CONVERSION OF STARCH BY MICROBIAL ENZYMES FOR PRODUCTION OF SYRUPS AND SUGARS[1]

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ABSTRACT

A new commercial amylogucosidase preparation has low activities of other enzymes, of particular importance being the very low level of transglucosidase. Conversions of corn and potato starches have shown that liquefaction of 30% starch pastes, prior to saccharification of amyloglucosidase, can best be accomplished by using a heat-stable bacterial alpha-amylase at a minimum concentration of 175,000 MW units per lb. of starch, a pH of 5.5 to 7.0, and stepwise heating cycles over the gelatinization range of the starch. Optimum conditions for saccharification of amyloglucosidase have been found to be a temperature of 60°C., a pH range of 3.35 to 4.4, and an enzyme concentration of at least 60 units per lb. of starch.

Enzyme liquefaction and saccharification give essentially quantitative conversions of starch to dextrose. Acid liquefaction and enzyme saccharification give about 5% lower dextrose yields than the all-enzyme system, with higher quantitities of nondextrose sugars, while the acid saccharification procedure gives at least 12% less dextrose than the all-enzyme process.

It has long been known that fungal amylase preparations produce some glucose from starch. The availability of enzymes of the amyloglucosidase type, which convert starch specifically and completely to glucose, is very recent. Kerr and co-workers (5,6) were probably the first to rather vaguely recognize the presence of enzymes of the amylo-

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glucosidase type in commercial fungal amylase preparations. Corman and Langlykke (2), Corman and Tsuchiya (3), and Pool and Underkofler (13) demonstrated the importance of the amyloglucosidase moiety in the production of fermentable sugars from starch. The enzyme activities designated in this earlier literature as "maltase," "glucogenic enzyme," and "limit dextrinase" are probably attributable to amyloglucosidase.

Phillips and Caldwell (11,12), who called the enzyme glucamylase, and Kerr, Cleveland, and Katzback (4) in 1951 produced amyloglucosidase preparations free from alpha-amylase and characterized the general mode of action of the amyloglucosidases. Very recently Pazur and Ando (8,9) and Pazur and Kleppe (10) have prepared highly purified amyloglucosidase from a commercial product, and have reported detailed studies on the mode of action of the enzyme. These workers have shown that the enzyme converts starch, amylose, amylopectin, amylodextrin, and a wide variety of glucosyl oligosaccharides having alpha–D–(1 → 4), alpha–D–(1 → 6), alpha–D–(1 → 3) linkages to glucose in essentially quantitative yields. The amyloglucosidase hydrolyzes the oligosaccharides by a multichain mechanism beginning at the nonreducing end of the molecule and proceeds at a faster rate if the terminal unit is linked by an alpha–D–(1 → 4) linkage rather than an alpha–D–(1 → 6) linkage to the remainder of the oligosaccharide molecule. The alpha–D–(1 → 4) linkage in maltose is hydrolyzed at about 15 times the rate for the alpha–D–(1 → 3) linkage in nigerose and about 28 times the rate for the alpha–D–(1 → 6) linkage in isomaltose.

Materials and Methods

The starch used in all experiments was either a commercial Buffalo[2] Pearl corn starch or commercial powdered potato starch.

The amyloglucosidase used was the commercial preparation, DIAZYME L30 obtained from *Aspergillus niger*, and the heat-stable bacterial alpha-amylase was the commercial preparation, HT-1000 obtained from *Bacillus subtilis*.[3]

The enzyme activities were determined, using solutions of Merck's soluble starch according to Lintner, special for diastatic power determination. A unit of amyloglucosidase activity (DU) is the amount of enzyme which will produce 1.0 g. of glucose in 1 hr. when incubated with a 4% solution of the soluble starch at 60°C. and pH 4.0. A Modified Wohlgemuth (MW) unit of amylase activity is the amount of

[2] Buffalo Brand is a registered trademark of Corn Products Co., Argo, Illinois.
[3] The designations DIAZYME and HT are registered trademarks of Miles Laboratories, Inc., for the amyloglucosidase preparations and bacterial enzyme preparations, respectively, marketed by Miles Chemical Co.

enzyme which will dextrinize 1 mg. of starch to the color end point of a Hellige alpha-amylase color disk in 30 min. when incubated with a 1% solution of the soluble starch at 40°C. and pH 5.4.

