Detection of saccharification products by thin-layer chromatography

In alcoholic fermentations using different kinds of raw materials containing starch as substrate, the saccharification process is generally followed by the disappearance of the colour formed with iodine. More accurate results may be obtained by the determination of the reducing sugars. Such methods only give total results without specifying which sugar is being formed. This paper is concerned with the detection of the saccharification products by thin-layer chromatography, using different saccharifying preparations.

Experimental

Qualitative analysis. Chromatoplates were prepared by coating glass plates (20 cm × 20 cm) with Merck's Kieselgur G according to STAHL[1]. 5 μl spots containing approximately 50 μg of sugar were applied 1.5 cm from the bottom of the plate. A mark was made 10 cm from the point of application so that the migration distance would be the same on all plates. The chromatoplates were irrigated by ascending migration of 5 ml of a solvent mixture. A number of different solvents were tried and a mixture of *n*-butanol–toluene–ethylacetate–water (10:2:5:3) was found to give the best results[2]. The separation was completed within 20 min. The chromatoplates were dried at 60° and then sprayed with anisaldehyde[1] and developed at 100° for 10 min.

Quantitative analysis. Reducing sugars were determined by the method of NELSON[3]. The disappearance of starch was followed by the method of BOWINE *et al.*[4].

Results and discussion

As a basic experiment, the hydrolysis of starch by α- and β-amylase was studied. 160 mg of soluble starch were dissolved in either 10 ml of 0.05 *M* phosphate buffer, pH 7.0, for α-amylase, or 0.05 *M* acetate buffer, pH 4.7, for β-amylase. 1 mg of the enzyme was added in 6 ml of water and the mixture incubated at 37°. Samples were taken at 5 min intervals for the determination of reducing sugar and starch, as well as for chromatography. They were immersed in a boiling bath for 3 min to stop the reaction. All blanks were prepared with an inactivated enzyme preparation.

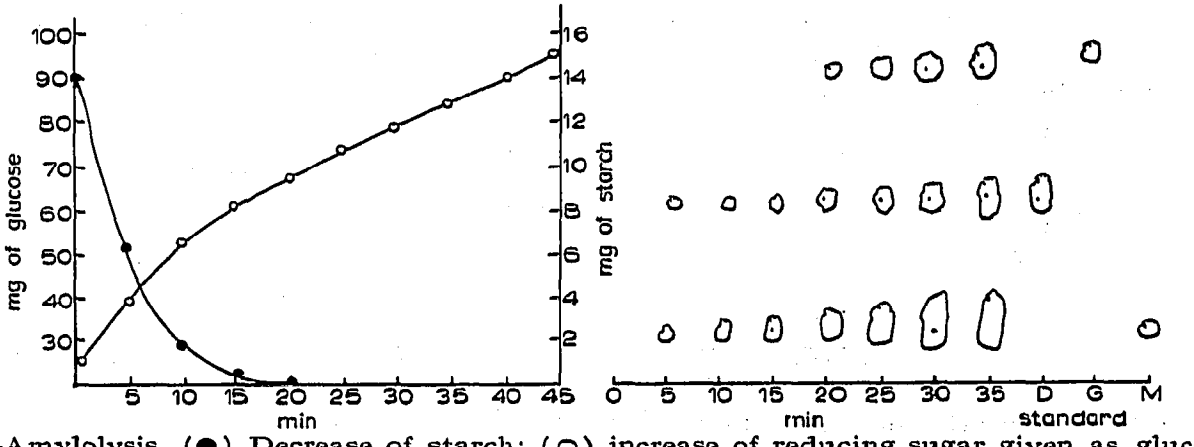

Fig. 1. α-Amylolysis. (●) Decrease of starch; (○) increase of reducing sugar given as glucose. Thin-layer chromatogram in minutes. Solvent system: *n*-butanol–toluene–ethyl acetate–water (10:2:5:3). The standards are: dextrins (D), glucose (G) and maltose (M).

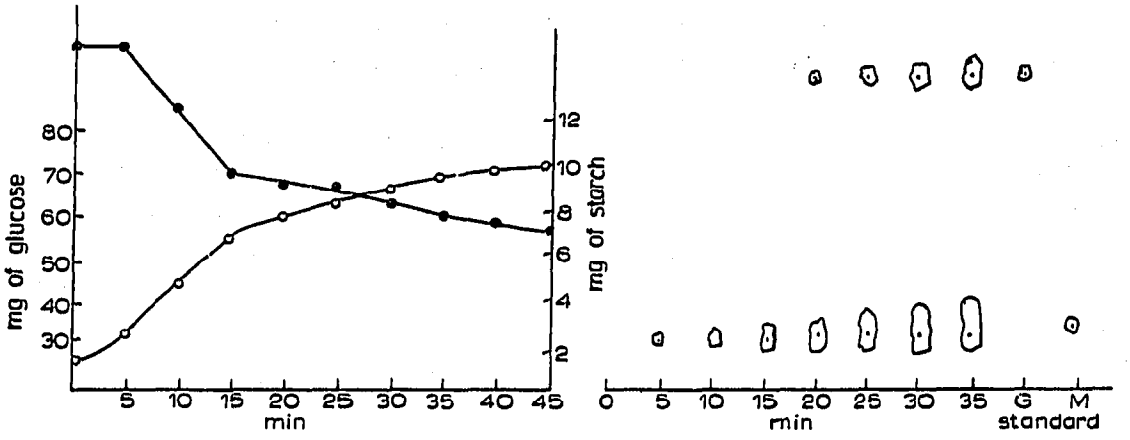

Fig. 2. β-Amylolysis. (●) Decrease of starch; (○) increase of reducing sugar given as glucose. Thin-layer chromatogram in minutes. Solvent system: *n*-butanol–toluene–ethyl acetate–water (10:2:5:3). The standards are: glucose (G) and maltose (M).

