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Note

Specificity of yeast (*Saccharomyces cerevisiae*) in removing carbohydrates by fermentation

Seung-Heon Yoon, Rupendra Mukerjea, John F. Robyt*

Laboratory of Carbohydrate Chemistry and Enzymology, 4252 Molecular Biology Building, Iowa State University, Ames, IA 50011, USA

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Abstract

The specificity of *Saccharomyces cerevisiae* yeast on the removal of carbohydrates by fermentation was studied. The common monosaccharides, D-glucose, D-fructose, D-mannose, and D-galactose were completely removed; D-glucuronic acid and D-ribose were partially removed; but D-xylose, D-rhamnose, and L-sorbose were not removed and were completely resistant. Of four glycosides, methyl and phenyl α- and β-D-glucopyranosides, three of the four were partially removed and methyl β-D-glucopyranoside was not removed. The disaccharides, maltose, sucrose, and turanose were completely removed, while cellobiose, lactose, and melibiose were completely resistant. Isomaltose and α,α-trehalose were partially removed. Maltotriose and raffinose were partially removed, but isomaltotriose and melezitose were completely resistant. The tetrasaccharides, maltotetraose, isomaltotetraose, and acarbose, were completely resistant. Further, the yeast enzymes did not alter any of the resistant carbohydrates by transglycosylation or condensation reactions or by any other types of reactions.

Keywords: Yeast; *Saccharomyces cerevisiae*; Fermentation; Carbohydrates; Monosaccharides; α- and β-D-Glucopyranosides; Disaccharides; Trisaccharides; Tetrasaccharides

1. Introduction

We have used the common bread yeast, *Saccharomyces cerevisiae*, to remove monosaccharides and disaccharide by-products in carbohydrate preparations for several years. It recently was brought to our attention that this technique was not commonly known among other investigators working with carbohydrates, and it was also questioned as to whether the technique might be deleterious to carbohydrate preparations by the action of the yeast enzymes on the desired carbohydrate products. With this communication, we show that *S. cerevisiae* has a high specificity for the removal of some common monosaccharides and disaccharides. It also was found that *S. cerevisiae* partially removed some di- and trisaccharides, but did not remove certain, less common monosaccharides, some common disaccharides, especially those with D-galactose at the nonreducing-end and/or those with β-glycosidic linkages. Oligosaccharides with four or more monosaccharide units were not removed by fermentation.

2. Experimental

2.1. Preparation of immobilized-yeast

Sodium alginate (1.25 g medium viscosity, A-2033 Sigma Chemical Co., St. Louis, MO.) was dissolved in 50 mL of hot deionized water with vigorous stirring. The solution was kept at 4 °C for 15 h. The final volume of the sodium alginate solution was adjusted to 50 mL with sterile water. One gram of dried bread yeast (Fleishman's yeast, Fenton, MO) was swollen in 5 mL of autoclaved water for 15 h and then mixed well with 50 mL of 2.5% (w/v) sodium alginate solution. The mixture was added to a dropping funnel, equipped with a No. 23 syringe needle, and was slowly added through the needle to 300 mL of 4% (w/v) $CaCl_2$ solution, forming gel beads of 2 mm diameter. The beads were then allowed to stand for 3 h at 4 °C to harden. The

* Corresponding author. Tel.: +1-515-2941964; fax: +1-515-2940453
E-mail address: jrobyt@iastate.edu (J.F. Robyt).

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immobilized-yeast in the Ca-alginate beads was washed with 300 mL of 20 mM pyridinium acetate buffer (pH 5.2) three-times to remove soluble material and then kept at 4 °C until use. One gram of dry yeast gave 26.3 g of wet, immobilized-yeast Ca-alginate beads.