Most of the studies involving starch liquefaction were carried out with a Brabender Amylograph, which gives good mixing and temperature control. After liquefaction, the starch solutions were transferred to flasks which were immersed in constant-temperature water baths during the saccharification periods. Unless otherwise indicated, the saccharification period was 72 hr.

The extent of saccharification achieved is expressed as dextrose equivalent (DE), the total reducing sugars expressed as dextrose and calculated as percentage of total dry substance.

Results and Discussion

Pertinent to the practical application of an enzyme system are such factors as the useful pH and temperature ranges, the storage stability, and the presence of possible interfering enzymes. The characteristics of the amyloglucosidase preparation were, therefore, determined. As shown in Fig. 1, the amyloglucosidase has a rather

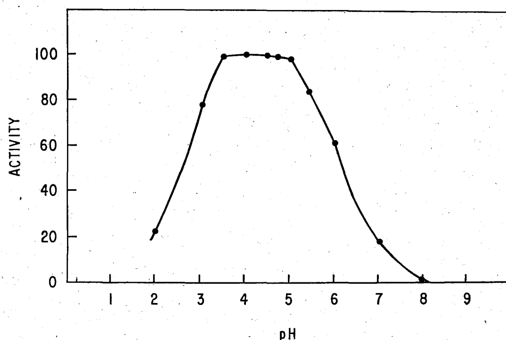

Fig. 1. Effect of pH on amyloglucosidase activity. The relative activities at the various pH values were calculated from the enzyme dilutions necessary to produce 20–30% hydrolysis of a 4% starch solution in 1 hr. at 60°C., maximum activity being referred to as 100.

broad pH optimum, maximum activity occurring over the range pH 3.5 to 5.0. Figure 2 shows that the maximum activity of the enzyme occurs over the temperature range 60° to 70°C. Storage stability is excellent, monthly assays over a period of 6 months showing no loss of amyloglucosidase activity in samples of the commercial product stored at room temperature.

Assays for other possible enzymes in the amyloglucosidase prepara-

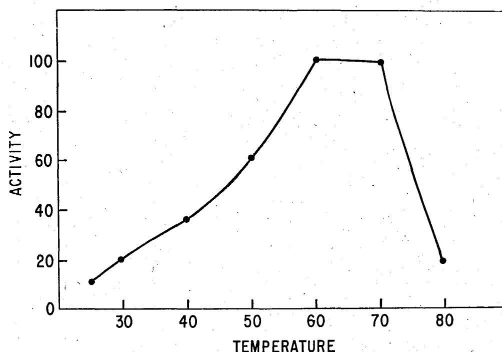

Fig. 2. Effect of temperature on amyloglucosidase activity. The relative activities at the various temperatures were calculated from the enzyme dilutions necessary to produce 20–30% hydrolysis of a 4% starch solution in 1 hr. at pH 4.5, maximum activity being referred to as 100.

tion were made, with the results shown in Table I. The preparation was found to contain no lactase, catalase, or glucose oxidase, traces of pectinase and hemicellulase too small to express in terms of units, and very low levels of the other enzymes.

TABLE I
ACTIVITIES OF OTHER ENZYMES PRESENT IN AMYLOGLUCOSIDASE PREPARATION

Enzyme	Activity
Alpha-amylase	128 SKB[a]/g.
Dextrinizing activity	39,000 MW[b]/g.
Invertase	0.13 g./100 g. sucrose/hr.
Lactase	none
Glucose oxidase	none
Catalase	none
Cellulase	480 CU[c]/g.
Hemicellulase	trace
Pectinase	trace
Protease (hemoglobin substrate)	440 HU[d]/g.
Protease (casein substrate)	1.95 NU[e]/g.

[a] SKB, Sandstedt, Kneen, and Blish units (14).
[b] MW, modified Wohlgemuth units (7).
[c] CU, cellulose units (7).
[d] HU, hemoglobin units (1).
[e] NU, Northrop units (7).

