

Note

Purification of commercial maltose and improved detection of oligosaccharides on paper chromatograms

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Commercial maltose hydrate, even the best grade presently available from several distributors, contains appreciable amounts of D-glucose, maltotriose, and other starch-derived oligosaccharides and dextrans (Fig. 1B). Recrystallization from aqueous ethanol does not remove them. A recommended purification procedure

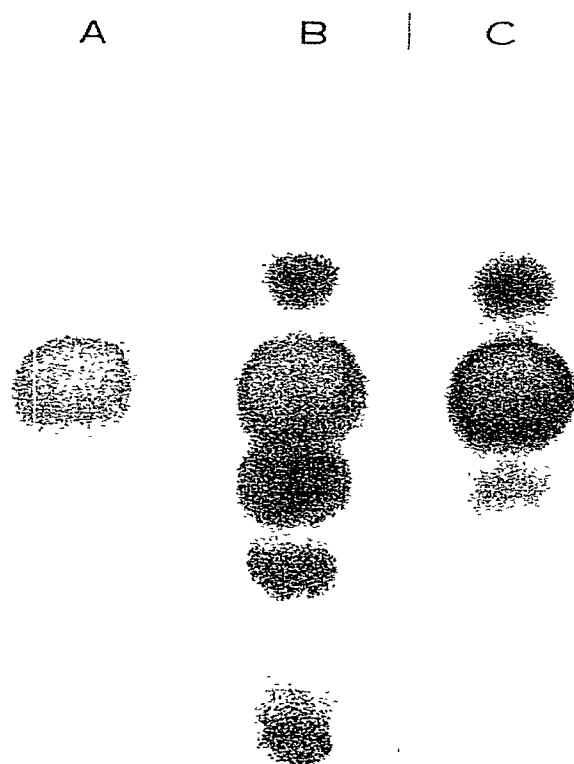


Fig. 1. Lane A, purified maltose, sprayed with amyloglucosidase after development; Lane B, commercial maltose sprayed with amyloglucosidase after development; Lane C, commercial maltose visualized without prespraying with enzyme.

requires acetylation, several recrystallizations of the octaacetate, deacetylation, and another recrystallization¹. Hodge *et al.*² isolated pure maltose from the limit-dextrins of amylopectin treated with β -amylase by a lengthy enzymic procedure. Pazur³ treated 100 g of soluble starch with β -amylase and isolated 30 g of maltose. In this procedure charcoal was used to adsorb maltose and other oligosaccharides from the reaction mixture. After the charcoal had been filtered off on a Büchner funnel, maltose was eluted with 10% ethanol in water. A similar, but shorter, procedure is now shown to be useful for the purification of commercial maltose.

Paper chromatography was used to check the purity of commercial maltose and purified maltose. A marked increase in sensitivity for the higher oligosaccharides and dextrins is achieved if the developed chromatogram is first sprayed with a 0.1% solution of amyloglucosidase. After the separated oligosaccharide spots have been hydrolyzed to D-glucose, their increased reducing power allows visualization by Trevelyan's procedure⁴, as shown in Fig. 1B as compared with visualization without the enzyme (Fig. 1C).

EXPERIMENTAL

Charcoal Darco-G60* (226 g) was washed several times with distilled water and then poured into a 12.5 cm Büchner funnel, and vacuum was applied to the filter flask. When the water level reached the surface of the charcoal, a 10% solution of maltose (500 ml) was poured onto the column. The D-glucose and maltose were eluted with 1,500 ml of water followed by 2,500 ml 5% ethanol in water. The aqueous effluent contained most of the maltose. Higher oligosaccharides can be eluted by raising the concentration of ethanol. The combined eluates were concentrated under vacuum in a rotary evaporator. The syrupy residue was dissolved in 100 ml of water, ethanol was added until the solution became cloudy, and the solution was refrigerated. The white precipitate of maltose hydrate (Fig. 1A) was filtered and dried; recovery 33.5 g (67%) $[\alpha]_D^{25} + 130.4^\circ$ (*c* 2.88, water).

Analytical chromatograms were developed on Schleicher and Schüll 2043-B paper (9 in. height \times 20 in. width) for 3 h at 75° by using 70% propyl alcohol in water as the solvent⁵. The paper was sprayed with a 0.1% solution of amyloglucosidase and incubated for 30 min at 25°. The dried chromatogram was dipped into a 1% solution of silver nitrate in acetone, dried, redipped in 0.5% KOH in ethanol, dried, and then washed with 4% aqueous sodium thiosulfate.

REFERENCES

- 1 M. L. WOLFROM AND A. THOMPSON, *Methods Carbohydr. Chem.*, 1 (1962) 334.
- 2 J. E. HODGE, E. M. MONTGOMERY, AND G. E. HILBERT, *Cereal Chem.*, 25 (1948) 19.
- 3 J. H. PAZUR, *Methods Carbohydr. Chem.*, 1 (1962) 335.
- 4 W. E. TREVELYAN, D. P. PROCTER, AND J. S. HARRISON, *Nature*, 166 (1950) 444.
- 5 D. FRENCH, J. L. MANCUSI, M. ABDULLAH, AND G. L. BRAMMER, *J. Chromatogr.*, 19 (1965) 445.

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