Assessing the relationship between flocculation ability of yeast and the presence of zymolectins throughout a lager fermentation

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BACKGROUND

- The yeast cell wall comprised mainly of mannoproteins (mannan and cell wall protein) in the outer layer which are essential for the flocculation mechanism.
  - Yeast mannoproteins in the outer layer of the cell wall are glycosylated polypeptides which are divided into long N- linked chains comprised of 10% protein and 90% carbohydrate, and short O- linked chains comprised of 50% protein and 50% carbohydrate[2,5].
- Flocculent yeast cells have FLO-genes that encode for zymolectins[8]. Zymolectins contain a N- terminal that have sugar recognition domains specifically for mannose molecules[9].
- As per the 'lectin-like' theory, yeast flocculation in a beer fermentation can be defined as the binding of zymolectins on flocculent cells to the mannose residues on the adjacent yeast cell wall, in the presence of cations such as calcium.
- Historically, it was believed that the onset of yeast flocculation was when most of wort sugars such as glucose (known to inhibit flocculation) had depleted[7].
- However, many instances have been recorded, where the fermentation is inadequate due to early flocculation or no flocculation[6]. Corrective actions such as applying shear, removal or sugars or changing charges were not effective.
- A lack of understanding the flocculation mechanism throughout the fermentation has led to misconceptions and lost opportunities.
- In this study, the flocculation of *S. pastorianus* was assessed for an ongoing fermentation using a to further understand the relationship between the flocculation ability of the yeast, zymolectins and mannose residues on the yeast cells.

OBJECTIVES AND HYPOTHESES

Method Development: Develop a modified to assess the flocculation ability of yeast throughout a fermentation process. A modified ASBC Yeast 11 method was developed that ensured all residual wort sugars were washed to prevent interference prior to the flocculation measurements.

- **Hypothesis**: The flocculation ability of the yeast changes over time and is independent of wort sugars

Zymolectin study: Assess the correlation between the flocculation ability of the yeast and the presence of zymolectins on yeast surface

- **Hypothesis**: The increase in flocculation ability of the yeast as the fermentation progressed was due to the increase in zymolectin concentrations on the cell surface

Mannose study: Investigate the correlation between the flocculation ability of the yeast and the mannose concentration in the yeast cells

- **Hypothesis**: There is a positive correlation between the flocculation ability of the yeast is dependent on the mannose residues in the yeast cells

EXPERIMENTAL PLAN

Multiple laboratory scale (200 mL) lager fermentations with *S. pastorianus* were carried out to assess changes in yeast flocculation characteristics at different stages in the fermentation process. Fermentation parameters such as total extract, pH, ethanol concentration were measured throughout each fermentation assay. Flocculation ability was measured using a modified ASBC Yeast-11 method (as shown in Figure 1) to observe changes in yeast flocculence throughout the fermentation for all assays. The assay was divided into top fraction (topmost 170 mL) and bottom fraction (bottommost 30 mL) and assessed separately for each assay. For the zymolectin study, a portion of the sample was mixed with Avidin-FITC fluorescent probes to measure the amount of bound fluoroprobes to the yeast cells using fluorescence spectrometry. A schematic diagram for the zymolectin quantification is illustrated in Figure 2. For the mannose study, a portion of the sample was analyzed to quantify the mannose concentrations on yeast cell. The yeast cells were mechanically ruptured to break open the cell wall, followed by acid hydrolysis to extract the mannose and enzymatically converted to d-glucose-6-phosphate which was quantified spectrophotometrically. A schematic diagram for mannose quantification is shown in Figure 3.

