Unfilterable Beer Haze Part II: Identifying Suspect Cell Wall Proteins

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ABSTRACT
The use of various diagnostic techniques has been previously utilized in the assessment of a commercially available India Pale Ale with cases of sporadically occurring unfilterable haze. The results from Part 1 suggested that β-glucans and proteins were the cause of the unfilterable haze and it was postulated that cell wall mannoproteins may also be a culprit of the unfilterable beer haze. In this follow-up study, proteins from high haze and low haze beer samples were precipitated and assessed using SDS-PAGE. Polyphenol interferences observed on the SDS-PAGE indicated that protein purification and targeted analysis was required. Proteins from high haze and low haze samples were fractionated and qualitatively identified via LC-MS. A library was built from FASTA sequences of targeted yeast proteins to qualitatively analyze the high haze and low haze samples. The protein fractionation was successful at purifying and isolating proteins from high and low haze samples. Two protein peaks were observed in the high haze sample, while one protein peak was observed in the low haze sample. The targeted LC/MS analysis discovered the presence of yeast cell wall mannoproteins and flocculation proteins, particularly Flo1 and Flo9. Understanding the source of these hazes can provide an opportunity for brewers to mitigate against their formation by adjusting brewing and yeast management practices.

Introduction

Beer is a complex matrix. Despite the increase in popularity of heavily turbid craft beer, clear, bright beer is still desirable in the majority of beers sold globally. Beer that is subject to the occurrence of sporadic, unfilterable haze represents a serious quality issue for breweries trying to meet technical specifications for various products.

Utilization of analytical instrumentation is a powerful tool available to brewing researchers to measure concentrations of various targeted analytes in the beer matrix. Common techniques include the following: gas chromatography mass/mass spectroscopy (GC/MS) to measure volatile aroma-active components, liquid chromatography (LC) to measure non-volatile beer components, and electron paramagnetic resonance (EPR) to assess reducing power in beer.

A UK brewery was experiencing repeated occurrences of a sporadic unfilterable haze, where particles were <0.45 µm in a core product. In Part 1 of this study, initial diagnostic investigations determined that cell wall mannoproteins, due to their small size, were a likely cause of this haze.

Several structural mannoproteins and flocculation proteins were selected for targeted analysis by LC/ESI-QTOF-MS analysis. Yeast cell walls are made up of a branched β-1,3-glucan and β-1,6-glucan network held together by hydrogen bonds. Glycosylated mannoproteins are linked to the cell wall β-1,3-glucan network. Two classes of glycosylated mannoproteins form the outer layer of the cell wall. First, glycosyl-phosphatidylinositol (GPI)-dependent mannoproteins are linked to β-1,3-glucan within the cell wall via β-1,6-glucan. Secondly, pir proteins (proteins with internal repeats) are directly linked to β-1,3-glucan by alkali-sensitive linkages. Expression of one GPI-mannoprotein regarding haze stabilization in beer has been studied and links between production of GPI-CWP, Cwp1, to cell wall stress have been found. A link between yeast cell wall proteins and increased beer turbidity has also been reported. However, a total fingerprint of excised cell wall mannoproteins present in high haze versus low haze beer is yet to be elucidated.

It was required to discover the source of the haze to determine what process adjustments and mitigations were
needed to reduce or prevent the occurrence of the sporadic haze for the product to be within set specifications.

**Experimental**

**Molecular determination of the origin of sporadic beer turbidity**

The contribution of yeast glucans and/or yeast cell wall proteins to observed sporadic haze was investigated utilizing intact protein precipitation, protein fractionation, LC/ESI-QTOF-MS analysis and a Megazyme® assay kit for D-mannose, D-fructose, and D-glucose (Megazyme Ltd, Bray, IRE).

**Intact protein precipitation**

Proteins were precipitated from a high haze sample (12.57 EBC) and a low haze sample (0.51 EBC) in triplicate using methods described by Pink et al.[8]

A 6 mL volume of sample was combined in a 50 mL centrifuge tube with 0.4 mL of ice-cold 50% (w/v) trichloroacetic acid (TCA) (Sigma Aldrich). Samples were vortexed and incubated on ice for 10 min after which, the tubes were centrifuged at 14,000 RCF for 5 min. The supernatant was carefully discarded, and the pellet was washed with ice-cold acetone. The tube was vortexed to mix, centrifuged at 14,000 RCF for 5 min, and the acetone-wash was repeated. After centrifugation and removal of acetone, the pellets were dried at 95°C on a heat block for approximately 5 min until the sample was dry.[8]

**SDS-PAGE analysis**

To determine the success of protein precipitation from the beer samples, an SDS PAGE gel analysis was performed. Samples were prepared from the air-dried pellets produced as described above. The pellets were resuspended in 50 µL of 0.5 M Tris-HCl, pH 6.8 buffer (Sigma Aldrich, Gillingham, GBR) and spun for 10 sec. A 50 µL volume of Laemmli sample buffer (Sigma Aldrich, Gillingham, GBR) was added to each tube and spun again for 5 min. The supernatant was cooled to room temperature in an ice bath, and spun again for 10 sec.

