COMMUNICATION II

Production of Cassava Syrup with Glucoamylase Immobilised to Acid-washed Charcoal

Key words: Cassava syrup, immobilized glucoamylase, acid-wash charcoal.

ABSTRACT

Glucoamylase immobilised to acid-washed animal charcoal was used to effect the saccharification of liquefied cassava starch. A 7 DE liquefied cassava starch solution (30% D.S.) was converted into a 69 DE cassava syrup when the substrate was fed at a flow rate of 1.0 ml min⁻¹ into a packed-bed column reactor of immobilized glucoamylase. The relationship between the flow-rate and the extent of saccharification was found to be asymptotic. Much of the brown colour that developed during the liquefaction of the substrate was removed when the flow rates was low, giving a fairly colourless syrup.

INTRODUCTION

Enzymes can be immobilised by binding to water-insoluble supports such as charcoal, porous glass beads, cellulose, chitin, ion-exchange resins and other biopolymers. Enzymes immobilised thus can effect the continuous conversion of substrate to product, allow re-use of the enzyme, and require smaller plant space than conventional processing plants.

Numerous enzyme supports or carriers and methods have been used to immobilise glucoamylase (E.C. 3.2.1.1). The general modes of immobilisation to support include adsorption, covalent attachment and entrapment. Immobilised glucoamylase was first reported by Wilson and Lilly (1969) for the production of sweet glucose syrup from dextrin. The enzyme was immobilised to DEAE-cellulose using the bifunctional reagent 3-amino-4, 6-dichloro-S-triazine. Since then other methods of immobilisation have been reported and include binding to activated Ammerlite resin (Park and Lima, 1978) cellulose beads (Chen and Tsoo, 1977), porous silica (Klyosov et al., 1979, Lee et al., 1970), gelatin (Hartmier, 1980), granular polyacrylonitrile (Handa et al., 1982) and chemically modified activated carbon (Cho and Bailey, 1984).

Various substrates for immobilised glucoamylase have been used. The most commonly used substrate is corn starch (Bachler et al., 1971; Park and Lima, 1973; Flynn and Johnson, 1978; Allen et al., 1979). Others include barley and wheat starches (Lindroos et al., 1980), wort (Hartmier, 1980) and cassava starch (Park and Lima, 1973).

It has been shown by a research group at the Phillip Lyle Memorial Research Laboratory (UK) that glucoamylase immobilised in a manner similar to that reported in this paper can be successfully used to produce glucose syrup of greater than 90 DE from commercial preparations of solubilized corn starch. However, a
similarly immobilized glucoamylase has not been used to effect the saccharification of cassava starch.

This paper reports on the activity of both soluble and immobilised glucoamylase in the saccharification of solubilised (liquefied) cassava (Manihot esculenta, L.) starch.

MATERIALS AND METHODS

Materials

Bacillus subtilis α-amylase (EC 5.2.1.1, 60 units/mg) was purchased from Sigma Chemical Co., St. Louis, U.S.A. Aspergillus niger 150 L glucoamylase (EC 3.2.1.3., 150 Novo units, Novo Industries, Denmark) was kindly donated by Dr. R. Alagaratnam of Enzyme Tekniks, Malaysia. Granular animal charcoal (10–18 mesh) and 50% glutaraldehydes were purchased from BDH Chemicals Ltd, Poole, England. Other chemicals were of analytical grade. Cassava (Manihot esculenta, L.) starch was obtained locally.

Immobilisation of Glucoamylase

Glucoamylase was immobilised according to the method developed by a research group at the Phillip Lyle Memorial Research Laboratory, U.K. The enzyme support or carrier that was used for immobilization was acid-washed animal charcoal and prepared according to Osborn et al., (1982) except that the acid-washed charcoal was air-dried at room temperature instead of under vacuum at 100°C for 12 hours. The charcoal was ground and sifted to obtain particles of sizes 710–1000 μm.

Glucoamylase was immobilised to the acid-washed charcoal by adding a 20% solution of glutaraldehydes in acetone to a beaker containing 40 g of charcoal mixed with 30 ml of charcoal enzyme solution. The reaction mixture was stirred and allowed to stand for about 1–2 hours. The mixture was then washed to remove any traces of the immobilization liquor. The immobilised glucoamylase was stored at 4°C in an air-tight container as a damp paste. The weight of immobilised glucoamylase used was expressed as gram wet weight (gww).

Preparation of Substrate

Prior to reaction with glucoamylase, starch must be solubilised or liquefied. For cassava starch, a two-step enzymatic liquefaction procedure was adopted. The first stage involved the addition of 60 units of α-amylase per ml of cassava starch slurry of different concentrations (20–40% dry substance, corrected for moisture content) at pH 6.8 and incubating the reaction mixture with shaking for 20 minutes at 85°C. The thinned starch solution was then cooked at 15 psi for 10 minutes.

The second stage involved a further addition of 60 units of α-amylase per ml of the thinned and cooked starch solution and incubating with shaking at 85°C. After cooling to room temperature, the pH was adjusted to 4.5 and the preparation was used as the substrate for saccharification by glucoamylase.

Saccharification of Substrate

Batch saccharification using soluble glucoamylase. Saccharification using soluble glucoamylase was achieved by adding the enzyme at a concentration of 15 units per ml substrate and incubating at 55°C with shaking. Samples were withdrawn at 30 minute intervals and the enzymic activity was inhibited by placing in an ice bath. Reducing sugar was analysed as soon as possible. It was found that placing samples in a boiling water-bath to terminate the reaction caused gelation.