The conditions for the immobilization of the yeast: 2.5% (w/v) medium viscosity alginate, 2.0% (w/v) yeast, and 0.36 M (4%, w/v) $CaCl_2$, were used to obtain minimum compression and breakdown of the alginate and minimum release of yeast cells into the reaction solution and maximum yeast viability with maximum flow of carbohydrates into the gel–yeast matrix.[1,2]

2.2. Yeast fermentation of carbohydrate solutions

Ten milligrams of each carbohydrate was dissolved in 1.0 mL of autoclaved water. Ten beads of immobilized-yeast (58.5 ± 0.8 mg or ~ 2 mg of yeast/mg of carbohydrate) were added to 100 μL of the carbohydrate solutions and allowed to react at 37 °C for 24 h. After the yeast treatment, the immobilized-yeast was removed by centrifugation or filtration. The immobilized-yeast was washed three-times each with 100 μL of deionized water and the washings were added to the supernatants of the digests. The supernatants were then taken to dryness in a SpeedVac and redissolved in 100 μL of deionized water for thin layer chromatography (TLC) analysis.

2.3. TLC analysis

Monosaccharides, glycosides, and disaccharides and their yeast-treated solutions (0.5 μL) were added to a 20 × 20 cm Whatman K5 TLC plate. The TLC plates for the monosaccharides and glycosides were irrigated two-

