

Importance of beer stabilization - Most brewers spend a lot of time and effort to produce beer that is bright and clear and remains so until it reaches the consumer. A lot of factors and process steps are to be managed before a beer can leave the brewery completely bright and stable. Beer brewing is a biological process involving raw materials with variable quality impacted on by the weather and season, but also dictated by the yeast strain and process.

Many causes of beer haze and product instability - Haze is in fact light scattering by compounds or even particulates that arise in the final product before, or even after packaging. Many substances that origin from malt, other cereals, hops or other ingredients can lead to haze, such as the (haze sensitive) proteins, polysaccharides including beta-glucans, arabinoxylans, starch, as well as polyphenols derived from both malt or hops. Also substances released by <u>yeast</u> in sometimes stressful process conditions may contribute.

Beer diagnostics - Various analytical techniques such as, but not limited to, microscopy and spectrometry, can be applied to diagnose the nature of the haze in 'defect' beer and propose steps to resolve issues.

Visual and instrumental haze determination

Beers are examined by looking though the bottles at a light source, or placing them into a black box with light coming from below. The light from below often exaggerates the haze perception.

Haze - Testing methods

- Haze at 20°C
- Chill haze: additional haze formed after 24h at o°C Forcing test: overnight at o°C, then 6 days at 60°C
- followed by 24 hours at o^oC Cycle test: overnight at o°C, then 2 days at 60°C followed by 24 hours at o^oC and repeat
- Real time storage test; Store at 20°C and measure monthly at 20'C and 0°C

Analytical assays

- Haze sensitive proteins; haze measurement after addition of tannic acid
- Haze sensitive polyphenols; haze measurement after addition of vinylpyrolidone
- Beta glucans EBC method
- Starch: lodine test

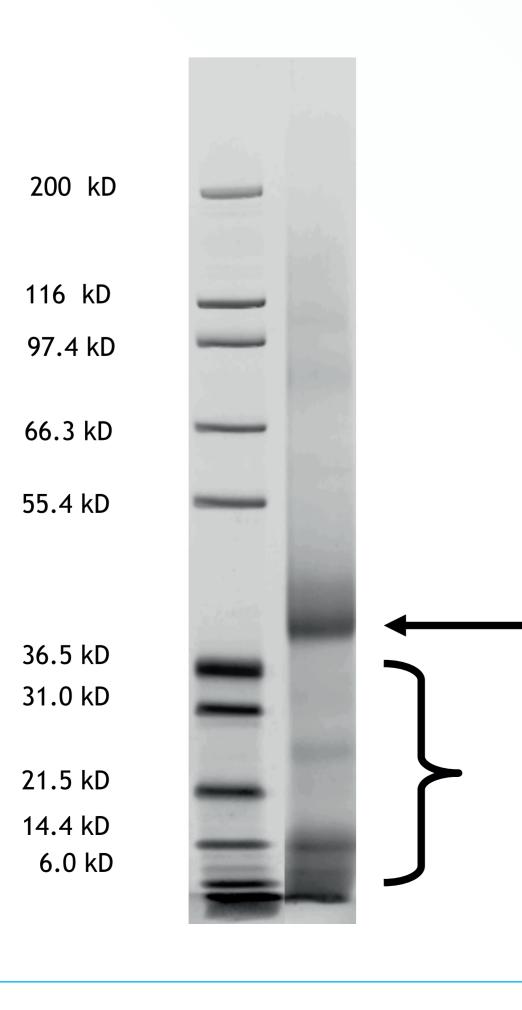


Haze terminology scale from Analytica EBC Measurement at 90° scatter angle: Brilliant Very clear Very slightly Hazy Slightly Hazy Hazy Very Hazy

SDS-PAGE gel electrophoresis – Proteins

sample.

In beer, several barley proteins form the majority, but also yeast proteins can be observed an indicative of issues.



WORLD BREWING CONGRESS 2016 Beer Haze Stabilization Made Simpler

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This separation method can give semi-quantitative indications of the most abundant proteins in the