To separate proteins based on molecular weight, a precast 4–20% Bio-Rad Mini-PROTEAN Tris-Glycine (TGX) polyacrylamide gel was used with a Bio-Rad Mini-Protein Tetra Cell System for precast mini gels (Bio-Rad Laboratories, Herts, GBR). A 10x concentrated Tris-Glycine running buffer (Bio-Rad Laboratories, Herts, GBR) was diluted 10-fold with ultrapure water (Merck-Millipore, Livingston, GBR) before use. Pre-cast gel cassettes were rinsed with distilled water, placed in the buffer tank, and filled with Tris-Glycine running buffer. Each well of the cassette was washed with 20 µL of running buffer three times. The outer wells (1 and 12) were loaded with 5 µL of 2–250 kD Precision Plus Protein Dual Extra Standard protein ladder (Bio-Rad Laboratories, Herts, GBR) and the remaining wells were loaded with 20 µL of sample. The tank lid and appropriate electrodes were attached, and the sample was run at 120 V for 75 min.

Following electrophoresis, the gel was removed from the cassette frame and rinsed three times with distilled water. The gel was placed into a weigh boat and covered with Colloidal Coomassie Blue stain (5% (w/v) aluminum sulfate hydrate (14–18 degree of hydration), 10% (v/v) ethanol, 0.02% (w/v) Coomassie Brilliant Blue G-250 and 8% (v/v) orthophosphoric acid) (Thermo Fisher Scientific, Perth, GBR) and was incubated overnight at room temperature with gentle agitation.

After staining, the gel was removed from the incubator and rinsed four times with distilled water to remove stain residue. The gel was placed back into the weigh boat and enough destaining solution (10% ethanol and 2% phosphoric acid) (Thermo Fisher Scientific, Perth, GBR) was added to cover the gel. The gel was destained with gentle agitation for 2 h, after which it was rinsed with distilled water until all background stain was removed. Finally, the gel was placed onto the white-backed gel reading tablet and visually analyzed with a Bio-Rad GelDoc EZ imaging system (Bio-Rad Laboratories, Herts, GBR).

**Protein fractionation utilizing an ÄKTA avant liquid-chromatography system**

To concentrate and quantitatively determine differences between protein fractions, an ÄKTA Avant Liquid Chromatography system was used to perform ion exchange chromatography (GE-Healthcare, Chicago, U.S.A.). Cation exchange was chosen as the pH of the beer samples was around pH 4, meaning that the vast majority of proteins present would be below their isoelectric point 9 pI, and therefore they would be positively charged.

A 1 mL HiTrap SP Sepharose FF (GE-Healthcare, Chicago, U.S.A.) cation exchange column was used to purify the proteins in the high and low haze beer samples. Specifications for the Hi-Trap SP FF column are listed in Table 1.

**Chromatography conditions**

To separate any contained beer proteins, the column was first equilibrated at pH 4 (broadly equivalent to the pH of the beer samples) for 5 column volumes (CV) using 0.1 M citrate buffer. A total of 50 CV of beer were loaded onto the column. The column was then washed for 2 CV in 0.1 M citrate buffer before a step elution method using NaCl was utilized, comprising of an initial step to 1.5 M NaCl. The steps were chosen based on data from a 0–1.5 M NaCl gradient elution (data not shown). Chromatography parameters and a sample trace are shown below (Table 2 and Figure 1). Fractions were collected in 5 mL aliquots during

| Parameter | HiTrap SP FF |
|-----------|--------------|
| Matrix    | 6% highly cross-linked beaded agarose |
| Chromatography | Cation exchange |
| Loading capacity | High |
| Column volume | 1 mL |

Table 1. Properties of the Hi-Trap SP FF chromatography column used to purify and isolate beer proteins.
elution. Each fraction was collected in a 10 mL tube (BD Biosciences, Franklin Lakes, U.S.A.) for further analysis.