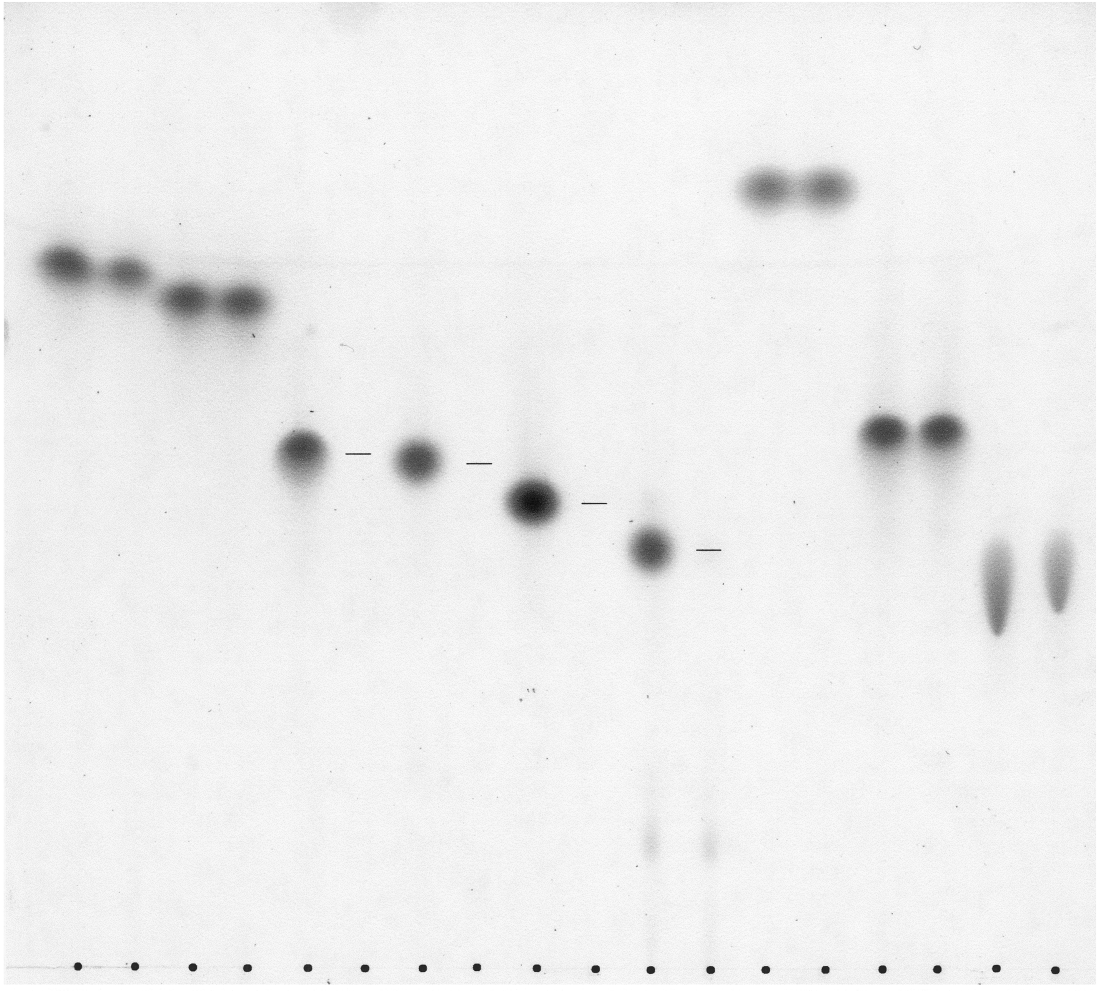

Fig. 1. TLC of monosaccharides treated 24 h with *S. cerevisiae* yeast in Ca-alginate at 37 °C. Rib, D-ribose; Xyl, D-xylose; Fru, D-fructose; Man, D-mannose; Glc, D-glucose; Gal, D-galactose; Rhm, D-rhamnose; Sor, L-sorbose; GlcA, D-glucuronic acid. + IY = treatment with Ca-alginate-immobilized-yeast. Whatman K5 TLC plate, 20 × 20 cm, was irrigated two-times with 85:15 volume proportions of MeCN–water, using an 18-cm path length.

times at 21 °C with 17:3 volume proportions of MeCN–water, using an 18-cm path length. The TLC plate for the disaccharides was irrigated three-times with the same solvent and conditions. The tri- and tetra-saccharides were added to a 20 × 20 cm Whatman K6 plate that was irrigated three-times with 17:3 volume proportions of MeCN–water and two-times with 85:20:50:30 volume proportions of MeCN–EtOAc–1-propanol–water at 21 °C, using an 18-cm path length.

The carbohydrates on the plates were visualized by dipping the plates into a MeOH solution of 0.3% (w/v) *N*-(1-naphthyl)ethylenediamine and 5% (v/v) H_2SO_4, followed by heating at 120 °C for 10 min.[3] The quantitative amounts of the carbohydrates were determined by TLC densitometry,[3,4] using a Bio-Rad scanning densitometer.

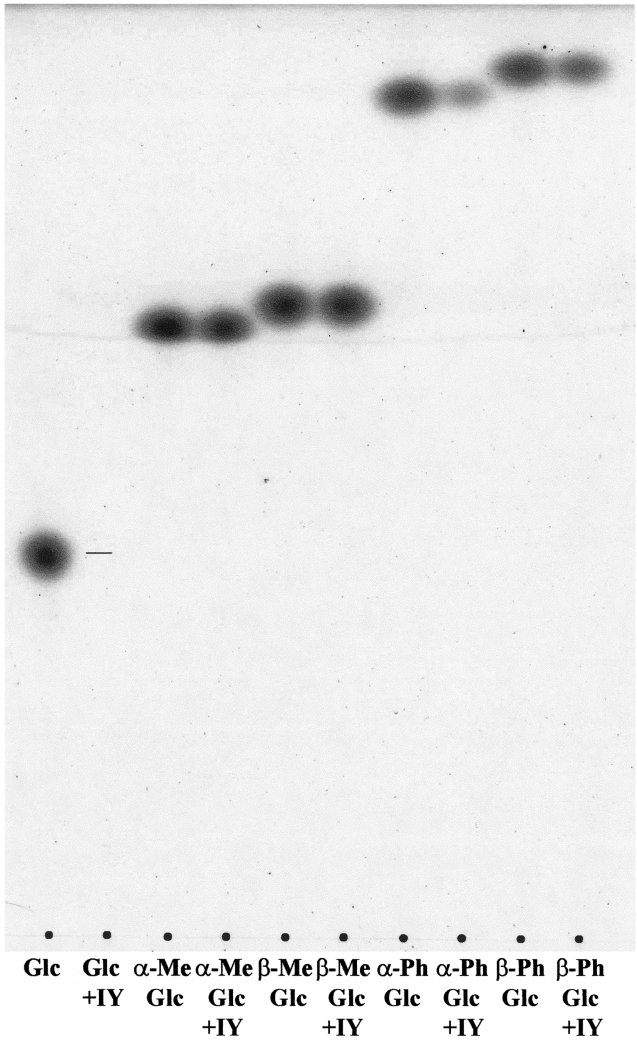

Fig. 2. TLC of methyl and phenyl α- and β-D-glucopyranosides treated 24 h with *S. cerevisiae* yeast in Ca-alginate at 37 °C. + IY = treatment with Ca-alginate-immobilized-yeast. Whatman K5 TLC plate, 20 × 20 cm, was irrigated two-times with 85:15 volume proportions of MeCN–water, using an 18-cm path length.

