California State University of Northridge

"Sugar Inhibition of Yeast Binding to Lectin Beads"

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For the degree of Master of Science in Biology

By

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Table of Contents

ABSTRACT

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Lectins, which are carbohydrate binding proteins, are used in the purification of glycan-containing molecules and in histochemical studies that have identified important cellular properties. Differences in cells with various carbohydrates on their surfaces influence malignancy and pathogenicity. Pathogenic organisms often bind to human cell surfaces via lectin-glycan interaction. Pharmaceutical companies are developing medications based upon their ability to inhibit carbohydrate mediated binding reactions.

In this study, I used microbeads derivatized with concanavalin A (Con A), a mannose-binding lectin, as a model in order to examine the binding properties of mannose-rich yeast, a model for pathogenic organisms. Ten sugars- including monosaccharides and oligosaccharides, at four concentrations each, were examined for their ability to inhibit cell-lectin binding in this model system. If a reagent causes disaggregation of yeast from lectin derivatized beads, that reagent might for example, block pathogen binding to cells. T- tests were performed to determine if any disaggregation of yeast from the Con A beads in the experimental samples was significantly different from the controls.

About 500 total trials were performed at each of the four concentrations of the ten sugars studied and controls, by counting the number of yeast that remain bound to the beads over a 60 minute time course. It is generally accepted that reagents that are most active are those that are effective at the lowest concentrations, suggesting that they bind most strongly to surface receptors. Here we found that Methyl-α-D-mannopyranoside, D(+)mannose and D(+)melezitose were most effective in disaggregating yeast from Concanavalin A at the lowest concentration tested (0.005M) at 60 minutes. A similar but slightly different ranking was observed at the 20 minute time. Most important is that the method used in this study is highly quantitative and can easily identify reagents that may be useful in anti-infection, anti-biofilm and anti-cancer venues.

Introduction

An assay has been developed to analyze the binding of mannose rich yeast to microbeads derivatized with concanavalin A (Con A), a mannose-blinding lectin.

Lectins are cell agglutinating proteins of non-immune origin that are broadly distributed in nature. They are found in microorganisms, plants and animals (15, 26). They bind mono- or oligosaccerides with significant specificity, similar to the way enzymes bind substrates and antibodies bind antigens (16, 26). This specific binding of lectins to saccharides on the surface of cells has provided a new tool for the investigation of the architecture of cell surfaces (26). This binding may involve several forces, mostly hydrophobic and hydrogen bonds, and may be inhibited by specific sugars (26). Lectins are oligomeric proteins composed of subunits with one sugar binding site per subunit (29). They are multivalent with at least two carbohydrate binding sites to allow crosslinking between cells or between sugar containing macromolecules (29). Experimental results in this study indicate that the binding of yeast to Concanavalin A can be blocked by addition of specific sugars at distinct concentrations.

Lectins differ in the composition of their amino acids, sugar content, molecular weight, number of carbohydrate binding sites per molecule and subunit structure (2, 7, 29). Much of those belonging to the same carbohydrate-binding class vary significantly in the specificity of their sugar-binding. Therefore, it is not surprising that the amino acid sequences involved in the carbohydrate-binding site of, for example, Con A, are poorly conserved in other lectins.

Concanavalin A is a lectin originally obtained from the jack bean *Canavalia ensiformis.* It is a member of the legume lectin family and binds specifically to specific structures found in various sugars, glycoproteins, and glycolipids, mainly internal and nonreducing terminal α-D-mannosyl and α-D-glucosyl groups (12, 26). Concanavalin A is a homotetramer globular protein composed of identical 26.5 kDA subunits and 235 amino acids. It has four binding sites, corresponding to the four subunits (30). Concanavalin A (Con A) binds to unsubstituted non-reducing α-D-glucose or α-D-mannose residues. The distinguishing feature of the substrate is that it contains hydroxyl groups at C-3, C-4 and C-6 (9, 19). The best fit for the Con A carbohydrate-binding site are expected to be oligosaccharides which contain three to four α-(1-2) mannosyl residues (20). The saccharide binding specificity of Con A has been shown to be determined by monosaccharaides glucose and mannose, which have similar hydroxyl group configurations at the C-3, C-4 and C-6 positions (6). Concanavalin A was the first lectin to be available commercially, and is widely used in biology and biochemistry to characterize glycoproteins and other sugar-containing entities on the surface of various cells (24).

Yeast are unicellular organisms with a clearly defined nucleus. *S. cerevisiae* is one of the most intensively studied eukaryotic model organisms in molecular and cell biology. *S. cerevisiae* cells appear "lemon-shaped" when viewed through a compound light microscope. This type of yeast has asci born on filaments that eventually burst to release spores during sexual reproduction. Under adverse conditions, the yeast cell will divide in order to make protective cells within a protective coat until conditions become more favorable.

The main studies on the biosynthesis of the polysaccharides of the yeast cell envelope were performed with *S. cerevisiae* as a model system (1, 5). Sugar units on biological membranes play an active role in a variety of cellular phenomena that include cellular adhesion, cellular recognition, and density-dependent inhibition of growth (12). Specific carbohydrate residues on many plasma membrane proteins may function in uptake of molecules by specific cells and tissues involving a recognition mechanism by membrane-bound glycoprotein receptors. Because of their strategic position, cell-surface carbohydrates have been implicated in cell-cell communication and in the interaction of cells within the environment (12, 32). Differential cell surface carbohydrate expression could be achieved by incomplete carbohydrate determinant formation, blockage of the binding site, and lowered residence time of cell surface carbohydrates on the cell surface (21).

The cell wall is also composed of many mannose residues (3). Mannans, polysaccharides rich in mannose residues, do not appear to contribute to the shape of the organism (1). They are covalently bound to protein and it has been proposed that their function is to hold together different components of the yeast cell wall (1).

In recent years carbohydrates have received more attention due to their involvement in human health and disease. Carbohydrates on cell surfaces serve as points of attachments for other cells such as toxins, viruses, bacteria, hormones and many other molecules. Therefore, carbohydrates are able to intervene with the migration of cells during the process of infection (12). Progresses in glycobiology have led to experimental approaches in treating viral and bacterial infections.

This study focused on the concentration dependent sugar inhibition of lectin binding to the yeast cell surface in a microdrop distilled water assay. Ten sugars, D(+)melezitose, D(+)trehalose, Maltotriose, D(+)glucose, Methyl-α-D-mannopyranoside, D(+)mannose, Methyl-α-D-glucopyranoside, L-rhamnose, D(+)xylose, and D(+)galactose, at four concentrations each, 0.05M, 0.025M, 0.01M and 0.005M were used. Considering the fact that Con A is a mannose binding lectin, it would not be surprising to find that the most effective sugars from the ten used in the experiment would be similar in structure to mannose. The molecular formula of mannose is $C_6H_{12}O_6$. D(+)mannose was one of the sugars being tested, so it was expected to be very effective in the amount of yeast bound to the bead after the 60 minute treatment . Two of the sugars used in the experiment, Methyl-α-D-mannopyranoside and Methyl-α-D-glucopyranoside are also seven carbon sugars which both have the formula $C_7H_{14}O_6$. D(+)xylose was the only five carbon sugar tested, with a molecular formula of $C_5H_{10}O_5$. D(+)glucose and D(+) galactose both have the molecular formula of $C_6H_{12}O_6$ and the third six carbon sugar, L-rhamnose, has a formula of $C_6H_{12}O_5$. The sugars whose formula is most different than that of mannose are D(+)trehalose, $C_{12}H_{22}O_{11}$, Maltotriose, $C_{18}H_{32}O_{16}$, and D(+)melezitose, $C_{18}H_{32}O_{16}$.

Table 1- Molecular formulas and molecular structure of the ten sugars used.

Sugar	Molecular Formula	Molecular Structure

D(+) mannose	$C_6H_{12}O_6$	
Methyl-α-D-mannopyranoside	$C_7H_{14}O_6$	
Methyl-α-D-glucopyranoside	$C_7H_{14}O_6$	
D(+) xylose	$C_5H_{10}O_5$	
D(+)glucose	$C_6H_{12}O_6$	
D(+) galactose	$C_6H_{12}O_6$	
L-rhamnose	$C_6H_{12}O_5$	
D(+)trehalose	$C_{12}H_{22}O_{11}$	
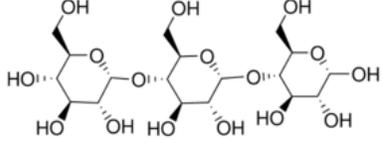
Maltotriose	$C_{18}H_{32}O_{16}$	
D(+)melezitose	$C_{18}H_{32}O_{16}$	
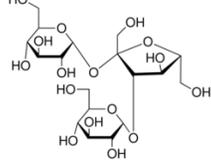
Materials and Methods

Yeast: Dry Baker's Yeast

Solutions: Distilled water was obtained from Arrowhead Co. (Los Angeles, CA)

Concanavalin A (immobilized): Purchased from Sigma Chemical Co.

Chemicals: D(+)melezitose, D(+)trehalose, Maltotriose, D(+)glucose, Methyl-α-D-mannopyranoside, D(+)mannose, Methyl-α-D-glucopyranoside, L-rhamnose, D(+)xylose, and D(+)galactose were all purchased from Sigma Chemical Co.

Formaldehyde 37%: Purchased from Sigma Chemical Co.

Toothpicks: Diamond brand flat wooden toothpicks

Microscope slides: Fisher brand plain microscope slides from Fisher Scientific (Pittsburg, PA) were used.