**Removal of salt by dialysis**

Dialysis was used to remove salt from the protein fractions collected from the ion exchange chromatography (ÄKTA Avant Liquid Chromatography system, GE-Healthcare, Chicago, U.S.A.). A dialysis buffer was made by preparing a 10 mM solution of NaOH (Sigma Aldrich, Gillingham, GBR) with 50 mM Tris (Sigma Aldrich, Gillingham, GBR) and fixed to a pH of 4.5 with a 1 M solution of citric acid (Sigma Aldrich, Gillingham, GBR) in distilled water. Dialysis tubing (Sigma Aldrich, Gillingham, GBR) was cut and rehydrated for 2 h in the dialysis buffer before sample was applied. Two-liter graduated cylinders, one for each sample, were filled with one liter of dialysis buffer. The tubing was folded, clipped at one end, and filled with sample. Bubbles were removed from each dialysis tube and the open end was clipped. Each fraction was dialyzed for 24 h at 4 °C with constant stirring.

After incubation, each dialysis tube was rinsed with distilled water and the content of each tube was decanted into a clean microcentrifuge tube (ThermoFisher Scientific) and snap-frozen with liquid nitrogen.

**Analysis of protein digests and fractions utilizing liquid chromatography-quadrupole time of flight-mass spectroscopy (LC-QTOF-MS)**

To resolubilize and denature the proteins, the precipitation method as described by Pink et al. was utilized by resuspending the air-dried pellets in 100 µl of urea (8 M) (Sigma-Aldrich) and incubated at room temperature for 2 h. The samples were then reduced by adding 5 µl of 1 M dithiothreitol (DTT) (Sigma-Aldrich) and incubated at room temperature for 30 min. A 500 µl volume of ammonium bicarbonate (50 mM) was added to each sample. Samples were alkylated by adding 30 µl of 0.5 M iodoacetamide (Sigma Aldrich, Gillingham, GBR) and incubated in the dark for 60 min. The alkylation reaction was then quenched by adding 15 µl of 1 M DTT. To each sample, 2 µl of trypsin (1 µg/µl) (Sigma Aldrich, Gillingham, GBR) was added, and the samples were vortexed for 30 sec before being transferred to a 37 °C heating block and incubated overnight.

The resulting peptide mixtures were purified using 100 µl C18 solid-phase tips (OMIX) (Agilent Technologies, Edinburgh, GBR) and desalted by washing with 0.1% formic acid (Sigma Aldrich). Peptide mixtures were eluted in 100 µl of a 60:40 (v/v) acetonitrile: water solution containing 0.1 M formic acid (Sigma Aldrich). The acetonitrile was then removed from the sample using a speed vacuum centrifuge. The sample was transferred to an amber glass vial (Agilent Technologies, Edinburgh, GBR) for analysis by LC-QTOF-MS.

The FASTA formatted sequences for proteins, specific to *Saccharomyces cerevisiae* were loaded into the coupled LC/
ESI-QTOF-MS software. By loading FASTA formatted sequences, the peptide fragments in samples could be matched to partial sequences of proteins suspected to be present in the samples (Table 3). Agilent Technologies Bioconform (Agilent Technologies, Edinburgh, GBR) software was used for post-run analysis to compare percent sequence coverage of proteins obtained from the trypptic digests in the high and low haze samples, respectively.

Samples were analyzed using an Agilent Technologies 1260 HPLC coupled to a 6530 qTOF mass spectrometer vial (Agilent Technologies, Edinburgh, GBR), in positive ion mode, with mobile phase A: water (0.1% formic acid) and B: acetonitrile (0.1% formic acid) (Table 4). The samples were separated using a Waters column (XSelect Peptide 100 Å, 2.5 µm, 4.6 × 100 mm) (Waters Corporation, Milford, U.S.A.) on a 45-min gradient (Table 5), at 0.5 mL/min flow rate. The same methods were applied to the dialyzed high and low haze fractionated proteins.

**Determination of D-mannose, D-fructose, and D-glucose**

A Megazyme® assay kit for D-mannose, D-fructose, and D-glucose (Megazyme Ltd.) was used to measure D-mannose, D-fructose, and D-glucose concentrations in the high and low haze beer samples. Suspensions, reagents, blanks, and samples were all prepared according to manufacturer instructions. The concentrations of the target molecules were calculated according to manufacturer instructions.