Of special interest in the practical use of amyloglucosidase is the amount of transglucosidase present. Transglucosidase catalyzes the formation (particularly from maltose) of unfermentable glucose polymers containing alpha-(1 → 6) linkages. If present in any substantial amount, this enzyme would catalyze the formation of unfermentable oligosaccharides and decrease the yield of glucose and fermentable sugars from starch. It has been ascertained that the amyloglucosidase

preparation used for these studies contains negligible quantities of transglucosidase.

Pazur and Kleppe[4] incubated a 0.2*M* solution of C^{14}-labeled maltose with high level of the amyloglucosidase preparation for 4 hr. and then separated the sugars by paper chromatography. They found that the amount of panose formed represented only 1.9% of the maltose hydrolyzed. Another preparation relatively high in transglucosidase gave under the same conditions 28% panose formed and hydrolyzed only 75% as much of the maltose. In our laboratory, maltose was incubated with the amyloglucosidase preparation for 72 hr., and then the hydrolysate was treated with yeast to remove fermentable sugars. Analysis showed that the nonfermentable sugars represented approximately 5% of the original maltose concentration. Maltose concentrations encountered during the enzymatic hydrolysis of starch with amyloglucosidase should be quite low; hence little reversion can be expected.

Since amyloglucosidase has but little activity against raw starch, the starch must first be rendered susceptible to amyloglucosidase action. This can be accomplished by heating under pressure with acid or by treating with an alpha-amylase at atmospheric pressure. Since we were interested in obtaining as nearly quantitative conversion of starch to glucose as possible, we decided to liquefy enzymatically. Studies were made of the effect of varying the pH, temperature, holding time, and bacterial alpha-amylase concentration during liquefaction of 30% starch slurries on the subsequent saccharification of corn starch with amyloglucosidase at a concentration of 80 units (DU) per lb. of starch.

Table II shows the effect of varying the pH of liquefaction on subsequent saccharification with amyloglucosidase. The concentration

TABLE II
EFFECT OF PH DURING ENZYMATIC LIQUEFACTION ON SUBSEQUENT SACCHARIFICATION WITH AMYLOGLUCOSIDASE

pH	DE	pH	DE
4.5	gelled	6.0	99.0
5.0	85.0	6.5	98.7
5.5	99.2	7.0	98.3

of bacterial amylase used for liquefaction was 175,000 MW units per lb. of starch. Liquefaction was carried out at the indicated pH by raising the temperature of the enzyme-starch slurry at the rate of 1.5°C. per min. to 85°C., then holding for 10 min. The liquefied starch

[4] Pazur, J. H., and Kleppe, K. Private communication, 1962.

was then cooled to 60°C. and the pH adjusted to 4.5. Amyloglucosidase was added at a concentration of 80 DU per lb. of starch and the mixture incubated at 60°C. for 72 hr.

The range from pH 5.5 to 7.0 appeared to be equally good for liquefaction with bacterial alpha-amylase, conversions of 98.3 to 99.2 DE being obtained. Upon subsequent saccharification with amyloglucosidase below pH 5.5, the starch was not completely gelatinized, and below pH 5.0 gelling occurred. Since the starch paste must be adjusted to pH 4.5 for conversion with amyloglucosidase, a pH of 5.5 was used for liquefaction in the following studies. This reduced to the minimum the amount of acid needed for adjustment.

Table III shows the effect of varying the liquefaction temperature on subsequent saccharification. The concentration of bacterial amylase used for liquefaction was 175,000 MW units per lb. of starch. Liquefaction was carried out at pH 5.5 by raising the temperature of the enzyme-starch slurry at the rate of 1.5°C. per min. to the indicated temperature, then holding for 10 min. The liquefied starch was then cooled to 60°C. and the pH adjusted to 4.5. Amyloglucosidase was added at a concentration of 80 DU per lb. of starch and the mixture incubated at 60°C. for 72 hr. The temperature range of 80° to 90°C. appeared to give the best results, showing conversions of 96.1 to 97.7 DE. For subsequent studies 85°C. was chosen, this offering less chance for inactivation of the enzyme than 90°C.