3. Results

Of the two pentoses studied, D-xylose was not fermented at all and 33% of the D-ribose was fermented (Fig. 1). All of the D-glucose, D-fructose, D-mannose, and D-galactose were removed by the yeast fermentation (Fig. 1); D-glucuronic acid was partially utilized to the extent of 28%; D-rhamnose and L-sorbose were completely resistant to yeast fermentation and were not removed.

Four glycosides were studied. Methyl α- and β-D-glucopyranoside and phenyl α- and β-D-glucopyranoside were treated with Ca-alginate–yeast (Fig. 2): 17.2% of the methyl α-D-glucopyranoside was removed; none of the methyl β-D-glucopyranoside was removed; 69% of the phenyl α-D-glucopyranoside was removed; and 27% of the phenyl β-D-glucopyranoside was removed.

Of the disaccharides, maltose, sucrose, and turanose were completely removed, and 43% of the isomaltose and 86% of the α,α-trehalose were removed (Fig. 3). Cellobiose, lactose, and melibiose were completely resistant to yeast fermentation and were not removed after 24 h (Fig. 3).

Of the trisaccharides, maltotriose was partially removed to the extent of 53% (Fig. 4). Raffinose was hydrolyzed to melibiose and D-fructose, which was removed by fermentation (Fig. 4). The melibiose was not fermented, as shown in Fig. 4. Isomaltotriose and melezitose were not removed and 100% remained after 24 h (Fig. 4). None of the tetrasaccharides, maltotetraose, isomaltotetraose, and acarbose, was removed and 100% remained after 24 h (Fig. 4).

For each of the carbohydrates, there was no production of any new kinds of carbohydrates or modifications of the carbohydrates by the action of the yeast enzymes, with the exception of raffinose, which was hydrolyzed to melibiose and D-fructose, the latter of which was removed by fermentation (Figs. 1–4).

4. Discussion

The primary reactions that take place in the removal of monosaccharides by yeast are anaerobic glycolysis in which the carbohydrate is converted into ethanol and CO_2. One of the end products of anaerobic glycolysis of carbohydrates is pyruvic acid, which is decarboxylated to CO_2 and acetaldehyde. The acetaldehyde is reduced to ethanol by reaction with NADH (reduced nicotinamide adenine dinucleotide), regenerating NAD^+, necessary for continuing the anaerobic glycolysis reactions.[5]

The common monosaccharides: D-glucose, D-fructose, D-mannose, and D-galactose were readily fermented and removed by *S. cerevisiae*. D-Rhamnose

(6-deoxy-D-mannose) was not utilized and it would, therefore, be expected that other 6-deoxyhexoses, such as D-quinivose (6-deoxy-D-glucose) and D-fucose (6-deoxy-D-galactose) also would not be expected to be fermented by yeast. The L-sugar, L-sorbose, also was not fermented and it is expected that L-sugars, in general, would not be utilized by *S. cerevisiae*.

Three of the glycosides were partially removed. The phenyl α-D-glucopyranoside was the most extensively removed to the extent of 69%; 27% of phenyl β-D-glucopyranoside was removed; 17% of methyl α-D-glucopyranoside was removed; but none of the methyl β-D-glucopyranoside was removed. This last result was somewhat of a surprise as the β-phenyl group was slowly hydrolysed, so that 27% was removed. The β-methyl group, however, was not hydrolyzed at all and was, therefore, completely resistant to removal by fermentation.

Of the disaccharides, maltose, sucrose, and turanose were completely removed by *S. cerevisiae* fermentation. Maltose was hydrolyzed to two D-glucose units by yeast α-glucosidase, turanose is also hydrolyzed by α-glucosidase to give the component sugars, D-glucose and D-fructose, that are then removed by fermentation. Sucrose is hydrolyzed by yeast invertase to D-glucose and D-fructose that also is then fermented. Isomaltose was slowly hydrolyzed by yeast α-glucosidase to two D-glucose units that are fermented to give incomplete removal in 24 h. α,α-Trehalose was also partially hydrolyzed by yeast trehalases to two D-glucose units and fermented. Very possibly, if the treatment of isomaltose and α,α-trehalose were extended for a longer period of

