

# COMPARATIVE SUSCEPTIBILITY TO AMYLASES OF STARCHES FROM DIFFERENT PLANT SPECIES AND SEVERAL SINGLE ENDOSPERM MUTANTS AND THEIR DOUBLE-MUTANT COMBINATIONS WITH *OPAQUE-2* INBRED Oh43 MAIZE<sup>1</sup>

H. FUWA, M. NAKAJIMA, and A. HAMADA, Department of Food and Nutrition, Osaka City University, Sugimoto-cho, Osaka, Japan, and D. V. GLOVER, Department of Agronomy, Purdue University, West Lafayette, IN 47907

## ABSTRACT

Cereal Chem. 54(2): 230-237

We confirmed that starch granules of potato, yamanoimo (tubers of *Dioscorea batatas* DECNE), and sweet potato were respectively in decreasing order more resistant to the attack of *Rhizopus* glucoamylase, pancreatin, and crystalline  $\alpha$ -amylase of *Bacillus subtilis* than were those of maize and rice. Several endosperm mutants, each nearly isogenic in the maize inbred Oh43 (*Zea mays* L.), their double-mutant combinations with *opaque-2*, and the normal counterpart, were studied for the relative susceptibility of their granular and gelatinized starches to amylases. When *opaque-2* was combined with each of the ten endosperm mutants, namely, *amylose-extender*, *brittle-1*, *brittle-2*, *dull*, *soft-starch*, *shrunken-1*, *shrunken-2*, *sugary-1*, *sugary-2*, and *waxy*, it was observed that the starch

granules of these double mutants were digested by *Rhizopus* glucoamylase, pancreatin, and *B. subtilis*  $\alpha$ -amylase to an extent very comparable to their respective nonopaque single-mutant counterpart. Starch granules of the *amylose-extender* mutant and its double combination with the *opaque-2* were much more resistant to the action of amylases than were those of the normal counterpart. Starch granules of the *sugary-2* mutant and its double combination with *opaque-2* were digested much faster than those of the normal counterpart by amylases. These differences among the endosperm mutants and their double-mutant combinations in susceptibility of starch granules to the action of amylases disappeared following gelatinization of starches with alkali.

Recently, Barbosa (1), Glover *et al.* (2), and Misra *et al.* (3) reported that several maize endosperm mutants in near-isogenic backgrounds increase the endosperm lysine as a percentage of protein substantially above the normal counterpart, and each gene when combined with *opaque-2* showed an enhanced effect on lysine content. Several of the endosperm mutants influence changes in pasting temperature, BEPT, viscosity, gel-stability of starch (4,5), starch-granule digestibility (5-7), and the production of amylose, amylopectin, water-soluble polysaccharides (WSP), and sugars (4, 8-13). In studies of endosperm mutants and their double-mutant combinations with the *opaque-2* or *floury-2* gene, Barbosa (1) showed that *opaque-2* or *floury-2* double combinations with other endosperm mutants did not affect the ratio of amylose to amylopectin except for the *amylose-extender opaque-2* and *sugary-1 opaque-2* combinations, which showed decreased amounts of amylose. Glover (2,14) showed that the *sugary-2 opaque-2* combination had an increased amylose content compared to the *sugary-2* counterpart.

This paper deals with a comparison of susceptibility of granular and

<sup>1</sup>Journal Paper No. 5654, Purdue Agricultural Experiment Station, West Lafayette, IN 47907. Supported in part by the Agency for International Development under the contract "Inheritance and Improvement of Protein Quality and Content in Maize," and the Eli Lilly Foundation. Reprint requests should be directed to D. V. Glover.

gelatinized starches obtained from several endosperm mutants, each nearly isogenic in the maize inbred Oh43, and the normal counterpart to fungal glucoamylase, pancreatin, and bacterial  $\alpha$ -amylase, with their respective double-mutant combination with *opaque-2*.

## MATERIALS AND METHODS

### Maize Mutants

Near-isogenic conversions of *opaque-2* ( $o_2$ ), *amylose-extender* (*ae*), *brittle-1* ( $bt_1$ ), *brittle-2* ( $bt_2$ ), *dull* (*du*), *soft starch* (*h*), *shrunk-1* ( $sh_1$ ), *shrunk-2* ( $sh_2$ ), *sugary-1* ( $su_1$ ), *sugary-2* ( $su_2$ ), and *waxy* (*wx*) to the maize inbred Oh43 (*Zea mays* L.) were recovered after six backcrosses to the recurrent parent. Each of these near-isogenic single mutants, their double-mutant combinations with  $o_2$ , and the normal counterpart (normal) were used in this study. The materials were grown at the Purdue Agronomy farm in 1970.