Auto pipette: Rainin R200 micropipette

Pipette tips: Fisher brand Redi-tip 1-200 μL specialty tips

Transfer pipettes: Fisher brand 1 ml disposable polyethylene plastic pipettes from Fischer Scientific   (Pittsburg, PA) were used

Microscopes: Olympus CK2 and Fischer Scientific inverted microscopes

Photography: Cannon Power shot SD750 digital camera was used

pH meter: Metler Toledo SevenMulti pH meter

Centrifuge: USA Scientific 115v~60Hz, 0.2A centrifuge

Microcentrifuge Tubes: Fisher brand 1.5 and 2 mL microcentrifuge tubes with flat top cap

Conical Tube- BD Falcon 15mL high-clarity polypropylene conical tubes

Gloves: Fisher brand latex gloves

Methodology:

Con-A preparation:

Using latex gloves, about 1/3 of the 1.5mL microcentrifuge tube was filled with immobilized Con A beads using a 1mL transfer pipette. Distilled water was added to the 1.5 mL mark on the microcentrifuge tube and the cap was closed. After balancing the centrifuge, the tube of Con A was centrifuged for 3 minutes. After 3 minutes, the supernatant was discarded using a 1mL transfer pipette, distilled water was added to the 1mL mark and centrifuged for 3 minutes. This washing of Con A beads is repeated for a total of 3 washes. On the third wash, the supernatant is removed and 2 drops of distilled water is added on top of the washed beads.

Yeast Preparation:

0.25 grams of yeast was added into a 2mL microcentrifuge tube. Next, 1mL of distilled water was added on top of the yeast. In order to fix the yeast with 1%

Formaldehyde, 0.027mL of the stock 37% formaldehyde was added to the yeast and left

for 30 minutes. After the yeast were fixed, the centrifuge was balanced and the tube of

the yeast was centrifuged for 3 minutes, the supernatant was removed using a 1mL

transfer pipette, and 1mL of distilled water was added onto the solution. This washing of

the fixed yeast was repeated for a total of 3 washes.

Sugar solutions:

   **Sugar solutions were made in 15mL conical tube using distilled

water- the amount of each sugar needed was added into the conical tube and 15mL of

distilled water was added on top and mixed to make stock solutions**

Table 2. Final concentrations of sugar solutions in each 0.2mL experiment will be:
0.05M, 0.025M, 0.01M, and 0.005M.

Sugar	MW	0.05M	0.025M	0.01M	0.005M
D(+)Melezitose	504.44 g/mol	0.757g	0.378g	0.151g	0.0757g
D(+)Trehalose	378.33 g/mol	0.567g	0.284g	0.113g	0.0567g
Maltotriose	504.44 g/mol	0.757g	0.378g	0.151g	0.0757g
D(+)Glucose	180.16 g/mol	0.270g	0.135g	0.054g	0.027g
Methyl-α-D-mannopyranoside	194.18 g/mol	0.291g	0.146g	0.0583g	0.0291g
D(+)Mannose	180.16 g/mol	0.270g	0.135g	0.054g	0.027g

Methyl-α-D-glucopyranoside	194.18 g/mol	0.291g	0.146g	0.0583g	0.0291g
L-Rhamnose	182.17 g/mol	0.273g	0.137g	0.0547g	0.0273g
D(+)Xylose	150.13 g/mol	0.225g	0.113g	0.0450g	0.0223g
D(+)Galactose	180.16 g/mol	0.270g	0.135g	0.054g	0.027g

Procedure:

On a clean slide, 3 separate drops of 0.1mL of distilled water were made in a row- the drop on the far left served for the experimental procedure, the middle drop was for the attachment of the yeast to the beads and the drop to the far right served for the control (no sugar). In the middle drop, Con A beads were added by dipping the flat end of the tooth pick into the prepared Con A solution and then mixing it into the drop of distilled water. Using the sharp end of the toothpick, the yeast cells were added to the Con A beads by dipping the toothpick into the yeast solution and then mixing it into the drop of the distilled water with the beads.

Using a micropipette tip, one bead with yeast attached to it was dragged into the drop of water for the control. Using another micropipette tip, another bead with yeast attached to it was added into the drop of water for the experimental procedure. Another 0.1mL drop of distilled water was added into the control and 0.1mL of the specific sugar at the concentration being tested was added into the experimental drop of water and timing of the experiment started. The number of beads attached to both the control and the experimental Con A

beads at 0, 20, 40 and 60 minutes were counted for a total of ten trials of each

sugar at its specific concentration.

Results

In this study, ten sugars at four specific concentrations were used to measure the effects on possible disaggregation of *Saccharomyces cerevisiae* from Con A, a mannose binding lectin. The sugar concentrations examined were 0.05M, 0.025M, 0.01M and 0.005M. The amount of yeast bound to the Con A bead was counted at 0 time and at 20, 40 and 60 minute intervals on both the treatment and control for a total of 10 trials for each sugar concentration.

The mean percentage of initial yeast remaining bound to the Con A beads after each experiment was calculated for each sugar concentration and also for all of the controls. Additionally, a 2-sample t-test was done to compare the average percentage of the initial yeast bound to the Con A beads of each sugar concentration and also from the control. P-values less than 0.05 ($p<0.05$) were considered statistically significant, indicating that there was a considerable difference between the disaggregation of the yeast treated with the sugars compared to the control, which didn't have the sugar treatment. Table 3 and Table 4 include the mean percentages of initial yeast remaining bound to Con A beads and also the p-values for each distinct sugar concentration. All values were rounded to the second decimal place.

Table 3. Mean percentage of initial yeast remaining bound to Con A beads after 60 minutes of specific sugar treatment. Results were determined from 10 trials for each sugar concentration. The column "C" represents the mean percentage of initial yeast remaining bound to Con A beads after 60 minutes of the corresponding control treatment.

Sugar	0.05M	0.05M "C"	0.025M	0.025M "C"	0.01M	0.01M "C"	0.005M	0.005M "C"
D(+)Melezitose	46.60%	87.24%	40.80%	90.99%	41.74%	94.51%	42.74%	97.58%
D(+)Trehalose	54.40%	95.20%	57.45%	88.78%	88.08%	98.30%	94.03%	96.20%
Maltotriose	64.44%	95.01%	58.23%	95.09%	55.72%	98.59%	66.90%	95.55405
D(+)Glucose	60.28%	96.93%	58.14%	97.73%	66.85%	99.18%	77.50%	96.58%
Methyl-α-D-mannopyranoside	77.10%	97.92%	60.18%	88.29%	48.11%	97.98%	54.53%	96.47%
D(+)Mannose	64.20%	96.51%	51.62%	94.08%	36.46%	96.69%	55.33%	97.31%
Methyl-α-D-Glucopyranoside	50.90%	97.52%	51.94%	96.85%	54.91%	99.04%	54.35%	95.60%
L-Rhamnose	65.66%	97.38%	76.51%	96.58%	86.62%	96.05%	84.58%	95.16%
D(+)Xylose	76.06%	95.85%	90.33%	96.08%	90.79%	95.52%	94.78%	96.93%
D(+)Galactose	78.95%	95.21%	91.60%	92.25%	93.30%	87.08%	94.38%	96.39%

Table 4. Results of statistical t-tests (p-values) after 60 minutes of specific sugar treatments. *p<0.05 is significant.

Sugar	0.05M	0.025M	0.01M	0.005M
D(+)Melezitose	<0.0001	<0.0001	<0.0001	<0.0001
D(+)Trehalose	<0.0001	0.00013	<0.0001	0.14
Maltotriose	<0.0001	<0.0001	<0.0001	<0.0001
D(+)Glucose	<0.0001	<0.0001	<0.0001	<0.0001

Methyl-α-D-mannopyranoside	<0.0001	<0.0001	<0.0001	<0.0001
D(+)Mannose	<0.0001	<0.0001	<0.0001	<0.0001
Methyl-α-D-Glucopyranoside	<0.0001	<0.0001	<0.0001	<0.0001
L-Rhamnose	<0.0001	<0.0001	<0.0001	0.0092
D(+)Xylose	<0.0001	0.0028	0.08	0.10
D(+)Galactose	<0.0001	0.60	0.04	0.11

Table 3 shows the mean percentage of initial yeast remaining bound to the Con A beads for each sugar concentration at the end of the 60 minute experiment. We see that there is no real correlation of the amount of yeast bound in regards to increasing or decreasing sugar concentrations. The treatment with 0.01M D(+) Mannose resulted in the lowest mean percentage of initial yeast remaining bound, 36.46%, (Table 3, Fig. 1), whereas the highest mean percentage of initial yeast remaining bound to Con A, 94.78%, occurred from 0.005M D(+) Xylose (Table 3, Fig. 2).

Fig. 1. Yeast bound to Con A in 0.01M D(+) Mannose and control at initial binding (0 minutes) and after 60 minutes of treatment. Large spheres are Con A beads, tiny spheres are yeast. Magnification 300x.

a) Yeast bound to Con A with 0.01M D(+) Mannose at time zero (left) and after 60 minutes of treatment (right). After 60 minutes of treatment, 36.46% of the initial yeast had remained bound to the Con A bead.
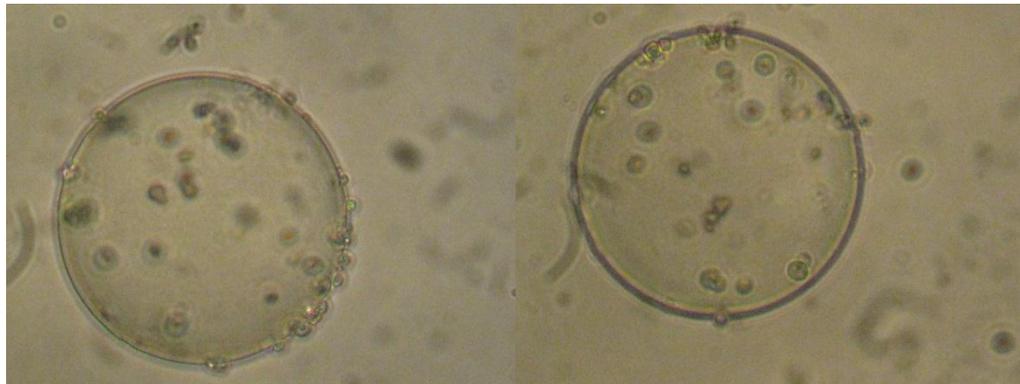
b) Yeast bound to Con A with distilled water at time zero (left) and after 60 minutes (right). After 60 minutes of treatment, 96.69% of the initial yeast had remained bound to the Con A bead.

