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IMPROVEMENT AND ESTIMATION OF ENZYMIC STARCH SACCHARIFICATION PROCESS

Yu-Yen Linko* and Xiao Yan Wu
Laboratory of Biotechnology and Food Engineering, Helsinki University of Technology, SF-02150 Espoo, Finland

SUMMARY : The effect on the dextrose equivalent value (DE) obtained in maltodextrin saccharification with a glucoamylase preparation containing various quantities of acid stable α-amylase isolated by anion exchange chromatography was investigated. When the acid stable α-amylase activity was increased 2.5-fold, a maximum DE value of 95.6% (glucose content, DX, 95.0%) was reached in 48 h, 1.4 % (DX of 1.1%) higher than that of the control (DE 94.2%; DX 93.9%).

INTRODUCTION

Agricultural surplus commodities, low quality grains and starch containing waste materials can be utilized to produce useful chemicals such as fuel ethanol (Linko et al., 1984), and lactic acid (Linko, 1985) for further production of biodegradable poly (lactic acid). The first step in the process is the liquefaction of starch by α-amylase (EC 3.2.1.1, 1,4-α-D-glucan glucanohydrolase), followed by saccharification with glucoamylase (EC 3.2.1.3, 1,4-α-D-glucan glucohydrolase) to obtain a high yield of glucose. Although the starch hydrolysis process is industrially well established, there is still room for improvement in the yield. Considering the large quantities of starch hydrolysed, even a small improvement in the yield is of marked economic importance. Therefore, great efforts have been made to search for improvements both in the liquefaction and saccharification stages. One of the first improvements was the elimination of trans-glucosidase activity from glucoamylase preparations in order to minimize the formation of α-1,6-linked oligosaccharides as by-products (Pazur et al,. 1960), thus increasing glucose yield. Inasmuch as glucoamylase is an exo-hydrolase which mainly attacks α-1,4-linkages at the non-reducing ends and α-1,6-linkages at a much reduced rate, the addition of debranching enzymes such as isoamylase (EC 3.2.1.68, Glycogen 6-glucanohydrolase) and pullulanase (EC 3.2.1.41, pullulan 6-glucanohydrolase) have also been used during saccharification for improved glucose yield and reduced reversion reactions (Reilly, 1985). More recently, the advantage of an adequate α-amylase activity in the

glucoamylase system employed in saccharification has been recognized (Labout, 1985). An increased rate of saccharification has been demonstrated both by applying immobilized enzyme technology (Linko et al., 1979), and by using continuous extrusion processing for the liquefaction (Linko et al., 1983). In the present work, saccharification of liquefied maltodextrin with glucoamylase containing a suitable level of acid stable α-amylase was studied to improve the glucose yield. The composition of a commercial glucoamylase preparation was modified by adding acid stable α-amylase obtained by BioPilot fractionation with Q Sepharose Fast Flow Chromatography.

MATERIALS AND METHODS

Materials : A commercial glucoamylase preparation (glucoamylase activity 3986 U ml^{1} and α-amylase activity 188 U ml^{-1}) was used in the present study. Maltodextrin (moisture 6.1 %, DE 12.8%) was obtained from Roquette Freres, France.

Enzyme separation system : BioPilot System (Pharmacia LKB Biotechnology, Uppsala, Sweden) equipped with LCC–500 PLUS controller, UV-M/1 control unit, conductivity meter control unit and pH meter control unit was used for the separation and collection of the components of a commercial glucoamylase preparation. All gels and ion-exchanger columns used in the present study were from Pharmacia.

Sephadex G-25 gel filtration : The crude enzyme solution was diluted 10-fold at room temperature and the diluted enzyme solution was applied to a SR 25 column packed with Sephadex G–25 gel filtration medium previously equilibrated with 0.05M sodium acetate buffer, pH 5.8, followed by eluting with the same buffer (3.5 bed volumes) at a flow rate of 15 ml min^{-1}. The peaks, determined at 280 nm, and exceeding a threshold value of 15% of the full UV scale (A_{280} = 2.0) were collected.