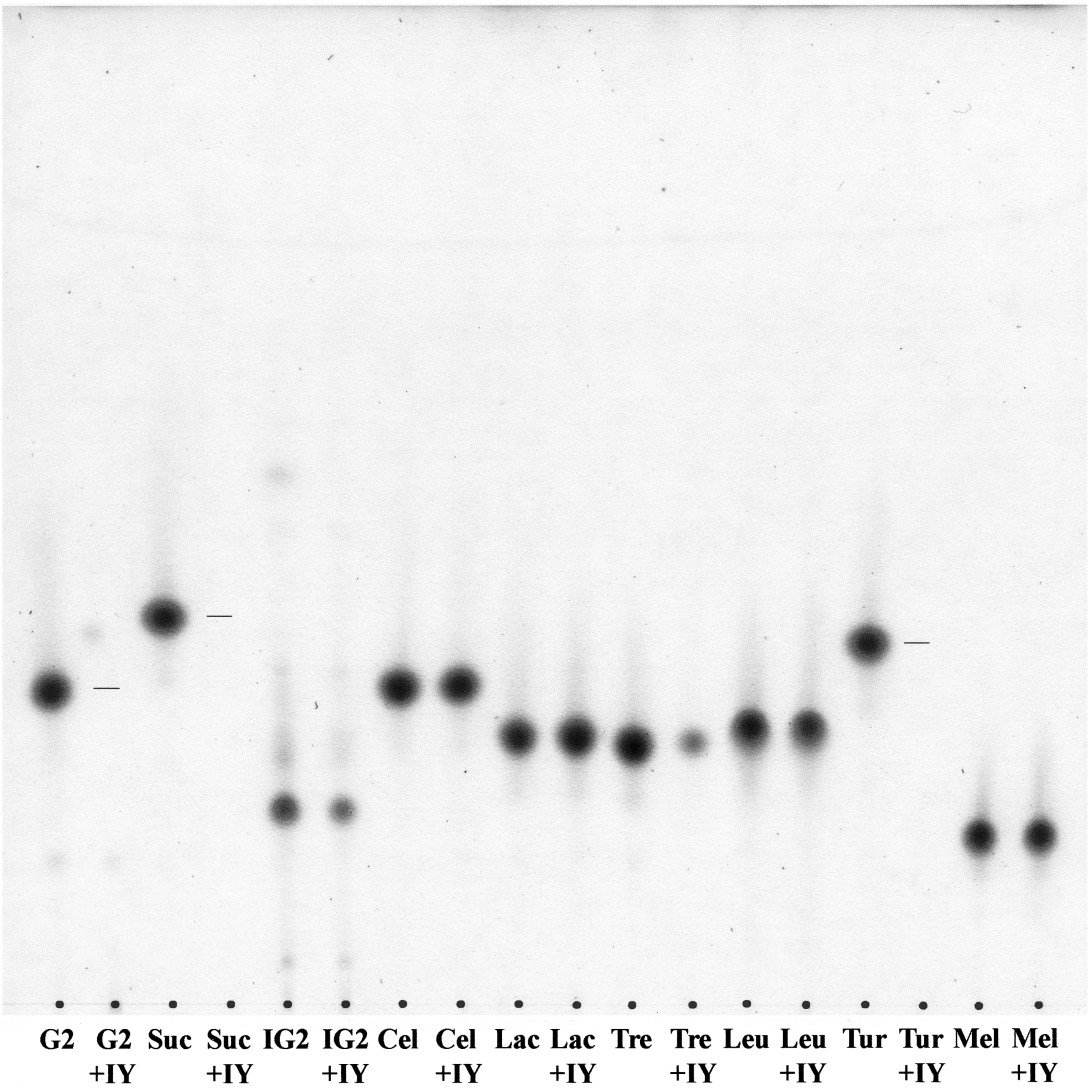

Fig. 3. TLC of disaccharides treated 24 h with *S. cerevisiae* yeast in Ca-alginate at 37 °C. G2, maltose; Suc, sucrose; IG2, isomaltose; Cel, cellobiose; Lac, lactose; Tre, α,α-trehalose; Leu, leucrose; Tur, turanose; and Mel, melibiose. + IY = treatment with alginate-immobilized-yeast. Whatman K5 TLC plate, 20 × 20 cm, was irrigated three-times with 85:15 volume proportions of MeCN–water, using an 18-cm path length.

time, they too might be completely removed by fermentation. Leucrose, a disaccharide with an unusual linkage, α-D-glucopyranosyl linked (1→5) to D-fructose, is not hydrolyzed by α-glucosidase and therefore its component sugars are not removed by *S. cerevisiae* fermentation.