Fig. 2. Yeast bound to Con A in 0.005M D(+) Xylose and control at initial binding and after 60 minutes of treatment. Large spheres are Con A beads, tiny spheres are the yeast. Magnification 300x.

a) Yeast bound to Con A with 0.005M D(+) Xylose at time zero (left) and after 60 minutes of treatment (right). After 60 minutes of treatment, 94.78% of the initial yeast had remained bound to the Con A bead.

b) Yeast bound to Con A with distilled water at time zero (left) and after 60 minutes (right). After 60 minutes of treatment, 96.93% of the initial yeast had remained bound to the Con A bead.

D(+) Melezitose

      D(+) Melezitose is a non-reducing trisaccharide sugar which is primarily produced by insects which eat plant sap. Treatment with 0.025M D(+) Melezitose had the lowest average percentage of yeast bound to Con A, 40.80% at 60 minutes (Table 3) and treatment with 0.05M D(+) Melezitose resulted with the highest amount of initial yeast bound, 46.60% (Table 3), of the D(+) Melezitose trials. After statistical tests were conducted, each concentration of D(+) Melezitose studied had a p-value less than 0.05 (Table 4 and 5), making it significantly different than the amount of initial yeast remaining bound to Con A beads on the controls. From the results of Fig. 3, it is evident that the four concentrations of D(+)Melezitose had a lower amount of initial yeast remaining bound than the control experiments they were compared to.

Figure 3. Mean percentage of initial yeast remaining bound to Con A over time. "E" is with D(+) Melezitose treatment and "Control" is the corresponding control treatment.

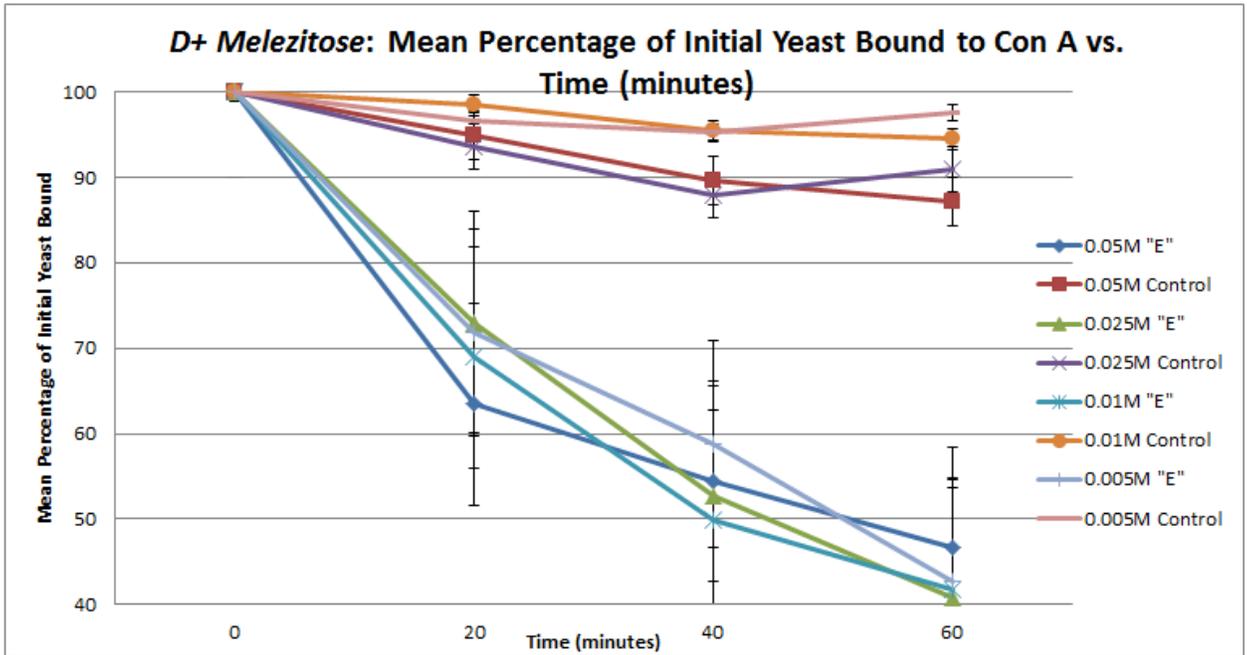**D+ Melezitose: Mean Percentage of Initial Yeast Bound to Con A vs. Time (minutes)**

Table 5. Results of statistical t-tests (p-values) after 20, 40 and 60 minutes of D(+)

Melezitose treatments. *p<0.05 is significant.

D(+) Melezitose Concentration	20 minutes	40 minutes	60 minutes
0.05M	0.00019	0.00019	<0.0001
0.025M	<0.0001	0.000086	<0.0001
0.01M	<0.0001	<0.0001	<0.0001
0.005 M	<0.0001	<0.0001	<0.0001

D(+) Trehalose

18

Trehalose is a non-reducing sugar which is formed by two glucose molecules which are bound by a 1,1 alpha bond. Trehalose is found vastly in nature among plants, animals and microorganisms. The lowest average of initial yeast remaining bound to the Con A, 54.40%, occurred with the treatment of 0.05M D(+) Trehalose whereas the highest mean percentage of initial yeast remaining bound, 94.03%, was seen with the treatment of 0.005M D(+) Trehalose (Table 3 and Fig. 4). Therefore, when looking at the statistical analysis, with a p-value of 0.14 after 60 minutes of treatment with 0.005M D(+) Trehalose, we can conclude that the treatment did not have a significant effect on the mean disaggregation of the yeast (Table 4 and 5). However, 0.05M, 0.025M and 0.01M D(+) Trehalose did have a significant effect on the amount of initial yeast which remained bound (Table 4 and 6). Although, 0.01M D(+) Trehalose had an average percentage of initial yeast remaining bound of only 94.03%, it was still significantly different than the control since the p-value calculated less than 0.05 (Table 3,4,6).

Figure 4. Mean percentage of initial yeast remaining bound to Con A beads vs. Time of D(+)Trehalose. "E" is with D(+) Trehalose treatment and "Control" is the corresponding control.

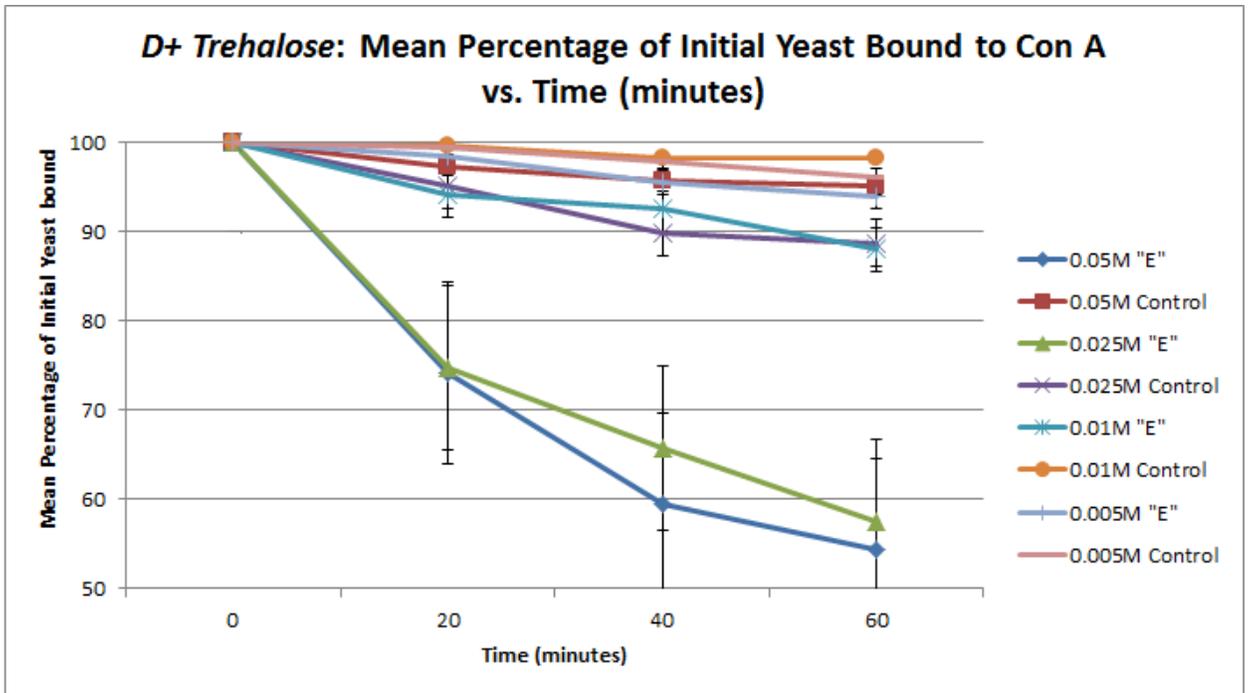D+ Trehalose: Mean Percentage of Initial Yeast Bound to Con A vs. Time (minutes)

Table 6. Results of statistical t-tests (p-values) after 20, 40 and 60 minutes of D(+)

Trehalose treatments. *p<0.05 is significant.

D(+) Trehalose Concentration	20 minutes	40 minutes	60 minutes
0.05M	0.00065	<0.0001	<0.0001
0.025M	0.00023	0.0038	0.00013
0.01M	0.00025	0.0028	<0.0001
0.005 M	0.16	0.07	0.14

Maltotriose

Maltotriose is a trisaccharide which consists of three glucose molecules. These molecules are bound by α-1,4 glycosidic bonds. Maltotriose is usually formed by the digestion of starch by amylase, a common enzyme in the saliva of humans. The lowest mean percentage of initial yeast still bound to Con A of Maltotriose, 55.72%, was evident with the treatment of 0.01M Maltotriose and the highest mean percentage of initial yeast still bound, 66.90%%, was seen in the presence of 0.005M Maltotriose (Table 3, Fig. 5). All concentrations of Maltotriose had p-values less than 0.05 at the end of the 60 minute mark, making the mean disaggregation of the yeast from the Con A significantly different than the mean disaggregation of the control (Table 4 and 7).

