occur in corn do not interfere with determination of amylose by this method. A simple procedure is followed, based on analysis of about 1-g. samples of corn in which extraction with DMSO and all other operations are carried out at room temperature. The method lends itself to analysis of large numbers of samples as required for genetic studies. Output per man-hour is one and one-half to two amylose analyses.

MATERIALS AND METHODS

Samples analyzed included varieties of corn ranging from waxy maize to amylomaize. Corn with ordinary starch included commercial yellow dent and flour corns. Most of the samples analyzed were amylomaize containing starch with

generally more than 50% apparent amylose. These were mostly selections submitted by corn breeders; however, numerous analyses were made of commercial amylomaize (Amicorn 5 and 7).

DMSO, industrial grade purchased in drum lots, was the solvent. It was diluted with distilled water to 90% DMSO (v./v.). This ratio of DMSO to water is within the optimal range for solubilizing starch (9).

Determination of Starch

Starch, dissolved in 90% DMSO, was determined polarimetrically. Optical rotation of the solution was measured with an electronic polarimeter equipped with a digital readout (Bendix automatic polarimeter, Model No. 143). Measurements were made in green light (interference filter transmitting 5,461 A ±15 A; half-band width, 100 A ±20 A). Optical rotation of starch solutions was read in a glass flow-through cell of 5-cm. path length. Concentration of starch, c, in the solution was calculated from equation 1:

$$c = \frac{100\,a}{[220°]\,P_{Pol}} \qquad (1)$$

where a = observed angular rotation, degrees;

$[220°]$ = specific rotation of starch in 90% DMSO in green light, 546 mμ;

P_{Pol} = path length of polarimeter cell, decimeters.

The polarimeter was calibrated against U.S. Bureau of Standards sucrose solution accurately made up in distilled water to contain about 1 g. of sucrose per 100 ml. of solution.

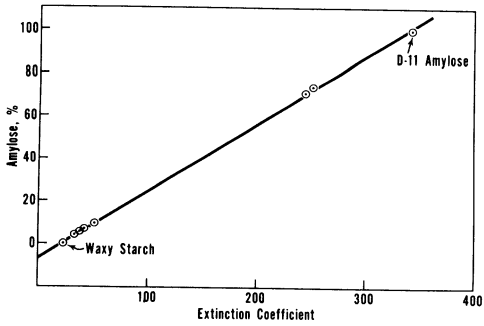
Determination of Amylose

Amylose content[3] (13) of the starch was determined spectrophotometrically by measuring absorbance of the amylose-iodine complex. The following reagents were used:

Hydrochloric acid, 0.5N	2.0 ml.
Potassium iodide, 0.016N	5.0 ml.
Potassium iodate, 0.0051N	5.0 ml.

To prepare the blue starch-iodine solution, about 50 ml. of distilled water was added to a 100-ml. volumetric flask. Five milliliters each of potassium iodide and potassium iodate was then added and an accurately measured aliquot (0.5 or 1.0 ml.) of the DMSO-starch solution was delivered into the flask with a calibrated Seligson pipet. Finally 2.0 ml. of 0.5N hydrochloric acid was added. The volume

[3]The term "amylose" as used here is equivalent to the term "apparent amylose" (13); the latter presupposes that a part of the iodine is sorbed by long side chains on the branched starch component. If this condition exists, amylose values of starches should be somewhat high on the basis of measurement of their total iodine sorption.



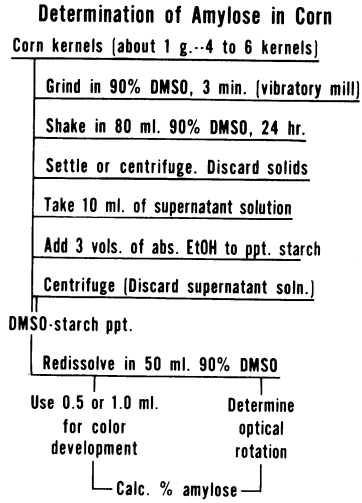


Fig. 1 (left). Calibration curve, showing linear relation between amylose concentration of starch and the extinction coefficient.

Fig. 2 (right). Steps followed in analyzing amylose in corn.

was adjusted to 100 ml. with distilled water.

The absorbance of this solution was read on a Beckman B Model spectrophotometer at 615 mμ.