TABLE III

EFFECT OF TEMPERATURE DURING ENZYMATIC LIQUEFACTION ON SUBSEQUENT SACCHARIFICATION WITH AMYLOGLUCOSIDASE

Temperature	DE	Temperature	DE
°C.		*°C.*	
70	93.5	85	97.2
75	93.5	90	97.7
80	96.1	95	92.3

A study was made on the effect of varying the holding time at 85°C. during liquefaction on subsequent saccharification. Holding times from 10 min. up to 60 min. appeared to have little effect, essentially complete conversions being obtained in all cases.

Table IV gives the results obtained by varying the concentration of bacterial amylase used for liquefaction. The lowest concentration capable of yielding quantitative conversion of the starch to dextrose in 72 hr. or less appeared to be 175,000 MW units per lb. of starch.

With a bacterial amylase concentration of 175,000 MW units per lb. of starch and a temperature of 85°C. for 20 min. for liquefaction,

TABLE IV

EFFECT OF CONCENTRATION OF BACTERIAL ALPHA-AMYLASE ON SUBSEQUENT SACCHARIFICATION WITH AMYLOGLUCOSIDASE

BACTERIAL ALPHA-AMYLASE	TIME OF SACCHARIFICATION	DE	BACTERIAL ALPHA-AMYLASE	TIME OF SACCHARIFICATION	DE
MW units × 10^{-4}/lb. starch	*hr.*		*MW units × 10^{-4}/lb. starch*	*hr.*	
70.0	48	100	17.5	72	100
52.5	48	100	14.0	96	96.6
35.0	48	100	10.5	96	58.6

the effects of varying the incubation temperature, pH of incubation, and amyloglucosidase concentration on saccharification of a 30% (w/w) starch mixture in 72 hr. were studied. The data presented in Tables V and VI indicate that the optimum temperature is 60°C. and the optimum pH range is 3.35 to 4.4 for this extended incubation period at high starch concentration.

TABLE V

EFFECT OF INCUBATION TEMPERATURE ON SACCHARIFICATION WITH AMYLOGLUCOSIDASE

(Conditions: pH 4.5; amyloglucosidase concentration 80 DU/lb. starch)

TEMPERATURE	DE	TEMPERATURE	DE
°C.		*°C.*	
40	90.1	70	88.3
50	90.8	80	34.0
60	99.6		

TABLE VI

EFFECT OF pH DURING INCUBATION ON SACCHARIFICATION WITH AMYLOGLUCOSIDASE

(Conditions: Temp. 60°C.; amyloglucosidase concentration 80 DU/lb. starch)

pH	DE	pH	DE
2.3	76.9	4.4	97.0
2.9	96.5	5.0	95.2
3.35	100	5.7	94.2
3.6	100	6.6	64.6
4.0	100		

Amyloglucosidase concentrations of 40, 50, 60, 70, and 80 DU per lb. of starch gave 88.0, 95.5, 99.8, 100, and 100 DE, respectively, after incubation for 72 hr. at 60°C. and pH 4.5. These results indicate an amyloglucosidase level of at least 60 DU per lb. of starch is necessary for essentially quantitative conversion of starch to dextrose.

Though the process which evolved from these studies gave nearly quantitative conversion of starch to dextrose, two disadvantages were evident: 1) the rather large viscosity increase during liquefaction, and 2) the relatively slow filtration rate of the converted digest.

To overcome the viscosity problem various methods of heating the starch were tried.

Figure 3 shows four curves obtained by liquefying corn starch in different ways. The liquefactions were carried out in an amylo-

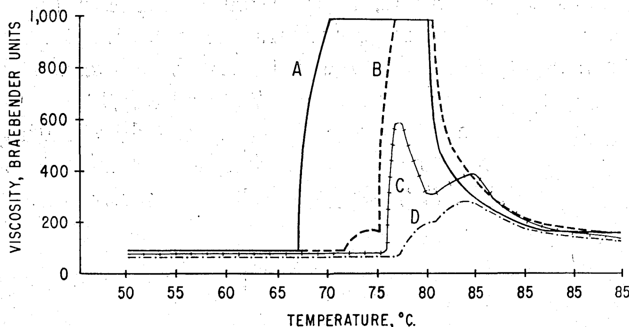

Fig. 3. Liquefaction of corn starch. Conditions of liquefaction: pH 6.0, starch concentration 30%, bacterial amylase concentration 175,000 MW units per lb. of starch. Curve A, heated to 85°C. and held for 20 min. Curve B, heated to 65°C. and held for 1 hr., then heated to 85°C. and held for 20 min. Curve C, heated to and held for 30 min. at 65°, 67°, and 70°C., then at 72°, 75°, 80°, and 85°C. for 10 min. each. Curve D, heated to and held for 30 min. each at 65°, 67°, 70°, and 72°C., then at 74° and 75° for 15 min. each, and at 77°, 80°, and 85° for 10 min. each.