**Results**

**Molecular determination of the origin of sporadic beer haze**

**Protein purification and fractionation with ÄKTA avant liquid chromatography**

When assessing the chromatograms of the protein fractionation utilizing ion-exchange chromatography, a difference in protein content between the high and low haze beers was evident. Two distinct peaks were observed at 280 nm in the high haze sample while only one peak was observed in the low haze sample (Figures 2 and 3). Proteins in each sample were collected in fractions, desalted with dialysis, and utilized in LC-QTOF-MS to identify proteins present (or absent) in each sample.

**SDS-PAGE analysis of protein fractions**

The protein fractions from the high and low haze samples were analyzed via SDS-PAGE to assess differences in protein content and abundance. Faint bands were observed in the SDS-PAGE gel, indicating that proteins were in low abundance. The normal/low haze fractions (wells 1 and 2) were not substantially different from the high haze samples (Figure 4). When the exposure of the image was increased, very faint bands were observed in the high haze sample at approximately 25 kDa (Figure 5). The eluted fraction in wells

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**Table 3.** Selected mannoproteins and flocculation proteins assessed in LC-QTOF-MS.

| Protein | Cellular function |
|---------|------------------|
| **Cell wall mannoproteins** | |
| Utb1 | Involved in aging, oxidative stress response, and regulation of mitochondrial biogenesis<sup>[9]</sup> | |
| Anchored to the cell by disulphide bridge<sup>[9]</sup> | |
| Involved in remodeling of cell wall during culture development and stress/degradation responses<sup>[9]</sup> | |
| **Sim1** | Cell wall remodeling during culture development<sup>[9],[10]</sup> |
| **Hfp1p** | Haze protective mannoprotein<sup>[12]</sup> |
| **Ecm33** | Required for cell wall integrity and assembly of the mannoprotein outer layer of the cell wall<sup>[9]</sup> |
| **Cwp1** | Linked to ß-1,3 and ß-1,6-glucan through a phosphodiester bond<sup>[9]</sup> |
| **Cis3** | Does not require GPl anchor<sup>[9]</sup> |

**Flocculation proteins**

| Flo1 | Flocculation inhibited by mannose residues<sup>[13]</sup> |
| Flo5 | Loss of gene FLO5, requires propagation culture to be replaced<sup>[11]</sup> |
| Flo9 | Small flocculation protein<sup>[13]</sup> |
| Flo10 | Small flocculation protein<sup>[13]</sup> |
| Flo11 | Involved in filamentous, chain formation growth and flocculation upon flocculation<sup>[13]</sup> |

**Table 4.** LC-QTOF-MS instrument parameters.

| HPLC conditions |
|-----------------|
| Column | Waters Xselect peptide 100 Å, 2.5 µm, 4.6 × 100 mm |
| Mobile phase A | LC-MS grade water (0.1 % formic acid) |
| Mobile phase B | LC-MS grade ACN (0.1 % formic acid) |
| Flow rate | 0.5 |
| Column temp | 35 °C |
| UV scan | 214 nm |
| Injection volume | 10 µL |
| Total run time | 40 min |

**Mass spec conditions**

| Ionization mode | POS |
|-----------------|-----|
| Gas temp | 300 °C |
| Gas flow | 41 l/min |
| Nebulizer | 35 psig |
| Sheath gas temp | 350 °C |
| Sheath gas flow | 10 l/min |
| Capillary voltage | 4000 V |
| Nozzle voltage | 500 V |
| Wash | First 2 min to waste as wash |
| Reference mass | 922.0481 |
| Fragmentor | 150 V |
| Skimmer | 65 V |

**Table 5.** Gradient composition throughout LC-QTOF-MS run.

| Time (min) | %A (H2O: 0.1% formic acid) | %B (Acetonitrile: 0.1% formic acid) |
|------------|-----------------------------|------------------------------------|
| 2.000      | 97                          | 3.0                               |
| 7.960      | 97                          | 3.0                               |
| 27.00      | 85                          | 15                                |
| 27.15      | 64                          | 36                                |
| 29.71      | 40                          | 60                                |
| 32.00      | 5.0                         | 95                                |
| 34.00      | 5.0                         | 95                                |
| 35.00      | 97                          | 3.0                               |
| 30.00      | 97                          | 3.0                               |
7 and 8 (6B6) only showed a faint band, observed at approximately 37 kDa. However, these bands were observed in all samples (Figure 4). Due to the faint bands in the high haze samples and the low concentration of proteins in the samples, LC/ESI-QTOF-MS was utilized to identify proteins and protein differences in the high and low/normal haze samples.