Protein Z or Serpin Z molecular weight 40 kD. also called 40 kD protein

Other proteins, mainly hordein fragments

Technique

Macroscopic: visual

Turbidity - haze

Light Microscopy + staining

HPLC - Chromatography

NMR / FTIR spectroscopy

Protein gel-electrophoresis

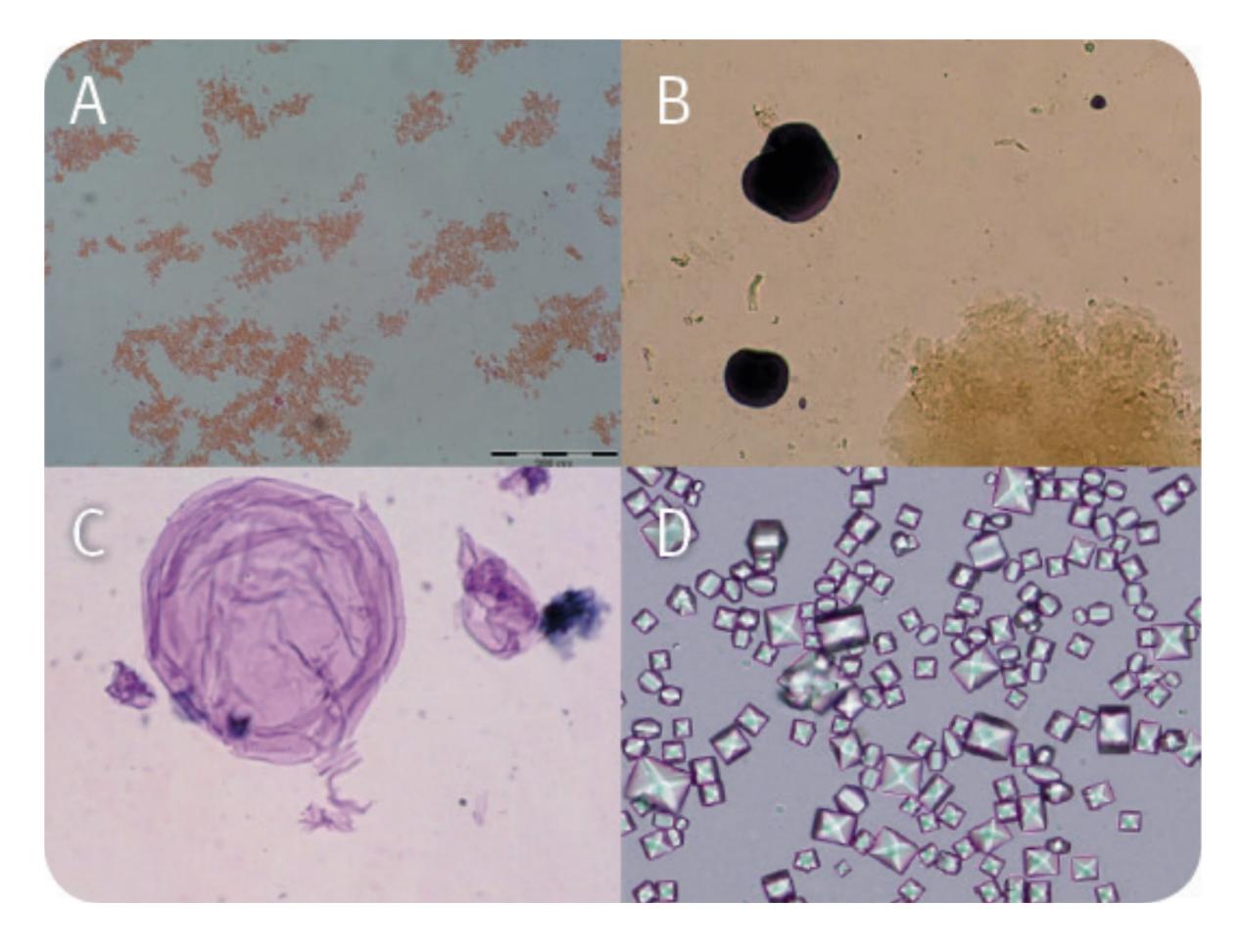
Proteomics, LC-MSMS

Light Microscopy + staining for specific compounds

Deposit or precipitate isolated for beer, egg. by centrifugation, can be looked at by light microscopy. A non quantitative detection of particular compounds can be done with specific staining agents

Stain	Color	Target component
Eosin Y	Pink	Proteins
Thionin	Purple	Neutral polysaccharides
Thionin	Pink	Acid polysaccharides
Congo Red	Orange-red	Beta-glucan
lodine	Red-brown	Erythrodextrin
lodine	Blue-black	Starch

Compounds detected	
Color, particles, haze	
(Chill) Haze	
Proteins, polysaccharides, filter aids,	
Oligo sugars	
Various	
Proteins, by size	
Proteins, identification	



Examples of beer deposits A. protein with eosin B: Starch with iodine C. 'skins' or ghost bubbles D. oxalate crystals



HPLC or HPSEC Chromatography Sugar spectrum analysis of wort or beer Oligosaccharides can be identified and quantified based on standards. Most occurring are glucose and maltose. Whenever mannose is observed in haze precipitate, this can only origin from yeast, not malt, and provides indications of yeast-derived haze substances

Proteomics – LC MSMS

Characteristic accurate mass determination of protein fragments can identify proteins by matching to reference database of yeast and barley proteins or even the entire genome

NMR / FTIR – unbiased identification

Characteristic resonance can be indicative for a particular substance, with a wide range of compounds detected, including proteins, sugars, acids, and also inorganic compounds, e.g.. silica or even silicones

Summary – 'Beer diagnostics'

A lot of factors and process steps are to be managed before a beer can leave the brewery completely bright and stable. Sometimes, the beer is hazy, unstable and not meeting the expectations.

We show many techniques that can be applied to diagnose the nature of the haze and may help to define and correct the cause of the issue in the brewery or choice of raw materials.

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