Q Sepharose Fast Flow Chromatography : After gel filtration, the enzyme solution was injected into a XK 16/20 column (1.6 × 6.0 cm) packed with Q Sepharose Fast Flow anion exchanger pre-equilibrated with 0.05M sodium acetate buffer, pH 5.8, and fractionated by stepwise elution with sodium acetate buffers of 0.05, 0.19, 0.33, 0.43, 0.60 and 1.00 M. Peaks exceeding a threshold value of 8 % of the full UV scale (here A_{280} = 1.0) were individually collected. The flow rate was kept constant at 5 ml min^{-1}.

Protein determination : Protein was determined by the method of Lowry et al. (1951).

Enzyme activity assays : Total α-amylase activity was determined by the method as described by Linko et al. (1988), except that potato starch covalently linked with Remazol Brilliant Blue R (Sigma) was used as substrate. One unit (U) of α-amylase activity was defined by absorbance (A) at 595 nm as 0.1 A min^{-1}, and the activity was reported per ml of the enzyme preparation.

Acid-stable α-amylase activity was determined after first inactivating the neutral amylase by incubation with 0.1M HCl at pH 2.5, 37 °C for 30 minutes according to the method described by Minoda and Yamada (1963).

Glucoamylase activity was determined according to the method described by Linko et al. (1988).

Dry matter : Dry matter was determined by heating samples mixed with pre-dried sand at 64 °C under vacuum of less than 0.07 kg/cm^2 for 18 h.

Dextrose equivalent (DE) value : Dextrose equivalent value (DE) was determined by the method described by Miles Laboratories, Inc. (1963).

Glucose determination : Glucose was determined by the hexokinase/glucose-6-phosphate dehydrogenase method (Boehringer Mannheim, GmbH, 1990) at 340 nm, and reported as DX (% of dry matter).

Saccharification of maltodextrin : A number of one litre covered stirred tank reactors were used for saccharification of maltodextrin as described by Linko et al. (1975). The stirring speed was about 250 rpm as determined by a stroboscope. The saccharification procedure was carried out at 60 °C in a water bath. The pH of the maltodextrin solution was adjusted to 4.5 using 0.1M HCl and commercial glucoamylase preparation (dosage equal to 268 U glucoamylase and 12.6 U acid stable α-amylase per 100g d.m.) with or without acid stable α-amylase was added. The dry matter concentration used in the present study was about 31 % (w/w). Samples were taken at certain time intervals, and the DE values were determined accordingly.

Modelling of saccharification process : Multiple variable regression analysis was employed to obtain a polynomial model for the DE-value as a function of the ratio (x_1) of total acid stable α-amylase activity to original acid stable α-amylase activity and saccharification time in hours (x_2).

Statistical treatment of data : The statistical methods given in Descriptive Statistics in Analysis Tools of MS-Excel 4.0 were used to analyze the DE values obtained. The significance was denoted as *** ($P < 0.001$).

RESULTS AND DISCUSSION

Isolation of acid stable α-amylase : As shown in Fig. 1, 6 protein peaks were obtained with stepwise Q Sepharose Fast Flow column anion exchange chromatography of a commercial glucoamylase. The protein content and enzyme activity of each fraction is listed in Table 1. It was found that peaks 1 and 2 had neither α-amylase nor glucoamylase activity, peaks 3 and 4 showed only α-amylase activity, and peaks 5 and 6 had only glucoamylase activity, but no α-amylase activity. Peak 3 retained more than 80% of its activity after incubation at 37 °C,

Table 1 Enzyme activities and protein content of the fractions

Fraction	1	2	3	4	5	6
α-Amylase activity (U ml^{-1})	0	0	0.87[a]	0.21	0	0
Glucoamylase activity (U ml^{-1})	0	0	0	0	14.37	3.1
Protein content (mg ml^{-1})	0.153	0.029	0.227	0.12	1.033	0.194

[a] 80 % acid stable α-amylase.