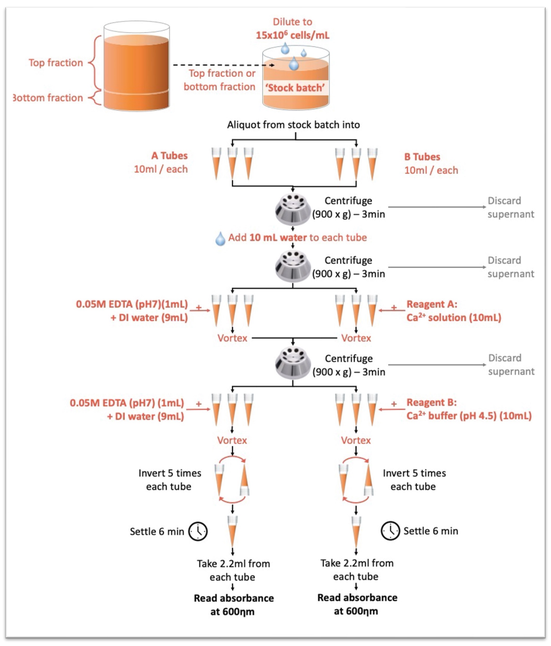

Figure 1. Modified ASBC Yeast-11 test to measure yeast flocculation.

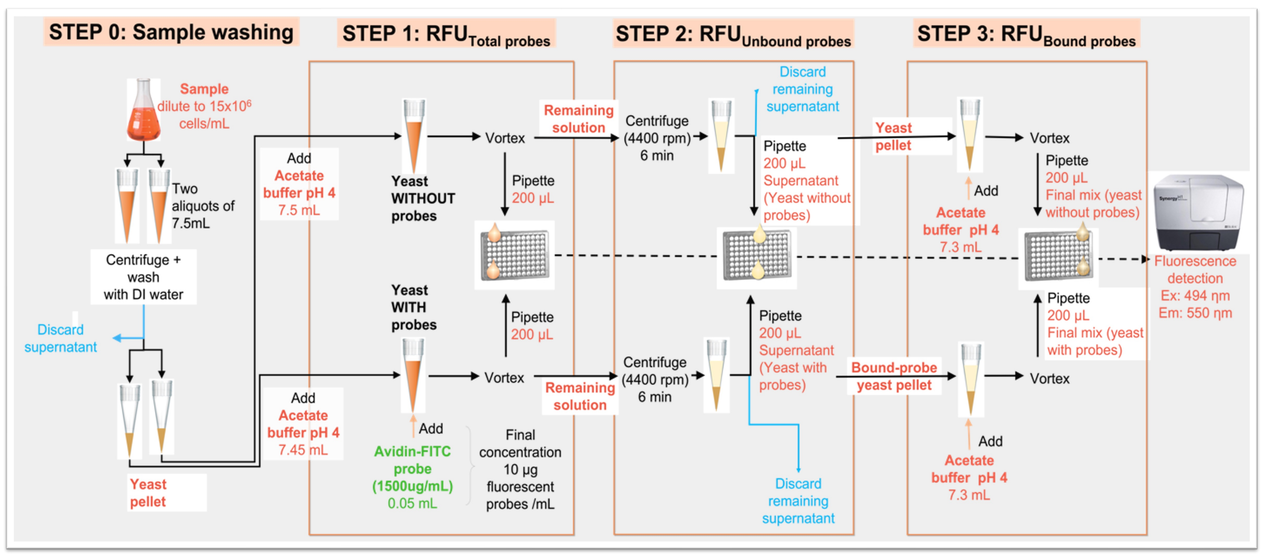

Figure 2. Schematic diagram of the method developed to assess the presence of zymolectins correlated to the bound Avidin-FITC fluorescent probes on yeast cells using fluorimetry