Figure 5. Mean percentage of initial yeast remaining bound to Con A beads vs. Time of Maltotriose. "E" is with Maltotriose treatment and "Control" is the corresponding control.

Maltotriose: Mean Percentage of Initial Yeast Bound to Con A vs. Time (minutes)
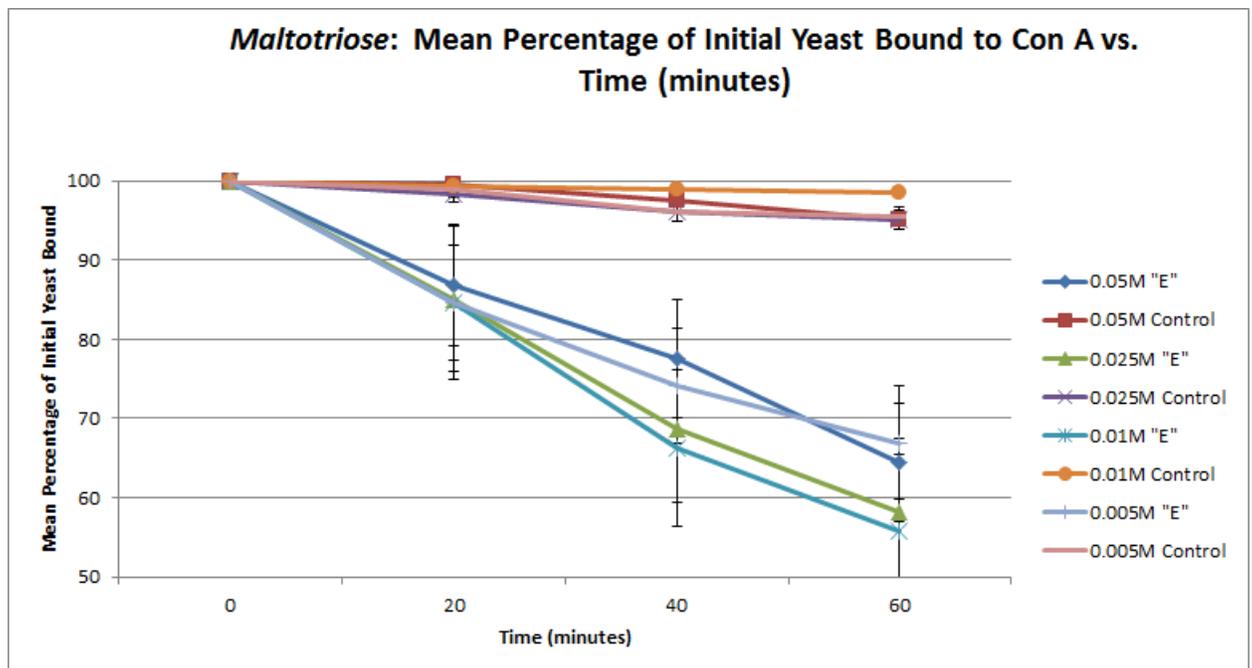
Table 7. Results of statistical t-tests (p-values) after 20, 40 and 60 minutes of Maltotriose treatments. *p<0.05 is significant.

Maltotriose Concentration	20 minutes	40 minutes	60 minutes
0.05M	<0.0001	<0.0001	<0.0001
0.025M	<0.0001	<0.0001	<0.0001
0.01M	0.00063	<0.0001	<0.0001
0.005 M	<0.0001	<0.0001	<0.0001

D(+) Glucose

D(+) Glucose, also known as grape sugar, is a monosaccharide and is an important source of physiological energy. In plants, glucose occurs as a product of metabolism following photosynthesis. In animals and fungi however, it is the result of the breakdown of glycogen or gluconeogenesis. The lowest mean percentage of initial yeast remaining bound to the Con A, 58.14%, was seen with the treatment of 0.025M D(+) Glucose and the highest mean percentage of initial yeast remaining bound, 77.50%, occurred after the treatment with 0.005M D(+) Glucose (Table 3 and Fig. 6). All concentrations of D(+) Glucose had p-values less than 0.05 at the end of the 60 minute mark, making the mean disaggregation of the yeast from the Con A significantly different than the mean disaggregation of the control (Table 4 and 8).

Figure 6. Mean percentage of initial yeast remaining bound to Con A beads vs. Time of D(+) Glucose. "E" is with D(+) Glucose treatment and "Control" is the corresponding control.

D(+) Glucose: Mean Percentage of Initial Yeast Bound to Con A vs. Time (minutes)
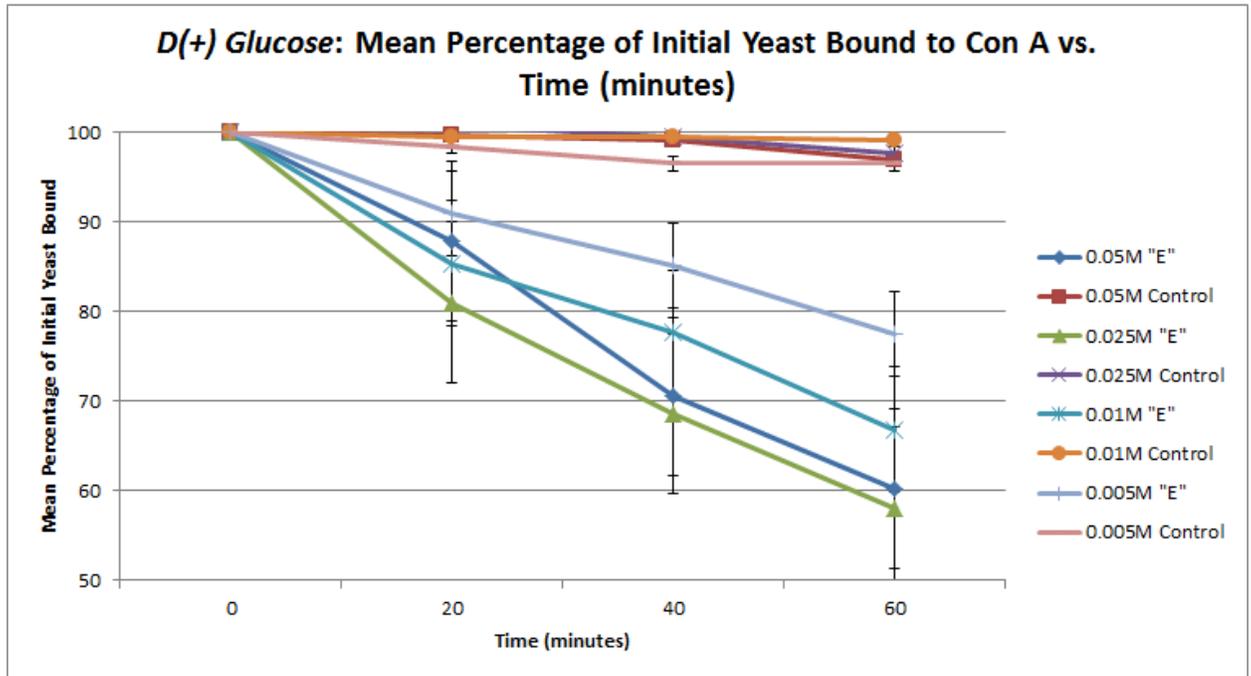
Table 8. Results of statistical t-tests (p-values) after 20, 40 and 60 minutes of D(+) Glucose treatments. *p<0.05 is significant.

D(+) Glucose Concentration	20 minutes	40 minutes	60 minutes
0.05M	<0.0001	<0.0001	<0.0001
0.025M	<0.0001	<0.0001	<0.0001
0.01M	0.01	0.002	0.000081
0.005 M	0.00023	<0.0001	<0.0001

Methyl-α-D-Mannopyranoside

Methyl-α-D-Mannopyranoside displayed the lowest mean percentage of initial

yeast still bound to Con A, 48.11%, with the treatment of 0.01M Methyl-α-D-

Mannopyranoside after 60 minutes and had the highest mean percentage of yeast still

bound, 77.10% after 60 minutes of treatment with 0.05M Methyl-α-D-Mannopyranoside

(Table 3 and Fig. 7), which is a distinct example where increased concentration does not

necessarily result in increased mean disaggregation. All concentrations of Methyl-α-D-

Mannopyranoside had p-values less than 0.05 after 60 minutes of treatment (Table 4 and

9), which makes the mean percentage of initial yeast remaining bound with all four

concentrations significantly different when compared to the amount of yeast bound to the

controls.

Figure 7. Mean percentage of initial yeast remaining bound to Con A beads vs. Time of

Methyl-α-D-Mannopyranoside. "E" is with Methyl-α-D-Mannopyranoside treatment and

"Control" is the corresponding control.