The β-linked disaccharides, cellobiose and lactose, were not hydrolyzed to their monosaccharides, and they were not removed by fermentation. Melibiose [α-D-galactopyranosyl-(1→6)-D-glucose] also was not hydrolyzed by yeast α-glucosidase because the hydrolysis requires an α-galactosidase, which *S. cerevisiae* lacks, and melibiose was not removed by *S. cerevisiae* fermentation.

Of the trisaccharides, maltotriose and raffinose, were partially utilized. Maltotriose was slowly hydrolyzed by yeast α-glucosidase to D-glucose, so that about 43% was removed. The D-fructose moiety of raffinose was hydrolyzed by yeast invertase more slowly than the hydrolysis of sucrose to give melibiose and D-fructose, the latter of which was fermented. Isomaltotriose and melezitose were not hydrolyzed and removed. The addition of a second α-(1→6)-linked D-glucose unit in isomaltotriose completely stops the hydrolysis by yeast α-glucosidase and isomaltotriose is not removed. Melezitose is a nonreducing trisaccharide in which D-glucose is linked α-(1→3) to the D-fructose moiety of sucrose. Invertase is not able to hydrolyze the D-fructose linkage to give D-glucose when a D-glucose unit is attached α-(1→3) to the D-fructose moiety of melezitose, and further the α-glucosidase is not able to hydrolyze the D-glucose attached to the D-fructose unit of sucrose in melezitose, making it completely resistant to fermentation. Turanose, the reducing disaccharide with D-glucopyranose linked α-(1→3) to D-fructose, is hydrolyzed by α-glucosidase to the component sugars, which are fermented, removing turanose.

None of the tetrasaccharides (maltotetraose, isomaltotetraose, and acarbose) are capable of being hydrolyzed by yeast α-glucosidase, and therefore were not removed. It has previously been recognized that as the size of an α-linked dextrin increases, the capability of yeast α-glucosidase decreases. In the case of isomaltodextrins and maltodextrins, yeast α-glucosidase will not hydrolyze the glycosidic linkages when the size of the dextrin reaches three or four or more D-glucose units, respectively. Acarbose is a pseudotetrasaccharide, having three carbohydrate units α-linked (D-quinivose