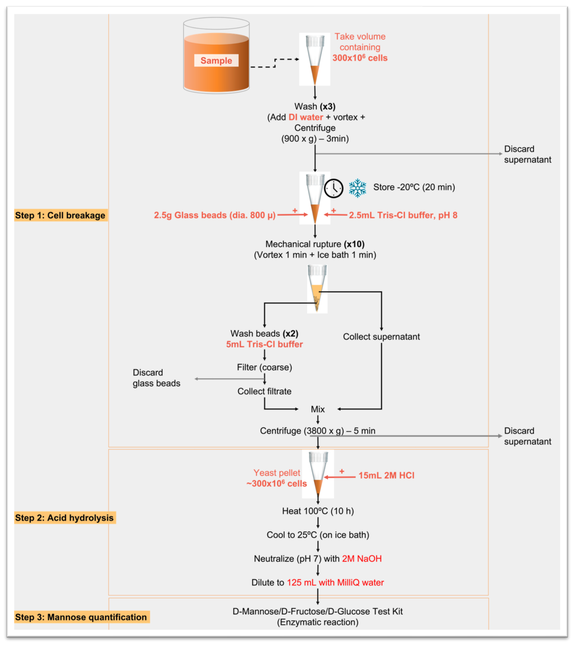

Figure 3. A schematic diagram for the mannose quantification on yeast cells using mechanical rupturing, acid hydrolysis and enzymatic reaction.

RESULTS AND DISCUSSION

- The total extract and ethanol concentration was fit using a single linear fit for all fermentations using the 4p-logistic model. All fermentations followed a similar trend as seen in Figure 4.
- Residual sugars were completely washed and did not interfere with the flocculation process, indicating the flocculation ability was measured independent of the sugars present in the wort.
- There was an initial drop in flocculation ability of the yeast at the beginning of a fermentation, followed by a gradual increase (Figure 5)

Zymolectin study

- Zymolectins were present on the yeast for the entire period of fermentation
- No correlation between flocculation ability and bound fluorescent probes was seen throughout the fermentation as seen in Figure 6. This disproved the zymolectin hypothesis

Mannose Study

- Mannose concentration followed similar trends to flocculation ability with time.
- A positive correlation was seen between mannose concentration and flocculation ability of yeast over time as seen in Figure 7.
- Yeast cells might not have sufficient mannose sites available for flocculation binding mechanism at the beginning of fermentation
- The results of this study complied with previous studies which showed that the yeast cell wall modifies in response to environmental conditions[4] and the carbohydrate linkages increase with progress in fermentations[1,3].

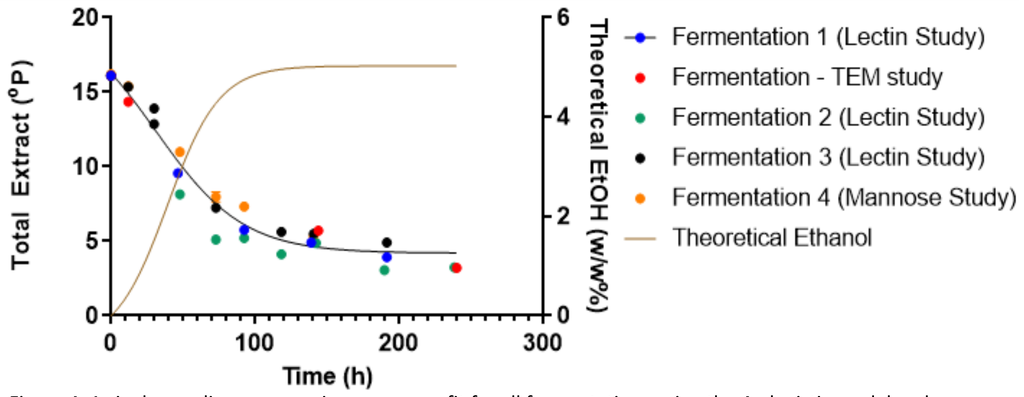

Figure 4. A single non-linear regression curve was fit for all fermentations using the 4p-logistic model and compared using the extra sum squares F-test ($p<0.05$).