Methyl-*α*-mannopyranoside: Mean Percentage of Initial Yeast Bound to Con A vs. Time (minutes)

Y-axis: Mean Percentage of Initial Yeast Bound

X-axis: Time (minutes)

Legend:
- 0.05M "E"
- 0.05M Control
- 0.025M "E"
- 0.025M Control
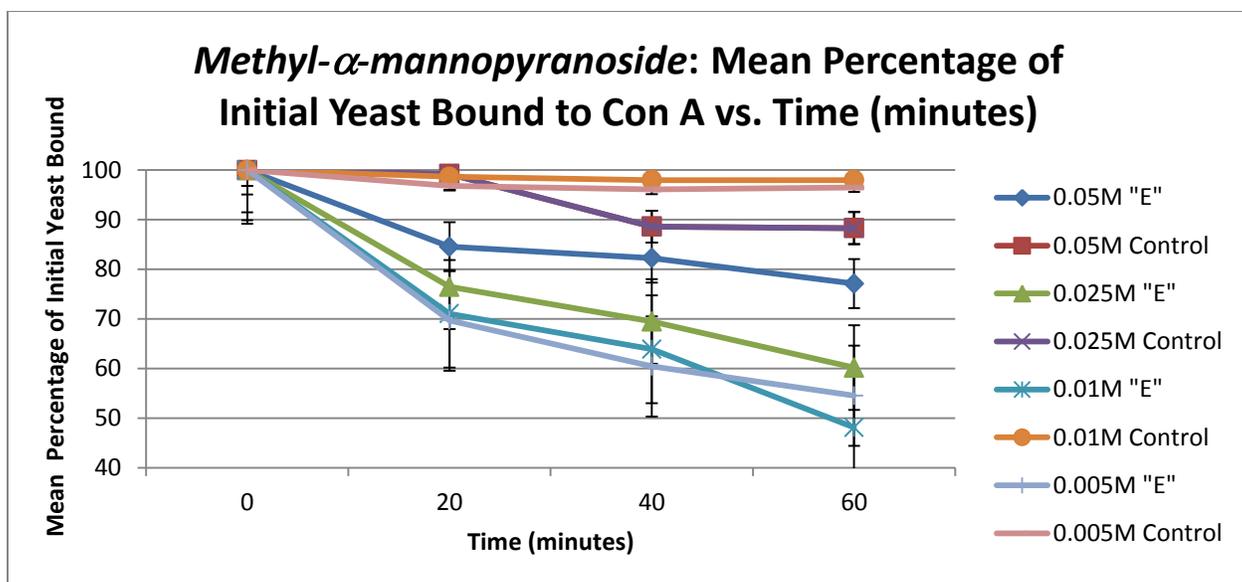- 0.01M "E"
- 0.01M Control
- 0.005M "E"
- 0.005M Control

Table 9. Results of statistical t-tests (p-values) after 20, 40 and 60 minutes of Methyl-α-D-Mannopyranoside treatments. *$p<0.05$ is significant.

Methyl-α-D-Mannopyranoside Concentration	20 minutes	40 minutes	60 minutes
0.05M	<0.0001	0.0032	0.000034
0.025M	0.00064	0.00023	0.000039
0.01M	<0.0001	<0.0001	<0.0001
0.005 M	0.00049	<0.0001	<0.0001

D(+) Mannose

D(+) Mannose is a C-2 epimer of glucose and has the same molecular formula, $C_6H_{12}O_6$ as the other monosaccharaides. D(+) Mannose is used a naturopathic remedy

for urinary tract infections and has been shows to work by inhibiting the bacteria from adhering to the urinary tract. The lowest mean percentage of initial yeast still bound after 60 minutes of treatment with both D(+) Mannose and also in all of the sugars tested, 36.46% was noted from 0.01M D(+) Mannose (Table 3 and Fig. 8). On the other hand, the highest mean percentage of initial yeast still bound to Con A, 64.20%, was seen from 0.05M D(+) Mannose. Again, this is a critical example which demonstrates that the amount of yeast bound has no correlation with concentration. All concentrations of D(+) Mannose examined had p-values less than 0.05, indicating that the amount of yeast remaining bound to Con a with the treatment of D(+) Mannose was significantly different than the amount of yeast remaining bound to the control (Table 4 and 10).

Figure 8. Mean percentage of initial yeast remaining bound to Con A beads vs. Time of D(+) Mannose. "E" is with D(+) Mannose treatment and "Control" is the corresponding control.

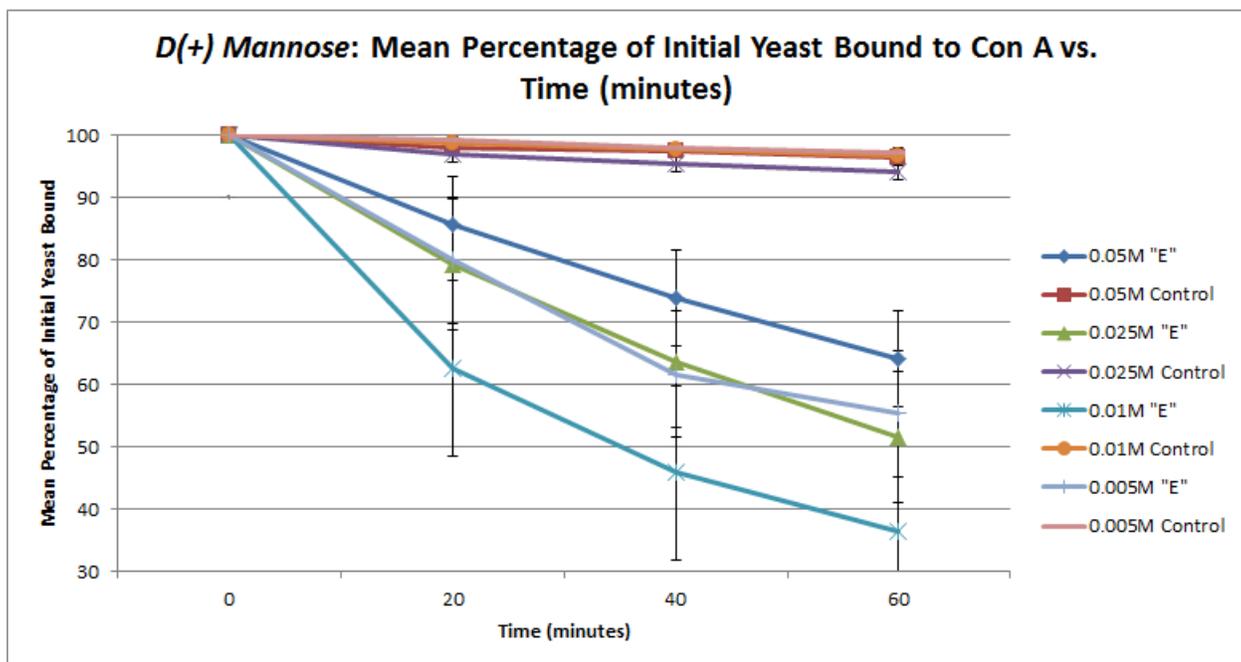D(+) Mannose: Mean Percentage of Initial Yeast Bound to Con A vs. Time (minutes)

Table 10. Results of statistical t-tests (p-values) after 20, 40 and 60 minutes of D(+) Mannose treatments. *p<0.05 is significant.

D(+) Mannose Concentration	20 minutes	40 minutes	60 minutes
0.05M	0.0011	<0.0001	<0.0001
0.025M	<0.0001	<0.0001	<0.0001
0.01M	<0.0001	<0.0001	<0.0001
0.005 M	0.00024	0.00048	<0.0001

Methyl-α-D-Glucopyranoside

Methyl-α-D-Glucopyranoside is a monosaccharide derived from glucose. The

lowest mean percentage of initial yeast remaining bound to the Con A beads with the

treatment of Methyl-α-D-Glucopyranoside, 50.90%, resulted after 60 minutes of

treatment with 0.05M, and the highest mean percentage of yeast remaining bound,

54.91%, occurred with the treatment of 0.01M Methyl-α-D-Glucopyranoside (Table 3

and Fig. 9). All four concentrations of Methyl-α-D-Glucopyranoside had similar mean

percentage of yeast bound at the end of the 60 minute experiments, ranging from 50.90%

to 54.91% (Table 3). Correspondingly, all concentrations of Methyl-α-D-

Glucopyranoside examined had p-values less than 0.05 (Table 4 and 11), indicating that

the amount of initial yeast remaining bound with the treatment of Methyl-α-D-

Glucopyranoside was significantly different than the amount of initial yeast remaining

bound of the control.

Figure 9 . Mean percentage of initial yeast remaining bound to Con A beads vs. Time of

Methyl-α-D-Glucopyranoside. "E" is with D(+)Methyl-α-D-Glucopyranoside treatment

and "Control" is the corresponding control.

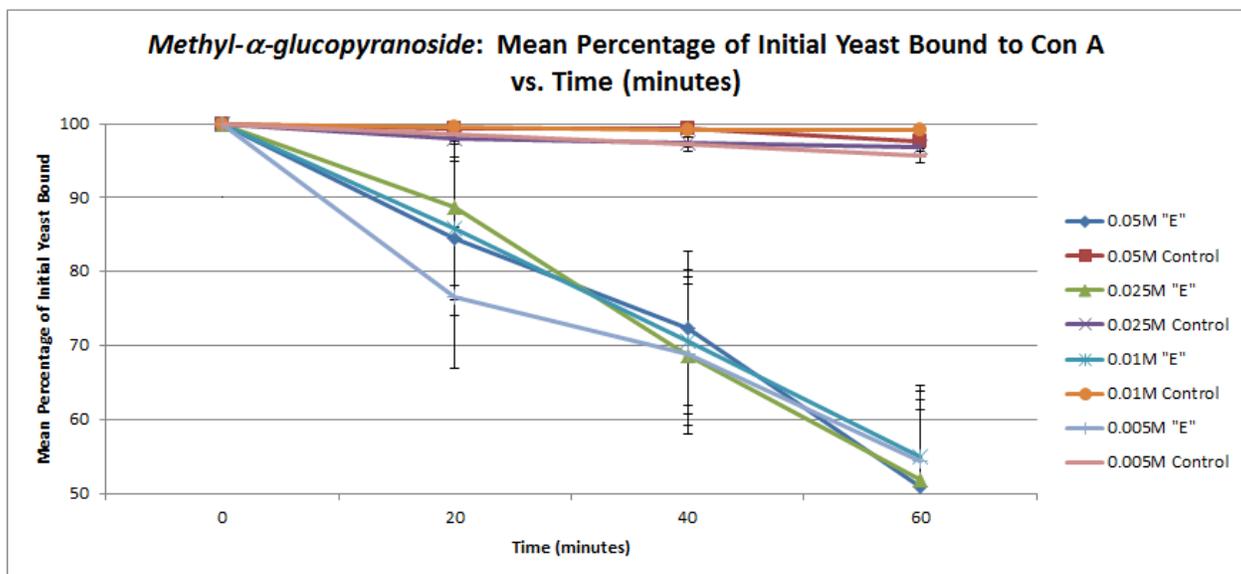Methyl-α-glucopyranoside: Mean Percentage of Initial Yeast Bound to Con A vs. Time (minutes)

Table 11. Results of statistical t-tests (p-values) after 20, 40 and 60 minutes of Methyl-α-D-Glucopyranoside treatments. *p<0.05 is significant.