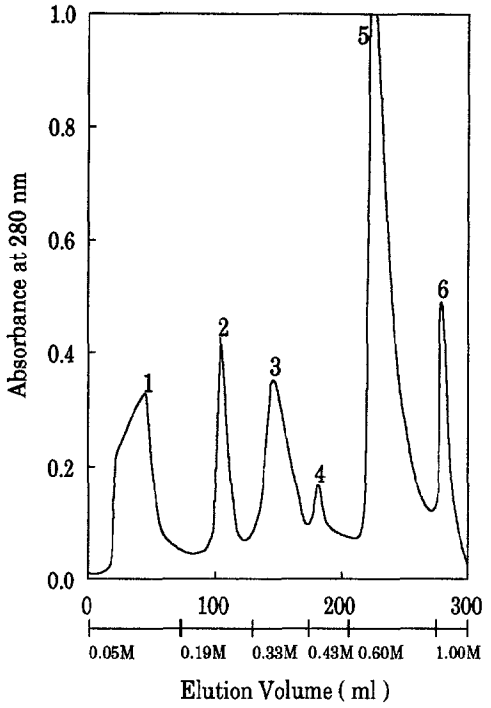

Figure 1. *Elution profile of glucoamylase with stepwise varying acetate buffer concentration (pH 5.8, Q Sepharose Fast Flow column (1.6 × 6.0 cm), flow rate 5 ml min^{-1}).*

pH 2.5 for 30 minutes, but peak 4 lost all of its activity under these conditions. This indicated that peak 3 represented an acid-stable α-amylase. The fractions of the acid stable α-amylase from several runs were combined and concentrated by ultrafiltration using a PM10 membrane (Amicon Corp., MA, U.S.A.) for further study.

Saccharification by glucoamylase with addition of various dosages of acid stable α-amylase : In a control experiment, a 31.6 % (d.m.) maltodextrin solution was saccharified at 60 °C, pH 4.5 using the commercial glucoamylase preparation only. A DE value of 94.2 % (DX 93.9%) was obtained in 48 hours. Fig. 2 illustrates the results when mixtures of commercial glucoamylase preparation and different amounts of added acid stable α-amylase at a constant glucoamylase activity were used in saccharification. During the first 14 h, an increase in the acid stable α-amylase clearly resulted in a marked increase in the DE value, with the highest increase obtained with the highest 5-fold increase in acid stable α-amylase activity. For example, a DE value of 89.3 % was reached in 14 h with the 5-fold addition of acid stable α-amylase to the commercial glucoamylase preparation, a value of 3.3 % higher than that of control (DE of 86.0 %). A maximum DE-value of as high as 95.6 % (DX 95.0 %), in 48 h saccharification was obtained with a 2.5-fold increase in α-amylase dosage (31.5 U per 100g d.m.), this was 1.4 % (DX 1.1 %) higher than that with the control.

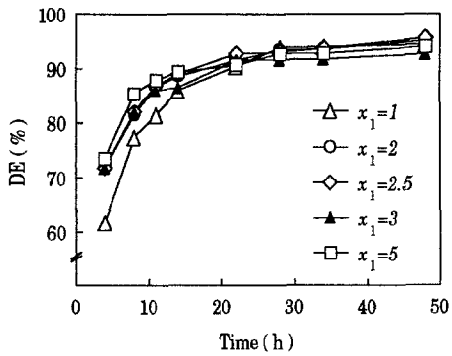

Figure 2. *Saccharification of maltodextrin (31.6 % d.m.) with the commercial glucoamylase in combination with varying amounts (x_1) of added acid-stable α-amylase at 60 °C, pH 4.5.*

Modelling and estimation of progress of maltodextrin saccharification process : The following polynomial model for DE value was obtained by multiple variable regression analysis of data showed in Fig. 2 with the various acid stable α-amylase activity additions of 1.0-, 2.0-, 2,5-, 3.0-, 5.0-fold of the original at constant glucoamylase activity:

$$DE = -0.30516x_1^2 + 6.085918x_1 - 1.26924x_1 \ln x_2 + 32.50459 \ln x_2 - 3.71342(\ln x_2)^2 + 23.43$$