### Sources of Starches

Starches of the maize mutants: Prepared by a modification (5) of Schoch's (15) method.

Potato starch: Reagent grade, Wako Pure Chemicals Industries, Ltd., Osaka, Japan.

Sweet potato starch: Kindly supplied by F. Goto, Aichi Prefectural Institute for Food Technology, Nagoya, Japan.

Yamanoimo (Chinese yam) starch: Prepared from round-shaped tubers of a variety of *Dioscorea batatas* DECNE and kindly supplied by K. Miyagawa, Department of Food and Nutrition, Osaka City University, Osaka, Japan. Details of the preparation method and properties of the starch are published elsewhere (16).

Rice starch: Prepared from polished rice grains of *Japonica* type by an alkali-steeping method (see footnotes to Table I and ref. 17).

### Sources of Enzymes

Pancreatin: N.F., Lot No. 0296-13, Difco Laboratories, Detroit, Mich.

*Bacillus subtilis* liquefying  $\alpha$ -amylase: Reagent grade, three times crystallized, NK-101, Osaka Nagase & Co., Osaka, Japan.

Glucoamylase: A concentrate from culture media of *Rhizopus amagasakiensis* and kindly supplied by K. Kusai, Nagase Industrial Co., Amagasaki, Japan. Crude glucoamylase is contaminated with other enzymes including  $\alpha$ -amylase. However, using crystalline glucoamylases of *Endomyces* sp. and *Rhizopus niveus*, which are free of  $\alpha$ -amylase by the method of Marshall and Whelan (unpublished data), we had relative susceptibility of starch granules comparable to those for crude glucoamylase.

Glucose oxidase: Reagent grade, "De-oxin" for analysis of glucose, Osaka Nagase & Co., Osaka, Japan.

Peroxidase: Type II, Control No. 7291162, Boehringer Mannheim, GmbH.

### Analytical Methods

Susceptibility of starch granules to pancreatin and bacterial  $\alpha$ -amylase was studied following the method of Fukui *et al.* (18) with a slight modification (see

Tables II and III). The contents of solubilized carbohydrates at pH 7.0 and 37°C were determined by the phenol-sulfuric acid method (19) and expressed as glucose equivalents. Susceptibility of alkali-gelatinized starches to pancreatin was studied by percentage decrease of absorbancy at the maximum wavelength of light absorption of starch-iodine complex according to Fuwa's (20) method at pH 7.0 and 37°C. Susceptibility of granular and alkali-gelatinized starches to glucoamylase were studied by a modification of the Toyama *et al.* (21) method at pH 4.8 and 37°C. The reaction mixture used to determine the susceptibility of gelatinized starches by glucoamylase contained 2.0 ml of starch solution, 2.0 ml

TABLE I  
Digestion of Various Starch Granules by Glucoamylase

Starch	Digestion Time (hr)			
	1	3	5	24
Potato	0.48 <sup>a</sup>	1.86	3.16	14.1
Yamanoimo	1.15	4.99	9.06	37.5
Sweet potato	2.73	9.49	16.0	53.8
Maize (Inbred Oh43 normal)	6.49 ± 0.44 <sup>b</sup>	21.1 ± 0.73	36.0 ± 1.18	93.7 ± 1.73
Rice	18.6	47.8	65.5	97.2

<sup>a</sup>Reaction mixture contained 40 mg (by dry weight) of starch granules, 0.32M acetate buffer (pH 4.8), and 0.2% *Rhizopus* glucoamylase preparation in a total volume of 5.0 ml. Two blanks were run simultaneously, one under identical conditions except for the starch granules and the other identical except for the enzyme. Incubation was at 37°C. At the indicated time intervals, 5.0 ml of 0.6M perchloric acid was added to the mixture. After stirring and centrifugation, an aliquot of the supernatant was used for assay of glucose colorimetrically by the glucose oxidase-peroxidase method. Data were expressed as percentage glucose equivalent. Each value is the average of two determinations.

<sup>b</sup>Mean ± standard deviation (n = 4). Each value is the average of four determinations of two separate experimental runs.