**Liquid chromatography-quadrupole time of flight-mass spectroscopy (LC-QTOF-MS)**

High and low haze protein fractions collected from the ÄKTA Avant Liquid Chromatography system (GE Healthcare, Chicago, U.S.A.) in addition to high and low haze intact protein precipitations were analyzed using LC/ESI-QTOF-MS.
As mannoproteins had previously been speculated to be unfilterable, structural mannoproteins and flocculation proteins specific to *Saccharomyces cerevisiae* were selected for targeted analysis based on the literature (Table 3). [1]

The data presented in Tables 6 and 7, demonstrates that there was a greater percent sequence coverage of mannoproteins in the high haze samples compared to the low haze samples. However, greater differences were observed in sequence coverage of the flocculation proteins between high and low/normal haze samples. Proteins Ecm33 and Uth1 contain relatively high sequence coverage for spectral data at 76.92% and 38.02% sequence coverage, respectively (Table 6). Despite the low sequence coverage, protein Flo9 was observed in all high haze samples with an average sequence coverage of 5.3% and was absent in the low haze samples analyzed (Table 7). The difference of protein Flo1 (>100%) between the high and low haze samples may have been an indication of the release of flocculation proteins related to a cell wall stress response (Table 7). In general, the high haze samples contained elevated cell wall protein concentrations when compared to the low haze samples (Tables 6 and 7).

**Determination of D-mannose, D-fructose, and D-glucose**

The D-glucose concentration found in the high haze and low haze samples were comparably close in values, with an average difference of 0.02 g/L (Table 8). However, D-fructose and D-mannose concentrations in the high haze beer samples were 1.22 g/L, nearly three times the concentration of 0.54 g/L noted in the low haze samples (Table 8). As the samples were the same color, color interference did not influence the assay. The average concentration of D-mannose in the high haze sample was significantly (\(p < 0.05\)) different from the concentration of D-mannose in the low haze sample (Table 8).

**Discussion**

While the impacts of haze-active proteins derived from barley have been extensively studied,[14–17] few definitive links between yeast protein influence on increased turbidity have been made.[1,5,7,12]
Table 6. Percent sequence coverage of cell wall mannoproteins in high and low haze samples obtained from LC-QTOF-MS analysis.

| Protein | Accession number (UniProt) | High haze (% coverage) | Low haze (% coverage) | % difference |
|---------|----------------------------|------------------------|-----------------------|--------------|
| Uth1    | B3LRC                      | 38.02                  | 26.52                 | 43.4         |
| Sim1    | P40472                     | 18.07                  | 6.93                  | 160.8        |
| Hpf1p   | A0A0L8IV9                  | 10.29                  | 8.48                  | 21.3         |
| Ecm33   | P38248                     | 76.92                  | 35.9                  | 114.3        |
| Cwp1    | YKL09                      | 13.81                  | 18.41                 | 25.0         |
| Cis3    | BSL27                      | 21.59                  | 14.54                 | 48.5         |

Table 7. Percent sequence coverage of targeted flocculation proteins in high and low haze samples obtained from LC-QTOF-MS analysis.

| Protein | Accession number (UniProt) | High haze (% coverage) | Low haze (% coverage) | % difference |
|---------|----------------------------|------------------------|-----------------------|--------------|
| Flo1    | P32768                     | 13.6                   | 0.91                  | 1394.5       |
| Flo5    | P38894                     | 9.21                   | 2.6                   | 254.2        |
| Flo9    | P39712                     | 5.3                    | absent                | 100          |
| Flo10   | P36170                     | 9.5                    | 5.05                  | 88.1         |
| Flo11   | P08640                     | 4.97                   | 4.39                  | 13.2         |

Table 8. Calculated concentrations of D-glucose, fructose, and mannose concentration in low and high haze beer samples. Samples were run in triplicate and the average value and standard deviations are reported.

| Carbohydrate | Low haze (g/L) | S.D. | High haze (g/L) | S.D. |
|--------------|----------------|------|-----------------|------|
| D-Glucose    | 0.43           | ±0.00| 0.45            | ±0.00|
| D-Fructose   | 0.54           | ±0.00| 1.22            | ±0.01|
| D-Mannose    | 0.54           | ±0.00| 1.22            | ±0.01|

Yeast flocculation requires the presence of flocculins. These are zymolectin proteins that extend outside of the cell wall to flocculent yeast cells to selectively bind mannose residues on neighboring yeast cells. The presence of mannose inhibits yeast flocculation as excess mannose in the growth medium binds to the flocculin receptors extending from the yeast cell. It is important to mention that yeast flocculation in the majority of ale brewing strains is inhibited by mannose, glucose, sucrose, and maltose. This flocculation characteristic is described as a NewFlo phenotype. NewFlo phenotypes are also sensitive to environmental factors such as pH, temperature, and nutrient content.