Reference Standards

The linear relation between the absorbance of amylose-iodine solutions and the concentration of amylose is shown in Fig. 1, and the equation for the curve is given under "Calculations" (eq. 2). Iodine affinity of reference standards, expressed as percent amylose, was plotted against the extinction coefficient ($E^{1\%}_{1\ cm.}$). Reference standards, all of known iodine titer, included a purified corn amylose, a waxy corn starch, and intermediate amyloses and amylopectins from corn. Iodine affinity was determined by the method of Bates et al. (14) as modified by Wilson et al. (15). The standards were dissolved in 90% DMSO, and 0.5- or 1.0-ml. aliquots were used to prepare 100 ml. of amylose-iodine solution as described under "Determination of Amylose."

The basic reference standard was a purified corn amylose, designated D-11, prepared from Iowa hybrid 939 corn starch by fractionation with butanol by the Schoch procedure (16). After purification by four successive precipitations from butanol, the final product had an iodine sorptive capacity of 200 mg. of iodine per g. This product was taken as 100% amylose. The wave length of peak absorption for D-11 amylose-iodine complex was 636 mμ; the extinction coefficient at this wave length, $E^{1\%}_{1\ cm.}$, was approximately 365.

Absorption maxima of starches ranging in amylose content from normal to high amylose varied between 593 and 625 mμ with an average value of 615 mμ. This

mean wave length was adopted for measurement of absorbance of all corn starches analyzed.

The waxy reference starch was obtained from Iowa 939 waxy corn. This starch was assumed to be 100% amylopectin (i.e., 0% amylose). The extinction coefficient of this waxy starch was about 26 at 615 mμ.

General Procedure

The scheme of analysis is outlined in Fig. 2.

About 1.1 g. of corn (representing about 1 g. of endosperm) is soaked overnight in distilled water in the refrigerator at 7°C. The entire sample may be ground or the germ and pericarp may be removed and only the endosperm processed. The sample is ground for 3 min. with 40 ml. of 90% DMSO on a vibratory mill (React-R-Mill, Udy Analyzer Co., Boulder, Colo.) in a nickel grinding chamber. The chamber is rinsed twice with 20-ml. portions of 90% DMSO.

Washings and ground sample are combined and shaken continuously for 24 hr. at room temperature. After the samples are removed from the shaker, cell walls, protein, and other cellular debris may be separated either by centrifugation or by gravity sedimentation.

Ten milliliters of the clear supernatant is added slowly, with constant agitation, to 30 ml. of ethanol in a polyethylene centrifuge tube, fitted with a polyethylene screw cap. The mixture is shaken for 2 hr. and the precipitate of DMSO-starch is centrifuged. The supernatant mixture of DMSO and alcohol is drained and the tube is inverted over paper toweling. Fifty milliliters of 90% DMSO is added and the centrifugate is dissolved on a continuous shaker. Either 0.5 or 1.0 ml. of this purified solution is taken for colorimetric estimation of amylose; optical rotations are measured on a separate portion of the same solution.

Calculations

The basic equation relating percent amylose and the absorbance of the blue amylose-iodine solution is:

$$\text{Percent amylose} = 0.296 \frac{A}{c_1 P_A} - 7.67 \qquad (2)$$

where A = absorbance of amylose-iodine solution;
P_A = path length of colorimeter cell, cm.;
c_1 = concentration of starch in the blue solution, g./100 ml.

Starch concentration, c, is determined polarimetrically in the purified DMSO-starch solution as shown in eq. 1. The final starch concentration, c_1, in the colored solution was derived from c by applying a dilution correction (eq. 3) in preparing 100 ml. of aqueous colored solution with a small volume (0.5 or 1.0 ml.) of DMSO-starch solution.

$$c_1 = \frac{c}{F_D} \qquad (3)$$

where F_D = dilution factor. For example, if 0.5 ml. of DMSO-starch solution is diluted to 100 ml., F_D has a value of 200.

Percent amylose is calculated by combining spectrophotometric and polarimetric data:

$$\text{Percent amylose} = \frac{0.296\, A[220^\circ] P_{Pol} F_D}{100\, a\, P_A} - 7.67 \qquad (4)$$

RESULTS AND DISCUSSION

Specific Rotation

An accurate value is required for the specific rotation of starch in DMSO at 546 mμ.