Methyl-α-D-Glucopyranoside Concentration	20 minutes	40 minutes	60 minutes
0.05M	<0.0001	<0.0001	<0.0001
0.025M	<0.0001	<0.0001	<0.0001
0.01M	<0.0001	<0.0001	<0.0001
0.005 M	0.0014	<0.0001	<0.0001

L-Rhamnose

Rhamnose is a deoxy sugar that occurs naturally. In nature, Rhamnose occurs in its L-form as L-rhamnose (6-deoxy-L-mannose). This is unusual, given that generally the naturally occurring sugars are in D-form. Rhamnose can be isolated from Buckthorn (*Rhamnus*), poison sumac, and plants in the genus *Uncaria*. Rhamnose is also a constituent of the outer cell membrane of acid-fast bacteria in the Mycobacterium genus, which includes the organism that causes tuberculosis. The lowest mean percentage of initial yeast still bound to the Con A beads after 60 minutes of treatment with L-Rhamnose, 65.66%, was noted with the treatment with 0.05M L-Rhamnose whereas the highest mean percentage of yeast still bound, 86.62%, was seen with the treatment of 0.01M L-Rhamnose (Table 3 and Figure 10). Even though the treatment of 0.01M L-Rhamnose indicated a mean percentage of initial yeast remaining bound of 86.62%, it still had a p-value less than 0.05 after 60 minutes of treatment, leading to the conclusion that it still had a significant effect on the mean percentage of initial yeast remaining bound when compared to the control (Table 4 and 12). After 60 minutes of treatment, all four concentrations of L-Rhamnose had p-values less than 0.05 (Table 4 and 12).

Figure 10 . Mean percentage of initial yeast remaining bound to Con A beads vs. Time of L-Rhamnose. "E" is with D(+) L-Rhamnose treatment and "Control" is the corresponding control.

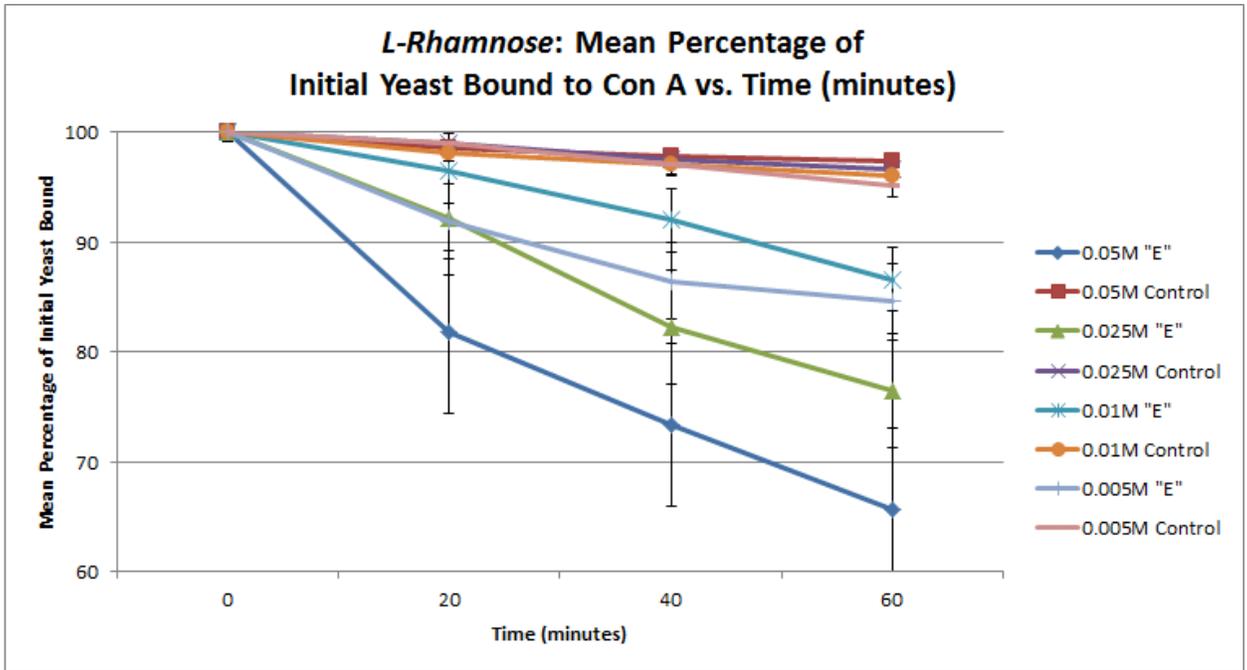**L-Rhamnose:** Mean Percentage of Initial Yeast Bound to Con A vs. Time (minutes)

Table 12. Results of statistical t-tests (p-values) after 20, 40 and 60 minutes of L-Rhamnose treatments. *p<0.05 is significant.

L-Rhamnose Concentration	20 minutes	40 minutes	60 minutes
0.05M	0.0043	<0.0001	<0.0001
0.025M	0.00060	<0.0001	<0.0001
0.01M	0.32	0.00088	<0.0001
0.005 M	0.0049	0.0095	0.0092

D(+) Xylose

Xylose is classified as a monosaccharide of the aldopentose type, which means that it contains five carbon atoms and includes a formyl functional group. It is the precursor to hemicellulose, one of the main constituents of biomass. The lowest mean percentage of initial yeast still bound to the Con A was seen after 60 minutes of treatment with 0.05M D(+) Xylose, 76.06%, whereas the highest mean percentage of initial yeast still bound, in both the D(+) Xylose trials and also from all of the sugar concentrations examined, 94.78% was noted with the treatment of 0.005M D(+) Xylose (Table 3 and Fig. 11). After the statistical tests were conducted, only 0.05M and 0.025M D(+) Xylose had a p-value less than 0.05, whereas 0.01M and 0.005M D(+) Xylose had p-values greater than 0.05 (Table 4 and 13). From this, it is concluded that the treatment with 0.05M and 0.025M D(+) Xylose had a significant effect on the mean percentage of yeast remaining bound to the Con A, although the treatment with 0.01M and 0.005M D(+) Xylose didn't have a significant effect when compared to the control.

Figure 11 . Mean percentage of initial yeast remaining bound to Con A beads vs. Time of D(+) Xylose. "E" is with D(+) Xylose treatment and "Control" is the corresponding control.

D(+) Xylose: Mean Percentage of Initial Yeast Bound to Con A vs. Time (minutes)
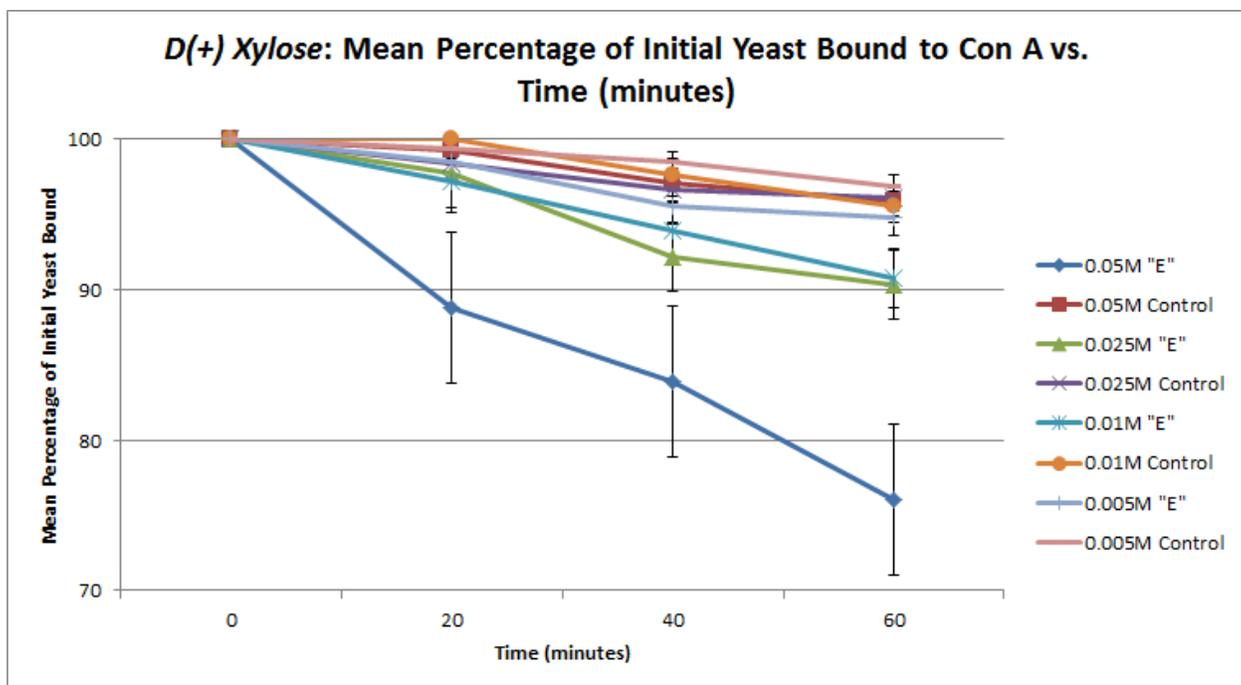
Table 13. Results of statistical t-tests (p-values) after 20, 40 and 60 minutes of D(+) Xylose treatments. *p<0.05 is significant.