Osmotic shock and shear cause the excision of yeast cell wall fimbrine, therefore, it was speculated that stress (be it chemical or physical) during storage, propagation, or fermentation conditions caused the Saccharomyces cerevisiae strain utilized to excise structural mannoproteins. Previously, it has been found that deletions of these proteins show a much less electron-dense outer cell wall. Additionally, mannan alone does not impair filterability as the hazes observed were unfilterable.

Following guidelines for proteomic data interpretation, the reported proteins were observed with 95% confidence that the protein was present in the sample with the given percent sequence coverage. Protein Sim1, which is required for remodeling the cell wall during culture development, was observed at approximately 18% sequence coverage in the high haze sample. This yeast-derived protein has been recovered in other proteomics studies and observed at a much lower low percent coverage in lager beers (9.1%).

Although this study cannot quantify protein concentrations, it can be speculated that the protein is present in relatively high abundance in the sample with a robust sequence coverage and 160% increase in coverage compared to the low haze sample (Table 6).

Protein Ecm33 was another structural cell wall protein with high sequence coverage. This mannoprotein is required for cell wall integrity and for the assembly of the outer mannoprotein layer of the cell wall (Table 6). In lager beers, a percent sequence coverage of 5.1% has previously been reported. In this study, high haze samples contained a 76.92% sequence coverage and a low haze percent sequence coverage of 35.9%, leading to 114% overall percent difference between the two (Table 6). As previously stated, absolute concentrations cannot be determined but it can be speculated that mannoproteins in the high haze samples were present in greater abundance than in the low haze samples.

The flocculation proteins reported large percent differences between the high and low haze samples. Flocculation protein 9 (Flo9) was of interest as the protein was absent in the low haze samples but present in the high haze samples. It is suspected that the GPI-anchored protein was cleaved off by β-1,6-glucanase but further research is required to prove this hypothesis. According to the zymolec tin flocculation theory, lectins extend from the cell wall, waiting for calcium ions to enable an active conformation for the zymolectins to bind with mannose residues on neighboring cells. As flocculation proteins/mannoproteins are located at the yeast cell surface, it is hypothesized that flocculation proteins are excised from the cell wall under stressed storage conditions, shearing by mechanical agitation, or by stressed propagation/storage conditions. This would increase turbidity in the propagation medium and in the beer produced. The data and literature support this current theory; however, confirmatory studies are required.

Finally, the results of the D-mannose, D-glucose, and D-fructose assay further confirmed the proposed hypothesis that cell wall mannoproteins contributed to increased turbidity. The mannose concentrations in the high haze samples were different at nearly three times the concentration when compared to the low haze samples. Overall, it can be speculated that yeast cell walls were altered due to stress or damage during the brewing process. The alteration of the yeast cell wall caused an increase in turbidity that was unfilterable as other factors of turbidity could be easily removed by separation aids.

**Conclusion**

The purpose of this study was to identify the ‘culprit’ of unfilterable turbidity in the production of an India Pale Ale. A multitude of factors in brewing, fermentation, conditioning, and packaging processes can affect the final turbidity of beer.
Diagnostic studies were utilized as a process of elimination to rule out the predominant causes of beer haze (Part 1). As the haze particles were unfilterable, it was evident that the particulates were small in size (<0.45 μm). Following enzymatic digestion and wet-chemical analysis, protein precipitation, protein fractionation, LC-QTOF-MS, and D-mannose analysis, it is suggested that the observed unfilterable haze was due to the presence of yeast cell wall mannoproteins.

It is important to note that during this study, changes to the yeast management plan in the brewery were implemented in the later months of sample collection. It was during this time that the turbidity levels of the high haze samples was reduced, and fewer batches were flagged as 'high' turbidity batches. This further proves the hypothesis and reinforces the importance of proper yeast management in a brewery. Appropriate propagation, storage, and handling conditions are essential to maintaining a healthy yeast strain. If brewing yeast are kept healthy, this should not affect the turbidity of the final product.

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