graph at pH 5.5 using 30% (w/w) starch slurries and 175,000 MW units of bacterial amylase per lb. of starch. Curve A was obtained by heating the starch slurry to 85°C. at the rate of 1.5°C. per min. and holding at that temperature for 20 min. The viscosity started to increase at 67°C. and reached a maximum between 70° and 80°C. It then started to drop and reached a minimum during the holding period at 85°C. A similar curve can be obtained by heating at any temperature above 70°C. However, at these lower temperatures gelatinization does not appear to be complete as judged by the lower dextrose equivalents obtained upon subsequent saccharification with amyloglucosidase.

Curve B was obtained by heating at 65°C., just below the gelatinization temperature, for 1 hr., then heating to 85°C. and holding for 20 min. The effect of this procedure was to raise the gelatinization temperature to about 77°C. and decrease the period of maximum viscosity from about 7 to about 2 min. A curve similar to curve B was obtained by raising the temperature of the slurry in a stepwise fashion, the temperature being raised to 65°C., then to 70°, 75°, 80°, and 85°C. with the mixture held for a period of 10 min. at each temperature.

Curve C was obtained by holding the temperature at 65°, 67°, and 70°C. for 30 min. each. The temperature was then raised stepwise to 72°, 75°, 80°, and 85°C., held for 10 min. at each step. This procedure produced two peaks, one at 77°C. with a viscosity of 600 B.U., the second between 80° and 85°C. with a viscosity of 400 B.U.

From curves A, B, and C, it appeared that the starch granules exhibited various degrees of susceptibility to gelatinization. If this is the case, then it should be possible to flatten the curves still more by increasing the number of steps between 72° and 82°C. This is exactly what happened in curve D. This curve was obtained by heating to 65°, 67°, 70°, and 72°C. for 30 min. each, then at 74° and 75°C. for 15 min. each, and finally to 77°, 80°, and 85°C. for 10 min. each.

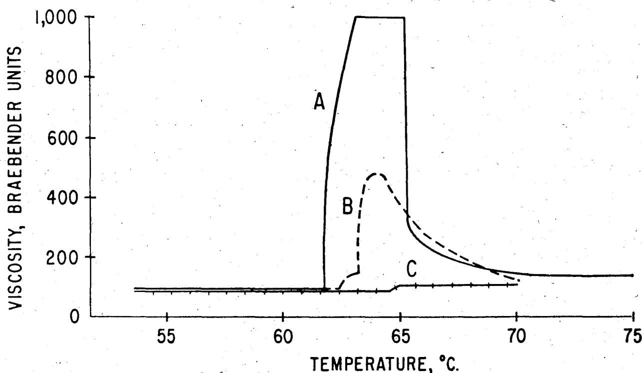

Fig. 4. Liquefaction of potato starch. Conditions of liquefaction: pH 6.0, starch concentration 30%, bacterial amylase concentration 175,000 MW units per lb. of starch. Curve A, heated to 85°C. and held for 20 min. Curve B, heated to and held for 10 min. each at 55°, 60°, 65°, and 70°C. Curve C, heated to and held for 30 min. each at 55°, 60°, and 65°C., then at 70°C. for 10 min.

Figure 4 shows the results of similar experiments with potato starch. Curve A was obtained by heating to 85°C. and holding for 20 min. The viscosity started to increase at about 62°C. and reached a maximum between 63° and 65.5°C. It then dropped rapidly, reaching a minimum at a little above 70°C. For curve B, the slurry was heated stepwise to 55°, 60°, 65°, and 70°C. and held for 10 min. at each temperature. Curve C was obtained by heating at 55°, 60°, and 65° for 30 min. each, then bringing to 70°C. for 10 min. As can be seen from this curve, liquefaction actually occurred, with virtually no increase in the viscosity of the starch.