Continuous saccharification using immobilised glucoamylase. Continuous saccharification of a 30% DS liquefied starch was carried out by feeding the substrate against gravity into a jacketed glass column (37 cm × 1 cm i.d.) containing 10 gww of immobilized glucoamylase. A peristaltic pump was then used to vary and regulate the flow rates of the substrate fed into the column. The temperature of the system was maintained at 55°C using a thermostatically controlled water circulator.
CASSAVA SYRUP BY IMMOBILISED GLUCOAMYLASE

Analyses

The initial amount of low molecular weight sugars in cassava starch was determined by the Luff-Schoorl method (Osborne and Voogt, 1978).

The amount of reducing sugar present at each stage of the liquefaction and saccharification processes was determined by the Nelson-Somogyi microanalytical method (Southgate, 1976). The extent of reducing sugar formation was expressed as dextrose equivalent (DE) where total reducing sugar (as glucose) was calculated as percentage of total dry substance (DS).

RESULTS AND DISCUSSIONS

Locally obtained cassava (*Manihot esculenta* L.) starch was found to contain about 10.6% moisture and 0.01% of low molecular weight sugars. Initial treatment of starch slurries of concentrations 20 - 40% D.S with α-amylase produced reduced sugar contents of between 2 - 4 DE. Subsequent treatment after cooking with the same enzyme for 1 hour at 85°C increased the reducing sugar to 2 - 7 DE (Fig. 1). The liquefied or solubilised substrate obtained was golden brown in colour, probably due to non-enzymic browning.

The effect of a fixed amount of soluble glucoamylase on liquefied cassava starch of various concentrations as a function of time is shown in Fig. 1. Addition of glucoamylase to liquefied starch solution increased the DE significantly. This was due to the fact that glucoamylase is a hydrolase that act upon 1, 4 - as well as 1, 6 -α-linkages in starch hydrolysates (solubilized starch). During saccharification, glucose units are removed in a stepwise manner from the non-reducing end of the substrate molecule. α-amylase, on the other hand, hydrolyses starch to produces fragments of more than or equal to two glucose units each.

The rate of conversion was greatest during the first 30 minutes of the reaction and then slowed down for all of the substrate concentrations tested. That the substrate concentration played a role in the effectiveness of the enzyme in the saccharification process is shown in Fig. 2. Increasing the substrate concentration decreases the DE obtained. The highest obtainable DE of 58 was achieved when the substrate concentration was 20% DS and at the conditions stated under Methods. When the extent of saccharification is expressed as g per litre substrate, the maximum concentration of reducing sugar obtained was when a 30% DS substrate was used. Increasing the substrate concentration decreased the amount of reducing sugar formed. This can be attributed to viscosity effects. Liquefied substrates containing higher concentrations of starch were found to be viscous and thus prevented effective mixing of the enzyme throughout the substrate. Based on the results obtained, the substrate concentration subsequently used for continuous saccharification with immobilised glucoamylase was 30% DS.

The particle size of the activated charcoal to which the glucoamylase was immobilized affects...
The extent of saccharification was found to be between 700 - 1000 μm even though immobilisation of glucoamylase to charcoal of smaller particle sizes gave a more active enzyme preparation. This is because when glucoamylase immobilised to charcoal of smaller particle size was packed in a bed column for continuous saccharification, the flow rate of substrate through the column became substantially reduced such that the process was no longer economical.

The relationship between flow rates and the degree of saccharification was found to be asymptotic as shown in Fig. 3. Increasing the flow rate reduced the contact or residence time of the substrate with immobilised enzyme in the packed-bed column. A 7 DE solubilised cassava solution was converted into a 69 DE cassava syrup at a flow rate of 1.0 ml per minute. Park and Lima (1973) obtained a 43 DE cassava syrup from a 10 DE substrate at 20% concentration and at a flow rate of 3.5 ml per min when using glucoamylase immobilized to Amberlite IR - 45 (OH) resin. Our result (Fig. 3) indicated that it is possible to obtain a syrup of much higher DE than 43 given the same flow rate and substrate concentration.

The colour of the cassava syrup obtained after continuous saccharification was influenced by the flow rate. The initial intensity of the colour of the liquefied starch was reduced significantly at low flow rates. It was also found that the colour was less intense for all of the flow rates tested. Packing activated charcoal in the enzyme column prior to packing the immobilised enzyme gave cassava syrup that was virtually colourless at the flow rate of 1 ml per min. Thus besides acting as an enzyme-carrier, activated charcoal also functioned as a decolourising agent.

Our report presents for the first time the use of glucoamylase immobilised as described in this paper, to convert solubilised or liquefied cassava starch into cassava syrup. Cassava syrup, in turn,
CASSAVA SYRUP BY IMMOBILISED GLUCOAMYLASE

can be used as a feedstock for the production of high fructose cassava syrup using the enzyme, glucose isomerase, and for alcohol fermentation.

Subhi Ba'i
Hasanah Mohd. Ghazali

Department of Food Science,
Faculty of Food Science and Technology,
Universiti Pertanian Malaysia,
43400 Serdang, Selangor.

ACKNOWLEDGEMENT

This work was supported by the Universiti Pertanian Malaysia through project no. 1718 – 1 – 424.

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(Received 23 May, 1985)