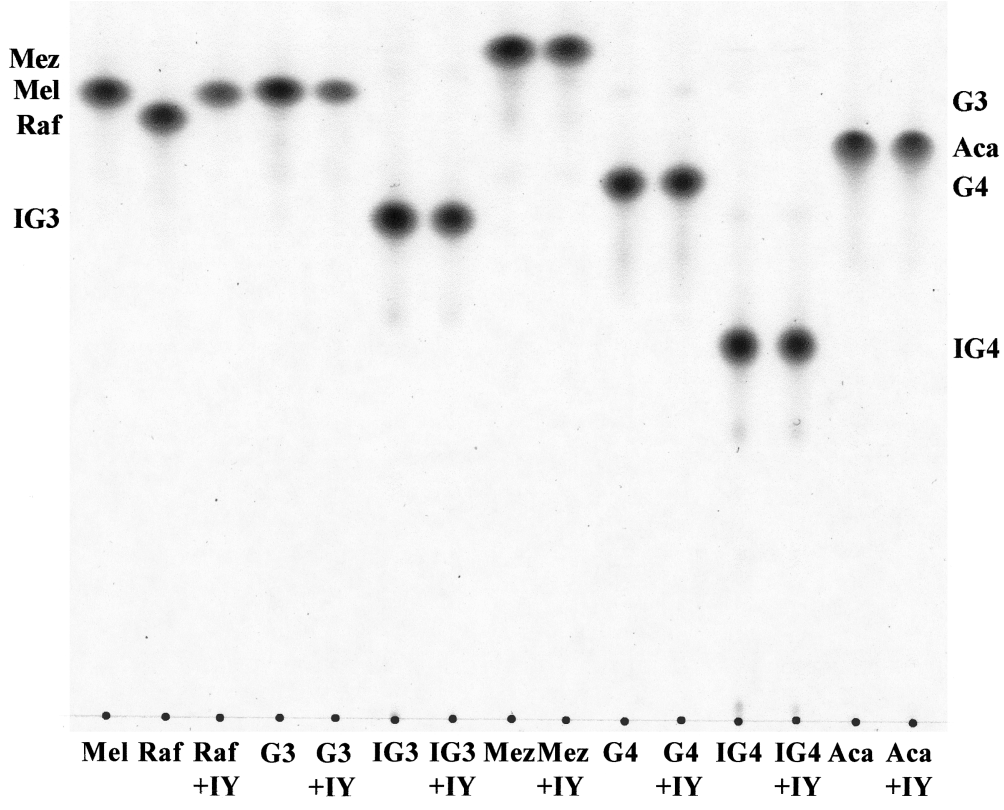

Fig. 4. TLC of tri- and tetra-saccharides treated 24 h with *S. cerevisiae* yeast in Ca-alginate at 37 °C. Raf, raffinose; G3, maltotriose; IG3, isomaltotriose; Mez, melezitose; G4, maltotetraose; IG4, isomaltotetraose; Aca, acarbose. + IY = treatment with alginate-immobilized-yeast. Whatman K6 TLC plate, 20 × 20 cm, was irrigated three-times with 85:15 volume proportions of MeCN–water and two-times with 85:20:50:30 volume proportions of MeCN–EtOAc–1-propanol–water, using an 18-cm path length.

and two D-glucose units) and a cyclohexenitol unit[3] attached to the C-4-amino group on D-quinivose.[6] Because acarbose appears to be a tetrasaccharide, the α-linkages are not hydrolyzed by yeast α-glucosidase. Further, because of the presence of the cyclohexenitol ring in a D-*gluco*-configuration and the presence of the *N*-glycosylic linkage between cyclohexenitol and 4-amino-4-deoxy-D-quinivose, acarbose acts as a transition-state analogue inhibitor for α-glucosidase and hence is not hydrolyzed by α-glucosidase.[6–9]

In this study, we have used alginate-immobilized yeast to remove carbohydrates from solutions. We have found that this decreases the leakage of colored materials and peptides from the yeast. The yeast, however, can be added directly when these materials are not a problem in the preparation.

In summary, we have shown that the yeast can be used to remove the common monosaccharides and the common α-linked disaccharides and some trisaccharides that are found as by-products in enzymatic carbohydrate syntheses. The β-linked disaccharides were not utilized by yeast and neither was methyl β-D-glucopyranoside because of the lack of hydrolysis by yeast β-glucosidase; phenyl β-D-glucopyranoside was partially hydrolyzed. The α-linked disaccharide melibiose is not hydrolyzed by α-glucosidase because it requires a α-galactosidase that the *S. cerevisiae* lacks. As the α-linked saccharides become larger than a disaccharide, the rate of hydrolysis by yeast α-glucosidase decreases and when the saccharide is a tetrasaccharide or larger, yeast α-glucosidase does not hydrolyze any of the α-linkages and the saccharides are not removed by yeast fermentation. Further, other yeast enzymes do not modify the carbohydrates by transglycosylation or condensation reactions or any other types of enzymatic reactions, with the exception of yeast invertase, which hydrolyzes raffinose to melibiose and D-fructose. Thus, *S. cerevisiae* can be conveniently used to remove the common mono- and di-saccharides that often constitute by-products found in enzymatic preparations of carbohydrates, such as the removal of D-fructose and unreacted maltose in the preparation of panose by the reaction of dextransucrase with sucrose and maltose; the removal of D-fructose and unreacted D-glucose in preparation of isomaltose by the reaction of dextransucrase with sucrose and D-glucose; and the removal of D-glucose, D-fructose, and various disaccharides in the modification of acarbose at the reducing-end and the nonreducing-end.[8–11]

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