TABLE II  
Digestion of Various Starch Granules by Pancreatin

Starch	Digestion Time (hr)	
	1	5
Potato	7.31 <sup>a</sup>	8.48
Yamanoimo	9.72	26.8
Sweet potato	15.2	43.3
Maize (Inbred Oh43 normal)	41.5	87.1
Waxy maize (Inbred Oh43 wx)	48.0	87.8
Rice	55.4	89.7

<sup>a</sup>Reaction mixture contained 50 mg (by dry weight) of starch granules, 6.7 mM phosphate buffer (pH 7.2), 10 mM sodium chloride, 10 mM calcium acetate, and 1% pancreatin in a total volume of 1.5 ml. Incubation was at 37°C. Two blanks were run simultaneously, one under identical conditions except for the starch granules and the other identical except the enzyme. At the indicated time intervals, 8.0 ml of ice-cold water was added to the mixture. After stirring and centrifugation, an aliquot of the supernatant was used for assay of solubilized carbohydrates by the phenol-sulfuric acid method. Data were expressed as percentage glucose equivalent. Each value is the average of two determinations.

of 0.4M acetate buffer pH 4.8, and 0.1 ml of 0.04% aqueous solution of *Rhizopus* glucoamylase. The starch solution was prepared by suspending 100 mg (by dry weight) of starch granules to 2.5 ml of water in a 10-ml volumetric flask and adding 0.25 ml of 10N sodium hydroxide. Standing overnight in a refrigerator, starch granules were completely gelatinized. The solution was neutralized with 1N acetic acid against phenolphthalein as an indicator and filled up with water. Incubation for the enzymatic reaction was 37°C. Two blanks were run simultaneously, one under identical conditions except 2.0 ml of water was used instead of the starch solution and, in the second blank, the other 1.0 ml of water was used instead of the enzyme solution. At appropriate time intervals, 0.5 ml of 3N perchloric acid was added to the reaction mixture. Glucose contents were determined by a colorimetric glucose oxidase-peroxidase method (22).

## RESULTS AND DISCUSSION

### Susceptibility of Various Starches to Glucoamylase

Table I lists starch granules of various types in order of decreasing resistance toward fungal (*Rhizopus* sp.) glucoamylase. Starch granules of maize were digested much more rapidly than those of potato. This agrees with the results of Leach and Schoch (23), who used glucoamylase from *Aspergillus niger*. Starch granules of tubers were more resistant to the attack of  $\alpha$ -amylases than were those of cereals (23–26). This was also the case for glucoamylase (Table I). Starch granules of *Japonica* rice are, in general, more susceptible to amylases than those of *India* rice. The alkaline steeping of polished *Japonica* rice was done in 0.2% solution of sodium hydroxide at low temperature (about 5°C), thus reducing chance artifacts of method of preparation. The differences in susceptibility of starch granules to glucoamylase completely disappeared following gelatinization of the starches with alkali. Since there were no differences, the data are not shown.

### Susceptibility of Starches of Various Genotypes of Inbred Oh43 Maize to Glucoamylase

As shown in Table IV, the *ae* starch was exceedingly resistant to digestion by glucoamylase. The *su*<sub>1</sub> and *su*<sub>2</sub> starches were digested much more rapidly than the normal maize starch. The *bt*<sub>1</sub>, *bt*<sub>2</sub>, *sh*<sub>2</sub>, *wx*, and *o*<sub>2</sub> starches were digested faster

TABLE III  
Digestion of Various Starch Granules by *Bacillus subtilis*

Starch	Digestion Time (hr)	
	1	2
Potato	1.92 <sup>a</sup>	4.98
Yamanoimo	4.28	10.8
Sweet potato	6.54	14.9
Maize (Inbred Oh43 normal)	16.9	51.5
Waxy maize (Inbred Oh43 wx)	22.6	67.1
Rice	27.5	52.9

<sup>a</sup>Crystalline  $\alpha$ -amylase of *B. subtilis* was used as the enzyme (final concentration 0.033%). For further details of the reaction conditions and assay, see footnotes to Table II.

