Beer Turbidity: Reasons, Analytics and Avoidance

Craft Brewers Conference Portland, Oregon

Beer Turbidity

- Beer Turbidity is a visual perception!
- Depending on the intensity it is visible to the human eye
- Danger: the consumer might get a negative impression

• Basic turbidity physics:

Light is deflected by particles and is scattered when passing through beer

Analytical evaluation: Nephelometry

The turbidity measurement is carried out with Nephelometers.

The requirements for Nephelometers are stated in the MEBAK Vol. II 2.15.1.2:

- Wavelength: 630 [nm] ±30 [nm]
- Measuring angles compared to the light axis:
 - 90° side scattering
 - 11° / 25° forward scattering
- Principle: Reflexion
- Units: Nephelometric Turbidity Unit [NTU]
 - Turbidity Unit / Formazine [TU/F]
 - [EBC]

Measurement angles

90° \rightarrow mainly (very) small particles

- Proteins
- Carbohydrates

11° / 25° \rightarrow mainly (large) particles

- Yeast cells
- Kieselguhr particles
- PVPP
- Inorganic material (e.g. CaOx-crystals)

Light Scattering



Causes for haze formation

Many substances are "blamed" with causing or inducing haze, e.g.:

- **Raw materials** (malt + adjuncts, yeast, water)
- Process aids
 - DE
 - Stabilization aids
- Microorganisms

Possible causes (I)

Malt: α-glucanes

- Milling
 - Wrong adjustment of the mill gap
 - Too high moisture of the kernels regarding wet milling
- Enzyme equipment of malt
- Insufficient enzymatic degradation
 - Mashing program
 - Acidification
- Mash-off temperature
- Sparging water temperatures

Malt: β-glucanes

Malt modification (Cytolysis)

Possible causes (II)

Malt: Arabinoxylans

- Arabinoxylans are suspected for inducing haze
- Ongoing research (Prof. Rath)

Malt: Proteins

- Certain protein fractions of malt → not the foam enhancing ones (30kDa ≥MW ≤ 50 kDa)
- Proteins forming particles during beer aging: < 30kDa
- Proteins forming colloidal haze: > 50 kDa

Malt: Oxalic acid

Dependent on the year / harvest

Possible causes (III)

Water: Residual Alkalinity

- Influences the pH during mashing
 - \rightarrow enzyme activity

Water: Content of Ca-ions

- React with oxalic acid to form Calcium-Oxalate which may precipitate in crystalline form
- Aim: total precipitation prior to filtration
- Brewing liquor and blending water (HG)!

Possible causes (IV)

Yeast: propagation yeast

- Smaller cells compared to yeast after several generations
- Slower sedimentation speed in CCV
- Bad clarification \rightarrow small proteins may not be removed by filtration

Yeast: Shear forces

 Due to very high shear forces it was reported that mannan, proteins and glucane may be removed from the yeast cell wall resulting or at least involved in haze formation

(Siebert et al., Technical Quarterly 24 (1987), p. 1-8)

Yeast: Re-use of beer recovered from yeast slurry

- Yeast releases proteins into the beer after a certain time period under unfavourable conditions (e.g. high temperatures)
- These proteins often do not react with silica gels
- May become even worse after flash pasteurization

Possible causes (V)

Yeast: Stress conditions

- Stress conditions:
 - high fermentation temperatures
 - Temperature shock
 - High sugar concentrations \rightarrow high gravity brewing
 - Acidification
- Excretion of Glycogen \rightarrow formation of "invisible haze"
- Extent of excretion is yeast strain dependent
- Cannot be filtered out
- Molecular structure of Glygocen is very similar to Amylopectin
- High molecular molecules, long chained, strongly branched

Main aspects of efficient yeast management regarding turbidity formation

Essentials:

- enough nutrients
- early cropping
- cold storage
- short storage times
- avoid stress conditions
- no contamination
- regular check of viability (> 95 %)
- Regular check of vitality (ICP value, short fermentation test)

Protein-Polyphenol haze

- Typical protein-polyphenol haze formation is influenced by:
 - Content and composition of proteins and polyphenols
 - Ethanol content
 - pH value
 - content of metal ions (filtration with DE!)
 - DO content (dissolved oxygen)

Haze types

Chill haze

- Proteins and polyphenols
- created when polyphenols polymerize and interact with the protein in the beer
- appears when beer is chilled to approx. 0°C
- returns to solution when beer is warmed to 20°C
- particles range in size from 0,1 μm to 5.0 μm
- Permanent haze
 - Other proteins and polyphenols involved compared to chill haze
 - Does not dissolve
 - particles range in size from 1 μ m to 10 μ m



Enzyme treatment

- Best solution to obtain a first indication
- Treatment with various enzymes
- Variation of incubation temperature and enzyme dosage possible

Example for enzyme treatment

Turbidity development (90°) after addition of enzymes



Carbohydrate haze

- Mostly caused by deposits / particles in beer
- Frequent reason: <u>α-Glucanes</u>
 - \rightarrow Problem in the brewhouse
 - Process (Mashing, Lautering \rightarrow turbidity!)
 - Raw materials
 - \rightarrow Problem with yeast management
 - Excretion of Glycogen
 - Autolysis

Methods for particle examination / identification

- Separation of the deposits from the product (if possible)
 - Centrifugation
 - Membrane filtration
- Microscopic analysis
 - Transmitted light
 - Polarization
 - Fluorescence