D(+) Xylose Concentration	20 minutes	40 minutes	60 minutes
0.05M	<0.0001	<0.0001	<0.0001
0.025M	0.59	0.0097	0.0028
0.01M	0.04	0.40	0.08
0.005 M	0.35	0.0090	0.10

D(+) Galactose

Galactose is a monosaccharide that is less sweet than glucose. Galactose is found in dairy products, sugar beets, other gums and mucilages. It is also synthesized by the

body, where it forms part of glycolipids and glycoproteins in several tissues. The lowest

mean percentage of initial yeast remaining bound to Con A after 60 minutes of treatment

with D(+) Galactose, 78.95%, was observed from 0.05M D(+) Galactose whereas the

highest mean percentage of initial yeast remaining bound, 94.38% was noted with

0.005M (Table 3 and Fig. 12). From the results of the statistical tests, after 60 minutes of

treatment, 0.05M and 0.01M D(+) Galactose had p-values less than 0.05, while 0.025M

and 0.005M D(+) Galactose had p-values greater than 0.05, which leads to the conclusion

that treatment with 0.025M and 0.005M D(+) Galactose did not have a significant effect

on the mean percentage of yeast remaining bound, whereas treatment with 0.05M and

0.01M D(+) Galactose did. (Table 4 and 14).

Figure 12. Mean percentage of initial yeast remaining bound to Con A beads vs. Time of

D(+) Galactose. "E" is with D(+) Galactose treatment and "Control" is the corresponding
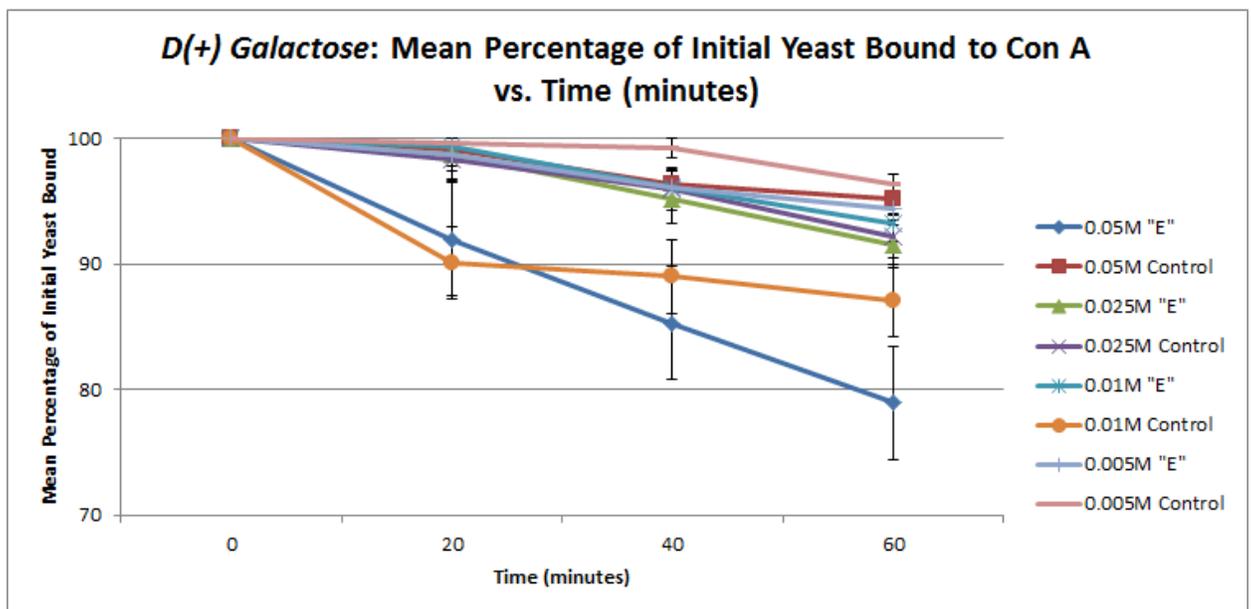
control.

Table 14. Results of statistical t-tests (p-values) after 20, 40 and 60 minutes of D(+) Galactose treatments. *p<0.05 is significant.

D(+) Galactose Concentration	20 minutes	40 minutes	60 minutes
0.05M	<0.0001	<0.0001	<0.0001
0.025M	0.73	0.49	0.60
0.01M	0.65	0.08	0.04
0.005 M	0.28	0.0048	0.11

After all ten sugars were tested and all statistical tests were conducted, it was evident that there was no correlation between the concentration of the sugars and the amount of initial yeast remaining bound to the Con A beads during the trials. The effectiveness of each sugar was ranked based on the amount of initial yeast still bound after 60 minutes. Treatment with the most effective sugars resulted in the least amount of yeast still bound to the Con A beads at the lowest concentration (0.005M). Methyl-α-D-Mannopyranoside was found to be the most effective sugar since at 0.005M there was an average percentage of initial yeast remaining bound to Con A beads of 54.53% whereas treatment with 0.05M resulted in an average of 77.10% of initial yeast bound (Table 3 and 15). The least effective sugar was D(+) Trehalose, resulting in an average of 94.03% of the initial yeast remaining bound to Con A after the 60 minutes of treatment with 0.005M and 54.40% of yeast still bound with the treatment of 0.05M (Table 3 and 15).

Table 15- List of sugars in order of decreasing effectiveness after 60 minutes of treatment.

1. Methyl-α-D-Mannopyranoside
2. D(+) Mannose
3. D(+) Melezitose
4. Maltotriose
5. Methyl-α-D-Glucopyranoside
6. D(+) Galactose
7. D(+) Glucose
8. D(+) Xylose
9. L-Rhamnose
10. D(+) Trehalose

Another critical point of time to take into consideration when looking at the effectiveness of each sugar is at the 20 minute mark. The sugars which show the least amount of yeast bound to the Con A beads at the lowest concentration (0.005M), at the fastest time (20 minutes), will likely indicate that those sugars bind most strongly.

Table 16. Mean percentage of initial yeast remaining bound to Con A beads after 20 minutes of specific sugar treatment at 0.005M. Results were determined from 10 trials for each sugar concentration. The column "C" represents the mean percentage of initial yeast still bound to Con A beads after 20 minutes of the corresponding control treatment.

Sugar	0.005M	0.005M "C"
D(+)Melezitose	71.95%	96.71%
D(+)Trehalose	98.43%	99.58%
Maltotriose	84.60%	99.02%
D(+)Glucose	90.94%	98.45%
Methyl-α-D-mannopyranoside	69.68%	96.80%
D(+)Mannose	79.99%	99.26%
Methyl-α-D-Glucopyranoside	76.52%	98.54%
L-Rhamnose	91.90%	99.01%
D(+)Xylose	98.47%	99.41%
D(+)Galactose	98.73%	99.66%

Table 17- List of sugars in order of decreasing effectiveness after 20 minutes of 0.005M treatment.

1. Methyl-α-D-Mannopyranoside
2. D(+) Melezitose
3. Methyl-α-D-Glucopyranoside
4. D(+) Mannose
5. Maltotriose
6. D(+) Glucose
7. L-Rhamnose
8. D(+) Xylose

| 9. D(+) Trehalose |
| 10. D(+) Galactose |

From the results of Table 16, we are able to compare the amount of initial yeast remaining bound after 20 minutes with the treatment of 0.005M of each sugar and compare it to the amount of initial yeast remaining bound to the control. As noted earlier, the sugars which show the least amount of yeast bound to the Con A beads at the lowest concentration (0.005M), at the fastest time (20 minutes), will likely indicate that those sugars bind most strongly. From the results of Table 16, it is evident that four of the sugars, Methyl-α-D-mannopyranoside, Melezitose, Methyl-α-D-Glucopyranoside and D(+)mannose resulted in less amount of the initial yeast remaining bound to the Con A with the treatment of 0.005M of each sugar when compared to the results from the treatment with 0.05M of each sugar. The remaining six sugars had less amount of yeast still bound to the Con A bead when treated with the 0.05M sugar when compared to the treatment with 0.005M of each sugar. The ten sugars were ranked based on their effectiveness after 20 minutes of treatment with each sugar at the lowest concentration, 0.005M (Table 17). As it was found at the end of the 60 minute trial- Methyl-α-D-Mannopyranoside, D(+) Melezitose and D(+) Mannose are amongst the most effective sugars. The remaining sugars seem to rank fairly similar when looking at their effectiveness after both 20 and 60 minutes of treatment (Table 15 and Table 17).

Discussion

Goldstein et. al defined a lectin as a sugar binding protein or glycoprotein of non-immune origin that agglutinates cells or precipitates glycoconjugates (13). Lectins are structurally diverse, varying in molecular size, amino acid composition, metal requirement and three-dimensional shape. Despite this variation, they can be grouped in families of structurally homologous proteins (29).

Lectins have been found to primarily react with the non-reducing end of oligo- and polysaccharides. Many lectins combine preferentially with a single sugar structure. There are however some lectins whose specificity is broader and includes several closely related sugars. Other lectins interact simply with complex carbohydrate structures such as those that appear in glycolipids, glycoproteins or on the surface of cells. The sugars that combine greatest with lectins are typical constituents of glycoproteins or glycolipids (29).

Microbial lectins not only have the ability to recognize various types of natural targets which contain carbohydrates, but they have the ability to also recognize differences in carbohydrate-containing targets within each type. Factors which possibly influence the affinity of a microbial lectin for complex glycans as constituents of carbohydrate-containing targets are: 1) types of both terminal and internal carbohydrate residues, 2) the presence of terminal residues of carbohydrates of the same type in clusters, which make it accessible to the lectin and 3) the degree of polysaccharide branching (9).

The agglutinating properties of lectins are analogous to those of antibodies. It is possible for specific sugars or sugar containing compounds to inhibit lectin activity (22, 27). Currently, lectins have become standard reagents in diagnostic microbiology (9, 16, 32). In a typical application, a suspension of a microorganism is mixed with a solution of lectin on a glass slide. A positive result, or agglutination, shows the presence of an organism or organisms with the appropriate sugar content. It may also provide some history of the organism in question.

Carbohydrates are a complex and large class of compounds which range from simple monosaccharaides to complex polysaccharides. It has been challenging for researchers to study carbohydrates due to their structural complexity. Up to date, carbohydrates have been extensively studied due to the growing field of glycobiology. The fact that carbohydrates are able to combine with proteins and lipids and form glycoproteins and glycolipids is what makes carbohydrates so important in cellular interaction. Cell Differentiation, infection, immune response and neural development are examples of crucial biological processes which are regulated by protein-carbohydrate interactions (10).