than the normal, and the *du*, *h*, and *sh*<sub>1</sub> were similar to normal in digestibility. These results are in good agreement with those of Sandstedt *et al.* (6) in regard to *ae*, *du*, *su*<sub>1</sub>, and *su*<sub>2</sub>. The differences in susceptibility of starch granules of various genotypes of maize to glucoamylase disappeared following gelatinization of the starches with strong alkali. Since there were no differences, the data for gelatinized starch granules are not shown. The results shown in Table IV and data from the gelatinized starch preparation indicated that, in general, the *o*<sub>2</sub> mutant has no apparent effect on altering susceptibility of the double-mutant endosperm starches to glucoamylase, compared to the respective single nonopaque mutant counterparts. An exception to this generalization is noted in the case of *su*<sub>1</sub> and *su*<sub>1</sub>*o*<sub>2</sub>, where the double mutant was less digestible. It is quite possible that this result may be spurious. In fact, results from other experiments

TABLE IV  
Digestion of Starch Granules of Various Genotypes of  
Inbred Oh43 Maize by Glucoamylase

Genotype	Digestion Time (hr)			
	1		3	
	Single mutant	Double mutant with <i>o</i> <sub>2</sub>	Single mutant	Double mutant with <i>o</i> <sub>2</sub>
<i>ae</i>	0.89 <sup>a</sup>	0.78	2.41	2.67
<i>bt</i> <sub>1</sub>	11.5	9.94	30.6	28.2
<i>bt</i> <sub>2</sub>	12.1	17.8	34.8	41.7
<i>du</i>	4.19	4.28	14.2	15.2
<i>h</i>	7.70	8.35	25.3	28.3
<i>sh</i> <sub>1</sub>	7.42	8.24	24.7	26.8
<i>sh</i> <sub>2</sub>	11.6	13.6	32.4	35.2
<i>su</i> <sub>1</sub>	29.4	8.99	54.2	29.2
<i>su</i> <sub>2</sub>	26.1 ± 1.44 <sup>b</sup>	31.4	58.3 ± 1.85	65.4
<i>wx</i>	8.20	16.6	30.6	48.8
<i>o</i> <sub>2</sub>	11.5	...	32.8	...
Normal	6.49 ± 0.44	...	21.1 ± 0.73	...

  

Genotype	Digestion Time (hr)			
	5		24	
	Single mutant	Double mutant with <i>o</i> <sub>2</sub>	Single mutant	Double mutant with <i>o</i> <sub>2</sub>
<i>ae</i>	3.85 <sup>a</sup>	4.22	14.4	17.0
<i>bt</i> <sub>1</sub>	45.3	43.9	95.5	91.1
<i>bt</i> <sub>2</sub>	52.6	62.8	95.0	96.8
<i>du</i>	24.0	26.6	75.5	85.8
<i>h</i>	41.3	46.6	90.5	94.7
<i>sh</i> <sub>1</sub>	43.0	45.9	93.9	93.8
<i>sh</i> <sub>2</sub>	49.2	52.0	94.0	93.6
<i>su</i> <sub>1</sub>	68.1	49.0	96.0	94.5
<i>su</i> <sub>2</sub>	77.8 ± 1.12 <sup>b</sup>	82.2	94.5 ± 0.74	93.3
<i>wx</i>	53.2	71.8	96.1	96.1
<i>o</i> <sub>2</sub>	50.3	...	95.5	...
Normal	36.0 ± 1.18	...	93.7 ± 1.73	...

<sup>a,b</sup>For details of the reaction conditions and assay, see footnotes to Table I.

(unpublished data) using kernels from different samples grown in different years and in several different inbred lines of maize indicate that starch granules of *su1o2* were digested as rapidly as those of *su1*.

#### Susceptibility of Various Starches to $\alpha$ -Amylases

Tables II and III list starch granules of various types in order of decreasing resistance toward pancreatin and *B. subtilis*  $\alpha$ -amylase, respectively. Starch granules of tubers were more resistant to the attack of  $\alpha$ -amylases than those of cereals, as has been reported by others (23–26). The higher resistance toward  $\alpha$ -amylase of tuber starch granules than that of cereals disappeared following gelatinization of starch granules with alkali, though the data are not shown.

#### Susceptibility of Starches of Various Genotypes of Inbred Oh43 Maize to $\alpha$ -Amylases

As shown in Tables V and VI, the relative susceptibilities of endosperm starch granules of various genotypes of inbred Oh43 maize to pancreatin and *B. subtilis*  $\alpha$ -amylase are almost identical or very similar to those of glucoamylase (Table II). These differences in susceptibility to  $\alpha$ -amylases among starch granules of the various endosperm mutants and their normal counterpart tended to disappear following gelatinization of starch granules with alkali, though the data are not shown. In general, it is concluded that the *o2* gene has no apparent effect in the double-mutant combinations on altered susceptibility of endosperm starches to  $\alpha$ -amylases (Tables V and VI), which was also the case for the glucoamylase studies.