Staining

- Staining with Diachromes
 - stain an object by absorbing some of the wavelengths of used light and reflect or transmitting others
 - e.g. Methylene Blue
- Staining with Fluorochromes
 - Stain an object by their fluoreszence
 - They absorb light of a characteristic wavelength and at the same time re-radiate it at a lower wavelength
 - e.g. 1,8-ANS (1-anilino-8-naphthalene sulphonic acid Mg)
- Staining solutions
 - with an affinity for proteinaceous particles
 - with an affinity for carbohydrate material
 - for adsorbents

Proteinaceous Particles and yeast cells



Proteinaceous Particles



Proteinaceous "Blurr"



BER

Carbohydrate Particle



Carbohydrate Particles (I)



Carbohydrate Particles (II)



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Starch



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Calcium-Oxalate crystalls



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Chemical-technical Analysis

- Requirement for quantitative statement: particle isolation possible!
- Many methods stated in literature, e.g.:
 - Determination of total protein content
 - Determination of total glucose content after hydrolysis
 - Photometric Iodine test
 - Modification possible by determining the extinction of the sample over the entire visual spectrum from 400-700 nm ((Hartmann, K., Diss. TU München (2006))
 - Differentiation between Amylose, Amylopectin and Glycogen
 possible
 - Gel permeation chromatography (as a preparation step)
 - Molecule separation according to their size

Foreign particles in beverages

- Only by accident
- Powerful countermeasures:
 - Rinsing of containers
 - Empty bottle inspection
 - Process control in packaging
 - others

Methods for foreign particle identification - I

Upon isolation of the haze by membrane filtration or centrifugation, different techniques can be used for the analysis of **foreign microparticles in complex fluids**:

➢ <u>Microscopy</u>:

>Bright-field illumination

absorbance of light in the sample.



Fig. 1: Glass-chip, bright-field.



Fig. 2: Glass chip, dark-field.

➤Dark-field illumination

contrast from the scattering of light by the sample.

Examples of applications:

- glass particles
- calcium oxalate crystals

Methods for foreign particle identification - II

-Polarized light

crystalline objects are able to rotate polarized light appearing shiny on a black background.



Fig. 1: Amorphous form of calcium oxalate, polarized light (source: Beer Deposits).

Examples of applications:

- calcium oxalate crystals
- cellulose fibers

-Fluorescence

some particles present autofluorescence others need to be



Fig. 2: Lining material, autofluorescence (source: Beer Deposits).



Fig. 3: Plastic fiber, stained with Nile Red.



Scence .

- laquer flakes
- lining material
- oily droplets
- plastic material

Nile Red.

-Scanning Electron Microscopy (SEM)

The sample is scanned by a beam of electrons that interact with the atoms of the sample, producing signals that are detected and can give two types of information:

- Topography of the surface

Through low-energy <u>secondary electrons</u> (emitted from close to the surface), a high resolution 3-Dimensional image of the surface of the object is generated, since irregularities on the surface produce shades.



Fig. 1: Surface of a membrane filter with residues of organic material.



Composition

By high-energy <u>back-scattering electrons</u> (BSE), it is possible to detect different chemical compositions, since heavy elements (high atomic number) backscatter electrons more strongly than light elements (low atomic number) and therefore appear brighter.



Methods for foreign particle identification - I

Energy-dispersive X-ray spectroscopy (EDX):

By the use of X-rays, the electrons in the atomic structure of a sample are excited and produce a characteristic set of peaks in the spectrum. Since each element has a different atomic structure, it is possible to determine the **elemental composition** of the analyzed specimen.



Case Study No. I Glass Corrosion

Analyzed sample: Beer, type: bottom-ferm. lager

Order: Identification of turbidity related/ turbidity causing particles

Results:

1. Macroscopic observation:

shiny needle like particles in suspension.

3. Microscopic observation:

layers of transparent plannar-shaped and rolled up material.



see next slide...

Case Study No. I Glass Corrosion

Results (cont.):

4. SEM image:

inside wall of the bottle, glass fragment.



SEM MAG: 796 x Det: SE Detector L . SEM HV: 7.00 kV PC: 6



5. EDX spectrum: isolated transparent particle.



Conclusion:

The shiny particles observed with naked eye proved to be glass (SiO2), that peeled off the inner wall of the bottle and came into contact with the product. The blue line is the spectrum of the microscope slide, which matches the spectrum obtained for the analyzed particle.



НR

Case Study No. I Glass Corrosion

Background Information:

- Glass corrosion is the attack on the glass surface by severe **atmospheric weathering**.
- In this process, alkali ions (calcium and sodium) are leached from the surface by interactions of the glass surface with humidity and carbon dioxide in the environment, originating carbonate crystals.¹
- This results in the degradation of the inner glass surface which peels away and diffuses glass flakes from the bulk glass into the product.
- Promoted by: high temperatures, high air humidity, low pH-value in the atmosphere (CO₂, acidic rain).²
- These crystals will typically occur as discrete particles that scatter light and give a hazy appearance.³

1 Freudenberger J. et al.(2009) Materials Science and Engineering. Springer Handbook of Mechanical Engineering. 73-222.

2 Papadopoulos N. et al. (2012) Influence of weather conditionson glass properties. Papadopoulos, C.A. Drosou. Journal of the University of Chemical Technology and Metallurgy, 47, 4, 429-439. 3 Gentaz L., et al. (2012) Impact of neocrystallisations on the SiO2-K2O-CaO glass degradation due to atmospheric dry depositions. Atmospheric Environment 55 459-466.

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