The results shown in Figs. 1 and 2 confirm the differences in action of the two amylases, showing the strong formation of dextrins with α-amylase, whereas β-amylase does not form them. On the other hand the hydrolysis of starch is much slower in the latter case. In both experiments we observe that the reducing power is due, at first, mainly to the presence of maltose, glucose being only formed in detectable amounts after 15 min of incubation.

For the saccharification of manioc by barley malt, 50 g of manioc meal were cooked with 1 % barley malt in 250 ml of water for 2 h at 1.7 atm. 10 % of malt were added to the solubilized mash and the pH adjusted to 5.0 with dilute H_2SO_4. The saccharification was followed at 50°, samples being taken for analysis.

As may be seen from Fig. 3, saccharification occurred in 10 min, dextrins, maltose and glucose all being responsible for the appearance of reducing power. Similar results were obtained using mold bran as saccharifying agent.

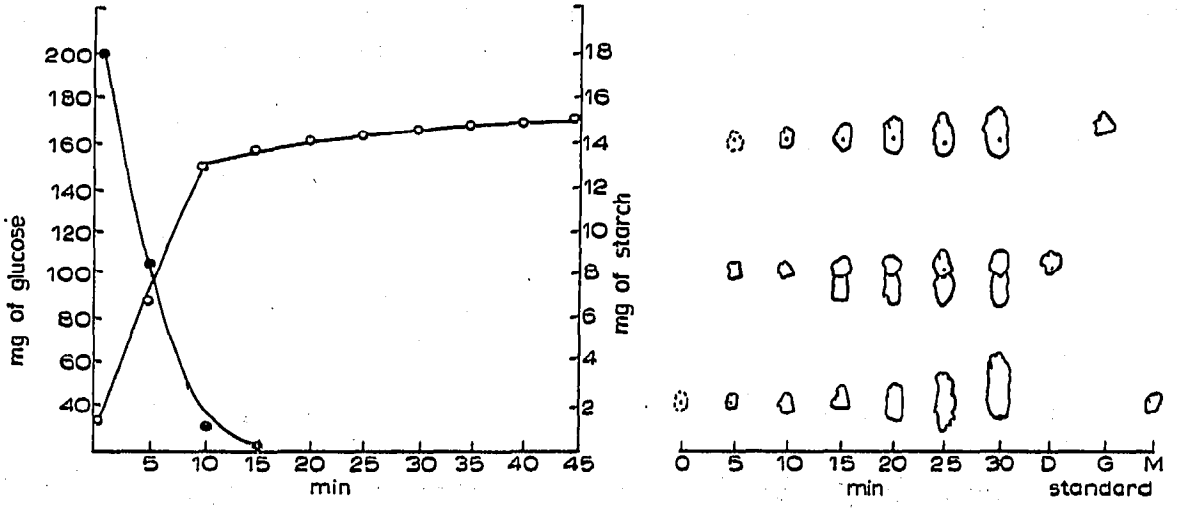

Fig. 3. Saccharification of manioc by barley malt. (●) Decrease of starch; (○) increase of reducing sugar given as glucose. Thin-layer chromatogram in minutes. Solvent system: *n*-butanol–toluene–ethyl acetate–water (10:2:5:3). The standards are: dextrins (D), glucose (G) and maltose (M).

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Sample application in preparative thin-layer chromatography

The successful resolution of mixtures on preparative thin-layer chromatoplates requires uniform application of the sample with respect to both concentration and shape of the starting zone. Uniformity of concentration has been attained by application of a solution of the mixture from a travelling syringe[1]. Recently a much simpler device was described[2] but this suffers from the drawback that a maximum volume of *ca.* 0.3 ml of solution can be added with a single application. Despite careful handling of the applicator, it is not possible to avoid slight irregularities in the shape of the starting zone on repeated application to the same position on a plate. These irregularities become exaggerated as the zones migrate so that separation of compounds with $\Delta R_F < 0.3$ is seldom efficient, especially with quantities greater than 20 mg of mixture per plate (20 × 20 cm). We have found that uniform migrating zones, and efficient separation of compounds with ΔR_F as low as 0.1, are obtained from the narrow, straight-edged bands prepared by the following procedure:

Two parallel cuts (A, Fig. 1), *ca.* 3 mm apart, are made through the adsorbent

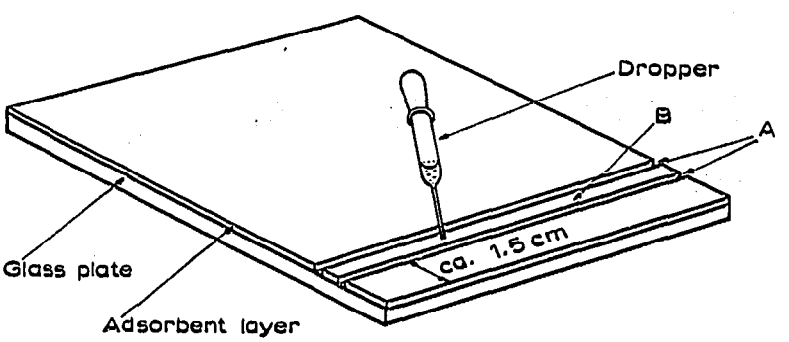

Fig. 1.