The final apparent viscosities of the enzyme-liquefied starch pastes differ little, regardless of the heating procedures. The stepwise heating-

holding method, as well as a slow continuous increase in temperature over a prolonged period, minimizes viscosity increases which cause agitation difficulties on a large scale. Under gradual heating the liquefying amylase is able to dextrinize the starch as rapidly as the granules swell and are gelatinized, with a minimum of viscosity increase.

Of the procedures studied in the hope of increasing filtration rates, heating under pressure was the only one which proved successful. Autoclaving the digests for 15 min. at 15 lb. pressure increased filtration rates better than fivefold.

Summarizing, the procedure which we have found up to the present time to give the best conversions of starch to dextrose is as follows:

1. Liquefy a 27–33% (w/w) starch slurry at pH 5.5–7.0, using 175,000 MW units per lb. of starch of a heat-stable bacterial alpha-amylase with stirring while heating in a stepwise fashion, particularly below the gelatinization temperature of the starch.
2. Cool to 60°C. and adjust to pH 4.0. Add amyloglucosidase in a concentration of 80 units per lb. of starch.
3. Incubate at 60°C. for 72–96 hr.
4. Heat at 230°F. at 15 lb. steam pressure for 15 min.
5. Cool and filter.
6. Treat the filtrate with 0.5% carbon at 70°C. for 20–30 min.
7. Filter and concentrate to about 80% solids.
8. For a slab sugar allow the concentrate to solidify in the cold.

With this procedure, a typical corn starch conversion gave 18.7 DE after liquefaction, and 95.0, 100, and 100 DE after 24, 48, and 72 hr. of incubation, respectively. A potato starch gave 22.1 DE after liquefaction, and 94.1, 100, and 100 DE after 24, 48, and 72 hr.

For the successful utilization of amyloglucosidase for converting high concentrations of starch, in the range of 30 to 50%, to dextrose, it has been found essential, both for handling the thick gelatinized pastes and for maximum yields of sugar, to thin the starch pastes prior to incubation with the amyloglucosidase. This thinning is conventionally done in plants using the enzymatic saccharification process by limited acid cooking under pressure. In Table VII are shown laboratory results obtained by 1) all-enzyme thinning and saccharification, 2) acid thinning by heating under pressure followed by enzyme saccharification, and 3) complete acid conversion. The latter was accomplished by adding hydrochloric acid to pH 1.5 and heating for 3 hr. at 20 lb. steam pressure.

As is well known, complete acid conversion never approaches quantitative dextrose yields because of side reactions resulting in produc-

TABLE VII

COMPARISON OF ALL-ENZYME (EE), ACID-ENZYME (AE), AND ACID (Ac) CONVERSIONS OF STARCH

METHOD	LIQUEFACTION DE	DE			FILTRATION TIME[a]	CAKE WEIGHT	FILTRATE DE
		After 24 hr.	After 48 hr.	After 72 hr.			
					min.	*g.*	
EE	12	93	100	100	3[b]	8	100
AE	10	78	85	91	18	13	99
AE	13	89	92	96	15	9	93
AE	19	84	87	93	2	7	93
Ac	. .	88[c]			5	4	. .

[a] Samples of 500 g. filtered through 24-cm. Büchner funnel fitted with Whatman No. 1 paper.
[b] Autoclaved at 15 lb. steam pressure prior to filtration. Filtration time of unautoclaved sample was 30 min.
[c] DE of hydrochloric acid hydrolysate converted at pH 1.5 for 3 hr. at 20 lb. steam pressure.

tion of reversion oligosaccharides. In the particular experiment given in Table VII, final conversion of 88 DE was obtained with the acid hydrolysis process. Acid thinning and enzyme hydrolysis give better conversions, in the range of 91 to 96 DE, whereas the all-enzyme conversion process under optimum conditions is essentialy quantitative. Filtration rates for the acid-enzyme and all-enzyme conversion processes are comparable if the all-enzyme digests are heated briefly under pressure following the conversion.