Cell surface carbohydrates are not only important factors in the attachment of animal cells with each other, but also for the binding of infectious bacteria, toxins, viruses, hormones and other molecules. Thus, medical research is concentrating on the use of sugars in medications and vaccines in order to inhibit and treat infections due to bacteria and viruses (28). Currently, carbohydrate based vaccines are being used for meningitis, typhus and pneumonia (18).

Bacterial adhesion is vital in infection and due to the fact that antibiotic resistance is on the rise, an excellent alternative would be to use drugs and vaccines which are carbohydrate based in order to prevent and treat infection (28). Infection often happens when bacterial lectins bind to the carbohydrates present on the host's cell surface and therefore, it is possible to block bacterial infection by designing drugs which include similar carbohydrates to those found on the surface of the host's cells. This carbohydrate based drug would then be capable of treating the bacterial infection by selectively preventing bacterial adhesion to the host cell by binding to the bacterial lectin (11). Therefore, carbohydrate based therapeutics may offer an effective way to battle human disease.

Additionally, scientists study glycoconjugates in order to see if they may be the basis of new vaccines. The vaccines that protect against meningitis (*Neisseria meningitidis*), pneumonia, blood infections (*Haemophilus influenzae type b*), and pneumococcal bacteria (*Streptococcus pneumoniae*) are mainly glycoconjugate in nature (15). These glycoconjugates represent part of the infectious bacteria's outer surface that are usually used to hide from its host . These vaccines assist the human immune system to identify these components on the bacterial surface and stop the infection before it starts. When administered to healthy adults, these vaccines lead to short term protection for about 90% of the infections by these microorganisms (33). Furthermore, genetic variation in pathogens as a result of selective pressures don't directly cause changes of the cell surface carbohydrate due to the fact that the expression of carbohydrates is not under direct genetic control (14). Due to the significant progress which has resulted from

the use of carbohydrate –based vaccines directed against bacterial infection, a large amount of clinical use has occurred.

Previously, in a study done by Zem et. al. in 2006, they analyzed the binding of the mannose-rich yeast to Con A, which is a mannose binding lectin, using an extensive list of thirty different sugars. At the time, they developed the most extensive saccharide inhibitor list ever developed for Con A. In their study, they kept the concentrations of all of the sugars constant at 0.05M and only used one time point, 4 minutes. At the end of their experiment, they ranked the saccharide inhibitors of yeast-Con A bead binding in order of decreasing inhibition effectiveness. After 4 minutes of treatment with 0.05M of each sugar, Zem et. al. concluded the most effective sugars to be D(+) Melezitose, D(+) Trehalose and Maltotriose. They found D(+) Glucose, Methyl-α-Mannopyranoside, D(+) Mannose and Methyl-α-Glucopyranoside to be somewhat less effective. Finally, the least effective sugars they found were D(+) xylose, L-rhamnose and D(+) Galactose. This list of effectiveness is based only on the treatment with 0.05M of each sugar after 4 minutes and has no other concentrations to compare the results to (34). Although thousands of replicates were examined, in their study, Zem et. al. based their sugar analysis solely on the observation of whether the sugar sample at a given sugar concentration of 0.05M at one time show more, less or the same cell binding to beads than the controls without sugar on the same slide. With this approach they were not able to answer how much less cell binding to beads occurred in the experimental versus control sample.

The focus of this study was to further examine the role of specific sugars in the inhibition of lectin binding. The protocol involved the use of ten of the sugars used in the

Zem et. al. experiment, D(+) Melezitose, D(+) Trehalose, Maltotriose, D(+) Glucose, Methyl-α-D-mannopyranoside, D(+) Mannose, Methyl-α-glucopyranoside, L-Rhamnose, D(+) Xylose and D(+) Galactose. Zem et. al. conducted their experiment using only 0.05M concentration of each sugar. In this study, however, I took it two steps further and analyzed the sugars at not only 0.05M, but also 0.025M, 0.01M and 0.005M and not only at 4 minutes, but over a 60 minute time course. It was important to see if there was any correlation between increasing concentration and a lower percentage of initial yeast bound to the Con A. Also, since in this study the amount of yeast bound to Con A was counted for each experiment and compared to the corresponding control experiment, I was able to calculate and answer the question that Zem et. al. was not able to answer, which was how much less yeast binding to the Con A beads occurred in the experimental versus control samples.

Considering that Con A is a mannose binding lectin, it was not surprising to see that the sugars which contain mannose or contain either a structure or molecular formula close to that of mannose, including Methyl-α-D-mannopyranoside and D(+) Mannose showed less average percentage of initial yeast bound to Con A after the 60 minute treatment with 0.005M when compared to the results after 60 minutes with 0.05M. Although D(+) Melezitose does not have a chemical structure or molecular formula similar to that of mannose, it was still a very effective sugar. Methyl-α-D-mannopyranoside, D(+) Mannose and D(+) Melezitose were found to be the most effective sugars when it comes to inhibition of yeast binding to Con A considering the general rule of thumb that if a sugar is most effective at its lowest concentration, it is more likely to strongly bind to cell receptors. Maltotriose and Methyl-α-D-

glucopyranoside had similar mean percentage of yeast bound to Con A from the treatment of both 0.005M and 0.05M. The least effective sugars, considering more yeast were bound to the Con A with 0.005M when compared to 0.05M were D(+) Trehalose, D(+) Glucose, L-Rhamnose, D(+) Xylose, D(+) Galactose.   Similar results of effectiveness were observed when looking at the results from the 20 minute time. The 20 minute time was analyzed since it is believed that the sugars which show the least amount of yeast bound to the Con A beads at the lowest concentration (0.005M), at the fastest time (20 minutes), will likely indicate that those sugars bind most strongly. The main difference between the results from this study and the Zem et. al study is the fact that Zem et. al. found D(+) Trehalose among the highly effective sugars, whereas in this study, it was found to be one of the least effective sugars at both the 60 and 20 minute time points. D(+) Melezitose, however, ranked near the top in effectiveness in all three lists: Zem et. al (0.05M) and the 20 and 60 minutes done in this study (0.005M)

Although Methyl-α-D-mannopyranoside, D(+) Mannose and D(+) Melezitose were found to be the most effective sugars, nearly all of the sugars at almost all of the concentrations tested showed a significant difference in the amount of yeast bound to Con A after treatment when compared to the controls. D(+) Trehalose at 0.005M, D(+) Xylose at 0.01M and 0.005M, and D(+) Galactose at 0.025M and 0.005M did not show a significant difference in the amount of yeast bound to Con A after treatment when compared to the controls since they had p-values greater than 0.05. Differences in mean percentages were still observed in sugar concentrations that were not considered to be statistically significant.

Statistical analysis of the data from this experiment did not always support the conclusions of the investigator. For example, since there was a control experiment for each sugar studied, and the result was sometimes low, the statistical significance did not always reflect the observation of binding. The unpaired t-test compares the experimental data of each sugar treatment to that of its corresponding control. When the t-test is calculated, certain sugar treatments will result in p-vales less than 0.05 and will be considered significant, even though there is not a large mean percentage difference observed. In contrast, a mean percentage difference may be seen, but not considered statistically significant.

The results generated here show some differences in sugar ranking compared with the Zem et al. results. We believe this helps support the fact that this highly quantitative approach using four concentrations of each sugar, rather than just one concentration, over a 60 minute time course instead of a single 4 minute time point, with statistical evaluation is an important improvement in helping to determine the effectiveness of specific sugars used in this experiment.

Yeast, *Saccharomyces cerevisiae,* is rich in mannose, and so it was chosen as a substrate in the same experiment for binding Con A, a mannose-binding lectin. Three sugars from the ten studied which may bind most tightly to Con A-lectin, based on the results of these experiments. These were Methyl-α-D-mannopyranoside, D(+) Mannose and D(+) Melezitose.

Microarray technology is becoming commonly used. In microarray technology, sugars or proteins are bound to small spots on solid surfaces or microchips. Its use is

important when there is a need to screen biological compounds, cell extracts, cells and various samples in order to examine the binding of the samples to the compounds which are bound to the chip (4). Microarray technology is also currently being used to produce possible vaccines for HIV and medications in order to prevent HIV infection, to detect bacteria present in food and also the blood, and to develop blood tests for diseases such as Crohn's disease (4). Although microarray technology is a promising tool for the future of human disease and treatments, it can cost hundreds of thousands of dollars. This study suggests that the use of both lectin and saccharide derivatized microbeads provide an inexpensive way of achieving several of the same experiments in the field of carbohydrate drug and diagnostic test advances. At this time, these experiments are being conducted using microarray technology which is quite expensive. If, for instance, the *Saccharomyces cerevisiae* model described here was actually a pathogen which bound to human tissue by means of its cell surface mannose residues, we might be able to create drugs which block binding. The sugars needed in these drugs might be based on the sugars which were inhibitors of binding found in this study. Alternatively, if it was not known which lectins or cell surface sugars were present on the *Saccharomyces cerevisiae*, it would be possible to run these cells through libraries of beads derivatized with various sugars or lectins in order to observe to which beads cell binding occurs. This would help to define cell surface molecules that may be involved in the attachment of the cells to human tissue.

Future studies will need to concentrate on the physical chemistry of the binding of not only the sugar to the Con A beads, but also, the sugar to the yeast in order to get a complete understanding of the role of the sugar. Also, it will be necessary to investigate

whether the sugar on the yeast surface is the actual cause of its binding to the Con A. This can be done by treating the yeast cells with various enzymes, for example glucosidases or mannosidases, before the binding experiment of the yeast to the Con A is conducted.

The method used in this study is an inexpensive substitute for microarrays. It is possible that this method can be used with different glycan derivatized beads, or as was done in this study, lectin derivatized beads in order to rapidly evaluate lectin-glycan interaction and the dynamics that influence it.

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