Specific rotation ($[\alpha]^{25^\circ}_{546\ m\mu}$) of a series of purified starches and starch fractions was determined in 90% DMSO. The values for $[\alpha]^{25^\circ}_{546\ m\mu}$ ranged from about +219° to +222° with an average of +220.48 ± 0.79 for a series of 13 starches and starch fractions. There are no significant differences in specific rotation between starches, amyloses, or amylopectins. Apparently alpha (1→6) branch points of amylopectins have no detectable effect on the main alpha (1→4)-linked chain. These results confirm earlier work of Neely (17), who likewise found no significant difference in specific rotation between amylose and amylopectin. Neely reported a value of +175°C. for the specific rotation of starch $[\alpha]_D$ in DMSO; he did not measure the rotation at 546 mμ.

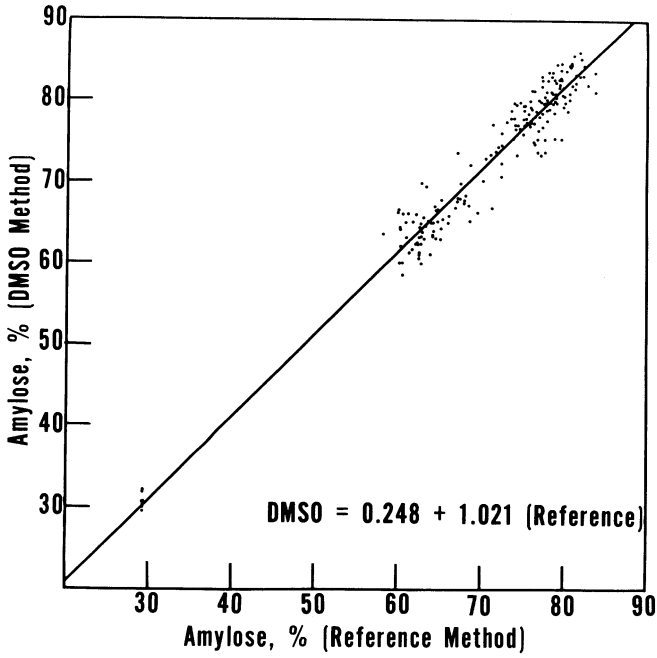


Fig. 3. Percent amylose in normal corn and amylomaize. Curve showing agreement in amylose determination between dimethyl sulfoxide (DMSO) method and a reference method.

Comparison of Methods of Amylose Analysis

Amylose analyses by the DMSO method are compared in Fig. 3 with analyses run on identical samples by a method similar to that of Williams et al. (18), who used it to determine amylose in rice.

The slope of the curve (Fig. 3) is 1.02, indicating good agreement between the two methods. As the slope shows, amylose values by the DMSO method are higher than those by the reference method. The absorbance of a given starch sample is the same regardless of whether the starch was originally dissolved in dilute alkali or in 90% DMSO. Consequently, high results with the DMSO procedure are due to more accurate determination of starch by direct polarimetric analysis of DMSO extracts than by the indirect gravimetric procedure used in the reference method.

Microscopic Examination of Ground Corn Residues

Effectiveness of starch extraction was checked by inspection of ground tissue residues. After 3 to 6 min. of grinding in DMSO, endosperm cells were completely broken down; starch granules were swollen or dispersed; no birefringent starch granules remained. Residual cell fragments with swollen starch trapped in the protein network were negligible in amylomaize residues; but in ordinary corn and in waxy corn, some swollen cell fragments originating from horny endosperm cells were found.

Endosperm cell walls are readily broken by grinding in DMSO and do not interfere with starch extraction. Some of the swollen starch, originating in the horny endosperm, is still held mechanically in the protein network of cell wall-free cell contents. Because DMSO readily dissolves the zein bodies, small voids are left in the protein network (19); the fibrous matrix protein does not appear to be disrupted by the solvent. McGuire and Erlander (11) considered it necessary to digest endosperm protein with Pronase to obtain quantitative starch extraction. However, residual starch may be freed from the protein network by thorough grinding or long shaking in DMSO without previous protein digestion. The protein network survives prolonged treatment.

After the ground corn was shaken in DMSO for 24 hr., residues of amylomaize were generally microscopically free of starch. Ordinary corn and waxy corn still showed small amounts of highly swollen undispersed starch material adhering to fragments of protein network.

In general, the rate of solubilization of starches by grinding different varieties of corn in DMSO was as follows: amylomaize> waxy maize>ordinary corn.

Effect of Precipitation

Starches ordinarily require thorough extraction with a polar solvent, such as methanol, to extract most of the fatty acids which interfere with iodine sorption. In our method, this time-consuming extraction is replaced by a rapid, simple procedure involving precipitation of starch from DMSO extracts. Fatty acids remained in solution and were discarded with the supernatnat liquid.