$R = 0.988^{***}$, $R^2 = 0.976$,

in which DE is the dextrose equivalent (%, d.m.); x_1 is the ratio of total acid stable α-amylase activity to the original acid stable α-amylase activity in the commercial glucoamylase preparation; x_2 is saccharification time in hours; R is multiple correlation coefficient, and R^2 is the coefficient of determination obtained by regression analysis based on the method of least squares. Fig. 3 illustrates the validation of the model obtained by saccharification using $x_1 = 1.5$ under otherwise standard conditions. As can be seen, the fit was excellent.

Fig. 4 gives the DE-value as the function of the ratio x_1 and saccharification time x_2. The addition of acid stable α-amylase at a ratio x_1 within the range of about 1.9 to 2.7-fold (shadowed area) results in a clear improvement. The time required for reaching the same yield of glucose was reduced, and the yield of glucose was increased.

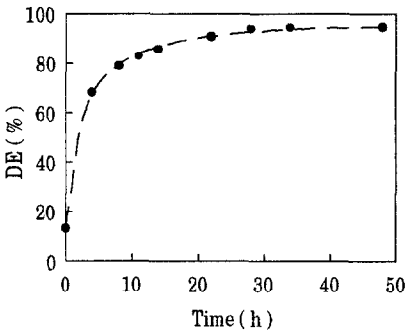

Figure 3. *Validation of the model for the DE-value using x_1 = 1.5. Estimated (---) and measured (•) DE values, when saccharification was conducted at 60 °C, pH 4.5 and 31.6 % d.m.*

Statistical analysis of the DE values obtained : Five maltodextrin saccharification runs (31-33 % d.m.) were carried out under the experimental conditions described above for the control. Fig. 5 shows the DE-value as the function of time for x_1 = 1.0 (Δ) and x_1 = 2.5 (◇) as obtained by exponential smoothing method. The multiple correlation coefficients were $R = 0.999^{***}$ and $R = 0.998^{***}$, respectively. The 95% confidence belts for the sample mean are also given, the standard deviation at 48 h was 0.22, and the significant increase in the DE value obtained with x_1 = 2.5 was 1.41. The results clearly indicate that the glucose yield (represented by DE value) could be improved by the addition of acid stable α-amylase.

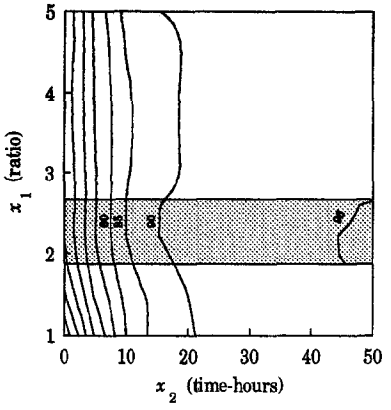

Figure 4. *DE-value of saccharified maltodextrin (expressed as response contour) as a function of x_1, ratio of total acid stable α-amylase activity to the original acid stable α-amylase activity in the commercial glucoamylase preparation, and x_2, saccharification time.*

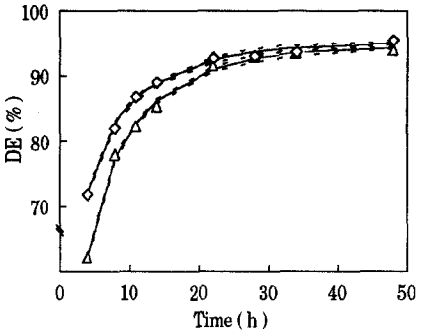

Figure 5. *DE value as the function of time(—), with 95% confidence belts (---) for DE values obtained from x_1 = 1.0 (Δ; $DE = 17.0 + 39.7\ ln(x_2) - 5.1\ ln(x_2)^2$) and x_1 = 2.5 (◇ ; $DE = 40.6 + 27.2\ ln(x_2) - 3.4\ ln(x_2)^2$), respectively.*

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