The sugar solutions resulting from conversions recorded in Table VII were carbon-treated and evaporated to 80% solids, and the syrups were poured into pans and allowed to solidify in the cold room. The results of analyses of the resulting slab sugars are given in Table VIII. The all-enzyme product has lower ash and higher dextrose content than the acid-enzyme-converted products.

One noticeable characteristic of the acid-enzyme conversions is the appreciably higher quantities of sugars other than dextrose present as

TABLE VIII

ANALYSIS OF SLAB SUGARS PRODUCED BY ACID-ENZYME (AE) AND ALL-ENZYME (EE) CONVERSIONS

	METHOD AND LIQUEFACTION DE			
	AE (10)	AE (13)	AE (19)	EE (12)
Color	off-white	white	off-white	white
Starch	neg.	neg.	neg.	neg.
Reducing sugars, %	87.6	87.9	86.4	90.8
Dextrose, %[a]	86.0	86.7	85.1	90.2
Other sugars, %[a]	3.7	3.4	4.3	0.6
Ash, %	0.856	0.218	0.600	0.118
Chloride, %	0.22	0.08	0.22	0.04
Moisture, %	9.2	9.6	8.9	9.1
Total, %	99.976	99.998	99.120	100.058

[a] Paper chromatography followed by elution and determination of reducing sugars.

compared with the products from all-enzyme conversion. Since exactly the same saccharifying enzyme system is operating in both cases, this difference cannot be ascribed to the presence of transglucosidase or other polymerizing enzymes in the amyloglucosidase preparation. Rather, the acid thinning treatment must produce glucosidic linkages which are not susceptible to attack by the saccharifying enzyme system.

Literature Cited

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis (9th ed.), Sec. 17, p. 231. The Association: Washington, D.C. (1960).
2. CORMAN, J., and LANGLYKKE, A. F. Action of mold enzymes in starch saccharification. Cereal Chem. **25:** 190–201 (1948).
3. CORMAN, J., and TSUCHIYA, H. M. Utilization of fungal amylase for alcohol production. Cereal Chem. **28:** 280–288 (1951).
4. KERR, R. W., CLEVELAND, F. C., and KATZBECK, W. J. The action of amyloglucosidase on amylose and amylopectin. J. Am. Chem. Soc. **73:** 3916–3921 (1951).
5. KERR, R. W., MEISEL, H., and SCHINK, N. F. Corn sirups of high fermentability. Ind. Eng. Chem. **34:** 1232–1234 (1942).
6. KERR, R. W., and SCHINK, N. F. Fermentability of cornstarch products — relation to starch structure. Ind. Eng. Chem. **33:** 1418–1421 (1941).
7. MILES CHEMICAL COMPANY. Enzyme assay procedures. (Mimeo copies available upon request.) Elkhart, Indiana.
8. PAZUR, J. H., and ANDO, T. The action of an amyloglucosidase of *Aspergillus niger* on starch and malto-oligosaccharides. J. Biol. Chem. **234:** 1966–1970 (1959).
9. PAZUR, J. H., and ANDO, T. The hydrolysis of glucosyl oligosaccharides with α-D-(1 → 4) and α-D-(1 → 6) bonds by fungal amyloglucosidase. J. Biol. Chem. **235:** 297–302 (1960).
10. PAZUR, J. H., and KLEPPE, K. The hydrolysis of α-D-glucosides by amyloglucosidase from *Aspergillus niger*. J. Biol. Chem. **237:** 1002–1006 (1962).
11. PHILLIPS, L. L., and CALDWELL, M. L. A study of the purification and properties of a glucose-forming amylase from *Rhizopus delemar*, gluc amylase. J. Am. Chem. Soc. **73:** 3559–3563 (1951).
12. PHILLIPS, L. L., and CALDWELL, M. L. A study of the action of gluc amylase, a glucose-producing amylase, formed by the mold, *Rhizopus delemar*. J. Am. Chem. Soc. **73:** 3563–3568 (1951).
13. POOL, E. L., and UNDERKOFLER, L. A. Fungal saccharifying agents — amylolytic factors of bran culture and submerged culture. J. Agr. Food Chem. **1:** 87–90 (1953).
14. SANDSTEDT, R. M., KNEEN, E., and BLISH, M. J. A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16:** 712–723 (1939).