Data are summarized in Table I, showing effectiveness of this procedure as compared with conventional solvent extraction of ground corn as a means of extracting fatty acids. Amylose values of precipitated DMSO starches were equivalent to those obtained by analysis of solvent-extracted corn.

TABLE I. EFFECT OF STARCH PRECIPITATION vs. SOLVENT EXTRACTION ON AMYLOSE DETERMINATION IN WHOLE CORN AND ENDOSPERM

Variety	Amylose				Part Analyzed
	Not Defatted		Defatted[a]		
	Precipitated %	Not Precipitated %	Precipitated %	Not Precipitated %	
Amicorn 5	60	49	61	59	Whole kernel
Amicorn 8	78	69	81	78	
Dent corn	27	23	28	27	
Amicorn 5	61	55	60	59	Endosperm
Amicorn 8	80	68	79	77	
Dent corn	27	24	28	27	
Average	55.5	48.0	56.2	54.5	

[a]Extracted with hot 85% methanol.

Analysis of Separate Parts of Kernel

Dissection of kernels to isolate endosperm for analysis not only is time-consuming, but also results in loss of some starch. However, grinding whole corn in DMSO, to avoid the work of dissection, may release optically active substances in addition to starch, excessive amounts of lipid, or other materials that might interfere with either measurement of the optical rotation or absorbance. Polysaccharides, sugars, proteins, and lipids concentrated in the germ or pericarp are possible sources of interference.

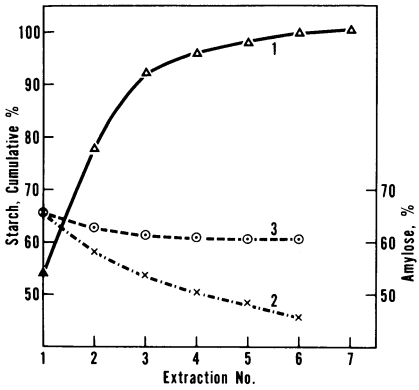
To test the over-all contribution of DMSO-extractable substances of germ and pericarp in amylose analysis, numerous assays were made of whole corn in comparison with isolated endosperm. A typical experiment with corn at three levels of amylose is shown in Table I. There was no difference in percent amylose when whole kernels or endosperm alone were assayed. Therefore, neither the germ, despite its high content of lipids and soluble sugars, nor the pericarp with its high pentosan content interferes with amylose determination in endosperm starch.

Noninterference of germ and pericarp with determination of amylose in endosperm starch was confirmed by analysis of DMSO extracts of hand-isolated germ and pericarp tissues. Pericarp extracts showed no optical activity, indicating that hemicelluloses, which make up about 50% of the pericarp, are not extractable with DMSO.

Fractional Extraction

Leach and Schoch (9) showed with pure starches that the linear fraction dissolved faster in DMSO than did the branched starch polymer. Similarly, we found in the fractional extraction of either amylomaize or ordinary corn that the first starch extracted was more linear than subsequent fractions (Figs. 4 and 5, curve 2). Because of this fractionation of starch during extraction, it was necessary to establish how much of the total starch (Figs. 4 and 5, curve 1) must be extracted to show an amylose content typical of that for the corn.

In amylomaize, amylose content of successive fractions extracted with DMSO



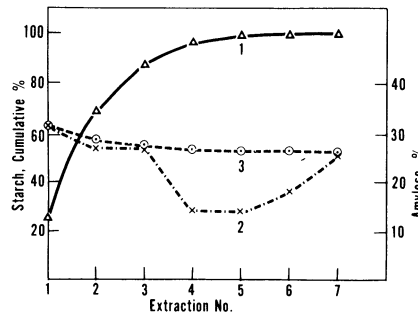


Fig. 4 (left). Fractional extraction of amylomaize. Triangles indicate starch, cumulative percent; X, percent amylose for respective extraction number; circle with dot, percent amylose, weighted average calculated from data for respective extraction plus previous extractions.

Fig. 5 (right). Fractional extraction of ordinary maize. Legend same as for Fig. 4.

varied from 65 to 46% (Fig. 4, curve 2). A weighted average of 61% amylose (Fig. 4, curve 3) was calculated from the entire series of individual fractions recovered in the experiment. This is in good agreement with an amylose content of 62% as determined by our procedure.