The physicochemical characteristics and  $\alpha$ -amylase susceptibility of starch granules are known to be dependent on endogenous and exogenous factors, especially genetic factors and the environmental temperature of the plant under which starch granules are formed (27).

The differences in susceptibility to the action of amylases on starch granules of the various genotypes of maize may also depend on these factors. These studies

TABLE V  
Digestion of Starch Granules of Various Genotypes of Inbred Oh43 Maize by Pancreatin

Genotype	Digestion Time (hr)			
	1		5	
	Single mutant	Double mutant with <i>o2</i>	Single mutant	Double mutant with <i>o2</i>
<i>ae</i>	7.4 <sup>a</sup>	7.4	16.8	23.4
<i>bt1</i>	41.3	38.5	79.0	77.0
<i>bt2</i>	47.2	57.8	81.2	89.4
<i>du</i>	26.4	33.0	61.7	65.5
<i>h</i>	41.1	42.8	82.9	85.1
<i>sh1</i>	46.3	45.1	85.5	84.2
<i>sh2</i>	46.0	46.4	76.5	78.5
<i>sh3</i>	58.3	49.6	79.6	81.7
<i>su2</i>	76.0	74.2	93.8	91.6
<i>wx</i>	44.3	52.9	84.6	88.4
<i>o2</i>	46.5	...	88.4	...
Normal	30.2	...	86.0	...

<sup>a</sup>See footnote to Table II for further details of the reaction conditions and assay.

TABLE VI  
Digestion of Starch Granules of Various Genotypes of  
Inbred Oh43 Maize *Bacillus subtilis*

Genotype	Digestion Time (hr)			
	1		5	
	Single mutant	Double mutant with $o_2$	Single mutant	Double mutant with $o_2$
<i>ae</i>	9.0 <sup>a</sup>	5.1	9.9	9.7
<i>bt</i> <sub>1</sub>	20.5	16.9	51.0	47.4
<i>bt</i> <sub>2</sub>	31.4	34.8	57.3	66.3
<i>du</i>	16.3	17.0	33.7	33.9
<i>h</i>	24.0	26.6	59.5	62.8
<i>sh</i> <sub>1</sub>	24.2	21.2	62.5	63.0
<i>sh</i> <sub>2</sub>	26.3	30.0	54.6	65.8
<i>sh</i> <sub>1</sub>	48.7	30.4	67.6	66.3
<i>su</i> <sub>2</sub>	68.0	72.2	86.8	90.7
<i>wx</i>	22.6	31.1	67.1	72.0
<i>o</i> <sub>2</sub>	24.4	...	57.1	...
Normal	22.1	...	50.9	...

<sup>a</sup>See footnotes to Tables II and III for further details of the reaction conditions and assay.

suggest that the genotype of the maize endosperm has a major influence on starch granule digestibility. We have not determined the effect of temperature on starch granule development and its influence on starch granule susceptibility to amylases.

From this study and others (1,2,3,7) it is apparent that there are several double-mutant combinations with  $o_2$  which may offer improved nutritional quality of maize with increased starch digestibility characteristics.