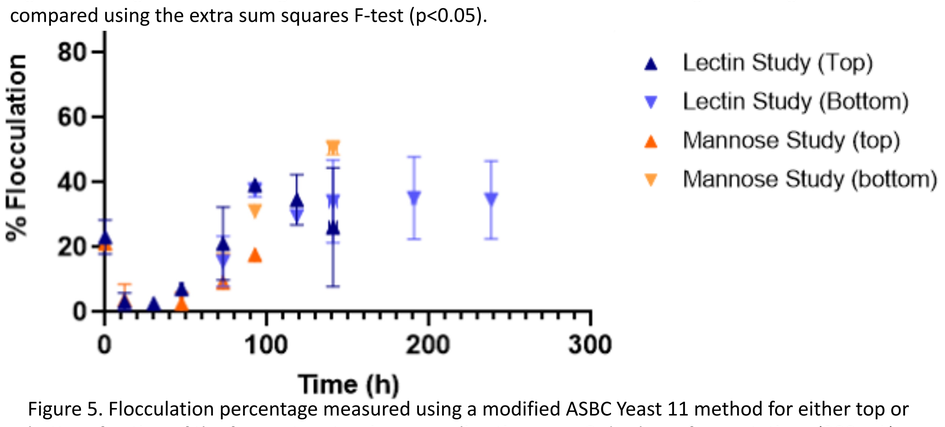

Figure 5. Flocculation percentage measured using a modified ASBC Yeast 11 method for either top or bottom fraction of the fermenter at a given sampling time over 8-day lager fermentations (200-mL).

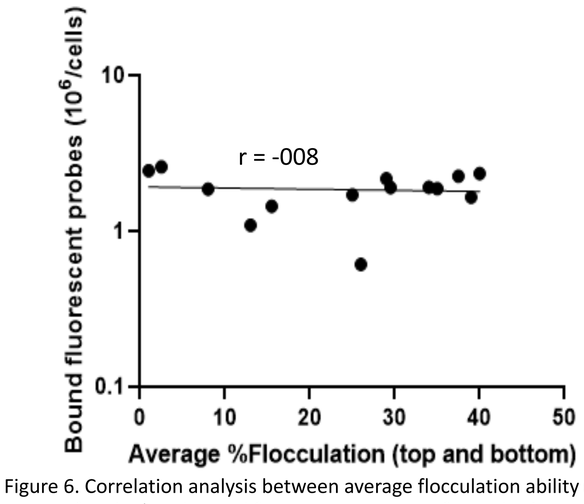

Figure 6. Correlation analysis between average flocculation ability (for both top and bottom fraction) and bound florescent probes performed using two-tailed Pearson's coefficient correlation (r=-0.08) (n=3).

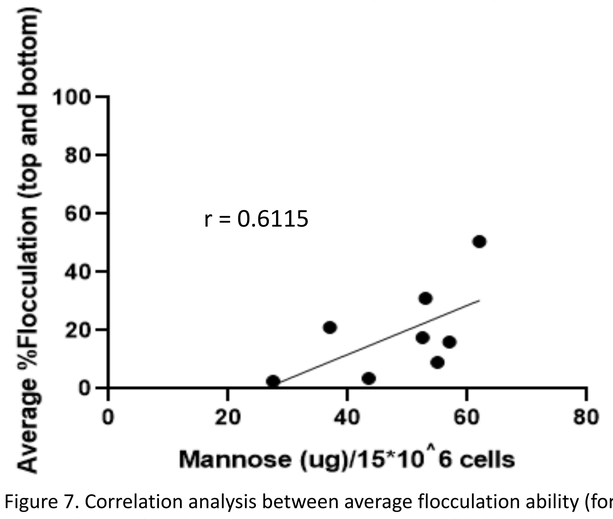

Figure 7. Correlation analysis between average flocculation ability (for both top and bottom fraction) and mannose concentration per 15E+06 cells performed using two-tailed Pearson's coefficient correlation (r=0.6115) (n=2).

CONCLUSION

- Flocculation ability of yeast changed throughout the fermentation process (lag, exponential & stationary phases)
- The loss of flocculation ability at the early stage of the fermentation process was not related to zymolectins as previously hypothesized
- Zymolectins were present throughout the fermentation process
- Increase in flocculation ability of yeast was correlated to increase in mannose concentrations (required for binding to zymolectins) as the fermentation progressed

FUTURE WORK

- Effect of different propagation regimes on flocculation mechanism of yeast
  - Time
  - Medium
- Understanding the causes of aberrant fermentations
- Mannose study in yeast species with different flocculation ability
- Fluorescent microscopy to study presence of zymolectins

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