In ordinary corn, the pattern of starch extraction differs from that in amylomaize (Fig. 5). Amylose content of most of the starch extracted is between 25 and 30% (Fig. 5, curve 2). However, most of the remaining starch has an amylose content of about 15% (Fig. 5, curve 2). The pattern of extraction shown in the curves of Fig. 5 is in agreement with microscopic observations of DMSO-extracted residues of ordinary corn. Unextracted starchy material stains reddish, which is typical of amylopectin.

The data show that quantitative recovery of starch from maize is not necessary. Amylose values typical of the corn sample can be obtained with about 70% starch recovery in ordinary corn and 90% in amylomaize. Actual starch recoveries in our method are in excess of 90 and 95%, respectively, for ordinary corn and amylomaize with only a 3-min. grinding period. By extension of the grinding period, quantitative starch recoveries are approached.

Acknowledgments

We are indebted to J. R. McCarthy for help in determining the specific rotation of starch and to Alvena Stoutenborough and Florence McClellan for performing many of the analyses.

Literature Cited

1. SENTI, F. R. Research to utilize amylose. Chemurg. Dig. 17(5): 7 (1958).
2. SENTI, F. R., and DIMLER, R. J. High amylose corn–properties and prospects. Food Technol. 13: 663 (1959).
3. SENTI, F. R., and RUSSELL, C. R. High amylose cornstarch–properties and prospects. Tappi 43: 343 (1960).

4. McCREADY, R. M., and HASSID, W. Z. The separation and quantitative estimation of amylose and amylopectin in potato starch. J. Am. Chem. Soc. 65: 1154 (1943).
5. KERR, R. W., and TRUBELL, O. R. Spectrophotometric analyses of starches. Paper Trade J. 117(15): 25 [T.S. 161] (1943).
6. SHUMAN, C. A., and PLUNKETT, R. A. [42] Determination of amylose content of corn starch, single kernel technique. Method. Carbohyd. Chem. IV. Starch, pp. 174-178 (1964).
7. EVERETT, W. W., and FOSTER, J. F. The subfractionation of amylose and the characterization of the subfractions by light scattering. J. Am. Chem. Soc. 81: 3459 (1959).
8. KILLION, P. J., and FOSTER, J. F. Isolation of high molecular weight amylose by DMSO dispersion. J. Polymer. Sci. 46: 65 (1960).
9. LEACH, H. W., and SCHOCH, T. J. Structure of the starch granule. III. Solubilities of granular starches in dimethyl sulfoxide. Cereal Chem. 39: 318 (1962).
10. EVERETT, W. W., and FOSTER, J. F. The conformation of amylose in solution. J. Am. Chem. Soc. 81: 3464 (1959).
11. McGUIRE, J. P., and ERLANDER, S. R. Quantitative isolation and dispersion of starch from corn kernels without degradation. Staerke 18: 342 (1966).
12. SHANNON, J. C. A procedure for the extraction and fractionation of carbohydrates from immature *Zea mays* kernels. Res. Bull. No. 842, pp. 1-8. Purdue University: Lafayette, Ind. (August 1968).
13. MONTGOMERY, EDNA M., SEXSON, K. R., and SENTI, F. R. High-amylose corn starch fractions. Staerke 13(6): 215 (1961).
14. BATES, F. L., FRENCH, D., and RUNDLE, R. E. Amylose and amylopectin content of starches determined by their iodine complex formation. J. Am. Chem. Soc. 65: 142 (1943).
15. WILSON, E. J., SCHOCH, T. J., and HUDSON, C. S. The action of *macerans* amylose on the fractions from starch. J. Am. Chem. Soc. 65: 1380 (1943).
16. SCHOCH, T. J. Fractionation of starch by selective precipitation with butanol. J. Am. Chem. Soc. 64: 2957 (1942).
17. NEELY, W. B. Optical rotatory dispersion studies on polysaccharides. III. Amylose, amylopectin and methylcellulose. J. Org. Chem. 26: 3015 (1961).
18. WILLIAMS, VIRGINIA R., WU, WEI-TING, TSAI, HSIU Y., and BATES, H. G. Varietal differences in amylose content of rice starch. J. Agr. Food Chem. 6(1): 47 (1958).
19. WOLF, M. J., KHOO, U., and SECKINGER, H. L. Distribution and subcellular structure of endosperm protein in varieties of ordinary and high-lysine maize. Cereal Chem. 46: 253 (1969).

[Received August 28, 1969. Accepted January 12, 1970]