#### Literature Cited

1. BARBOSA, H. M. Genes and gene combinations associated with protein, lysine, and carbohydrate content in the endosperm of maize (*Zea mays* L.). Ph.D. Thesis, Purdue University (1971).
2. GLOVER, D. V., CRANE, P. L., MISRA, P. S., and MERTZ, E. T. Genetics of endosperm mutants in maize as related to protein quality and quantity. In: High-quality protein maize. Dowden, Hutchinson and Ross: Stroudsburg, Pa. (1975).
3. MISRA, P. S., JAMBUNATHAN, R., MERTZ, E. T., GLOVER, D. V., BARBOSA, H. M., and McWHIRTER, K. S. Endosperm protein synthesis in high-lysine maize mutants. *Science* 176: 1425 (1972).
4. KRAMER, H. H., PFAHLER, P. L., and WHISTLER, R. L. Gene interactions in maize affecting endosperm properties. *Agron. J.* 50: 207 (1958).
5. SANDSTEDT, R. M., HITES, B. D., and SCHROEDER, H. Genetic variations in maize: Effects on the properties of the starches. *Cereal Sci. Today* 13: 82 (1968).
6. SANDSTEDT, R. M., STRAHAN, D., UEDA, S., and ABBOT, R. C. The digestibility of high-amylose corn starches compared to that of other starches. The apparent effect of the *ae* gene on susceptibility of amylase action. *Cereal Chem.* 39: 123 (1962).
7. GLOVER, D. V., and BARTELOME, L. *In vitro* digestibility characteristics of endosperm mutants and their double combinations with *opaque-2*. Annual Report in "The inheritance and improvement of protein quality and content in maize." Contract csd/2809. US-AID: Washington, D.C. (1972).
8. KRAMER, H. H., and WHISTLER, R. L. Quantitative effects of certain genes on the amylose content of endosperm starch. *Agron. J.* 41: 409 (1949).

9. DVONCH, W., KRAMER, H. H., and WHISTLER, R. L. Polysaccharides of high amylose corn. *Cereal Chem.* 28: 270 (1951).
10. DUNN, G. M., KRAMER, H. H., and WHISTLER, R. L. Gene dosage effects on corn endosperm carbohydrates. *Agron. J.* 45: 101 (1953).
11. CAMERON, J. W., and TEAS, H. J. Carbohydrate relationships in developing and mature endosperms of brittle and related maize genotype. *Amer. J. Bot.* 41: 50 (1954).
12. FUWA, H. Phosphorylase and Q-enzyme in developing maize kernels. *Arch. Biochem. Biophys.* 70: 157 (1957).
13. CREECH, R. G. Genetic control of carbohydrate synthesis in maize endosperm. *Genetics* 52: 1175 (1965).
14. GLOVER, D. V. The inheritance and improvement of protein quality and content in maize. Annual Report. Contract csd/2809. US-AID: Washington, D.C. (1972).
15. SCHOCH, T. J. Preparation of starch and starch fractions, p. 5. In: *Methods in enzymology*, III, ed. by S. P. Colowick and N. O. Kaplan. Academic Press: New York (1954).
16. SUGIMOTO, Y., TAKAYA, T., and FUWA, H. *In vivo* digestion of starch granules of potato, yamanoimo, high-amylose corn, and corn by rats. *J. Jap. Soc. Starch Sci.* 22(4): 103 (1975).
17. KOBAYASHI, T. Preparation of starch, p. 198. In: *Starch handbook* (in Japanese), ed. by Z. Nikuni and S. Suzuki. Asakura Shoten, Ltd.: Tokyo, Japan (1961).
18. FUKUI, T., FUJII, M., and NIKUNI, Z. Digestion of raw starch granules by the action of amylase. Particularly on rice starch granule. *J. Agr. Chem. Soc., Japan* 38: 262 (1964).
19. DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and SMITH, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28: 350 (1956).
20. FUWA, H. A new method for microdetermination of amylase activity by the use of amylose as substrate. *J. Biochem.* 41: 583 (1954).
21. TOYAMA, T., HIZUKURI, S., and NIKUNI, Z. Estimation of starch gelatinization by means of glucoamylase. *J. Jap. Soc. Starch Sci.* 13: 69 (1966).
22. LLOYD, J. B., and WHELAN, W. J. An improved method for enzymic determination of glucose. *Anal. Biochem.* 30: 467 (1969).
23. LEACH, H. W., and SCHOCH, T. J. Structure of the starch granule. II. Action of various amylases on granular starches. *Cereal Chem.* 38: 34 (1961).
24. BALLS, A. K., and SCHWIMMER, S. Digestion of raw starch. *J. Biol. Chem.* 156: 203 (1944).
25. BOOHER, L. E., BEHAN, I., and McMEANS, E. Biological utilization of unmodified and modified food starches. *J. Nutr.* 45: 75 (1951).
26. GALLANT, D., MERCIER, C., and GUILBOT, A. Electron microscopy of starch granules modified by bacterial  $\alpha$ -amylase. *Cereal Chem.* 49: 354 (1972).
27. HIZUKURI, S. The effect of environment temperature of plants on the physiochemical properties of their starches. *J. Jap. Soc. Starch Sci.* 17: 73 (1969).

[Received September 9, 1974. Accepted June 4, 1976]