All About Proteins

or

How to Consistently Get Good Head Without a Lot of Hassle

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Beer Foam Physics

Four key processes:

- Bubble formation – smaller bubbles last longer
  - Dispensing, nucleation, low beer surface tension, gravity
- Drainage – dry bubbles tend to collapse
  - Higher surface viscosity (foam positive)
  - Bulk viscosity has no effect (ex. higher beta glucan content)
  - Higher alcohol (foam negative)
- Coalescence – bubble coarsening via rupture
  - good surface viscosity & elasticity properties
  - hydrophobic interactions (foam positive)
  - lipids and detergents (foam negative)
- Disproportionation – bubble coarsening via gas diffusion
  - gas composition (N2 can’t diffuse as well as CO2)

Beer Foam Physics

- Stable foam with polyhedric, honeycomb-like structure of well-drained foam
  - Source: Prins and van Marle 1999.

- The faster the foam drains, the thinner the bubble walls become, and the faster the bubbles coalesce.
Barley Does Not Want To Make Beer

- It wants to be pollinated. (Don’t we all)
- In order to be pollinated, it needs to grow from a seed into a plant.
- The seed is equipped with a protein carbohydrate matrix called the endosperm, and enzymes (proteases) that will break that endosperm down into useful growth building blocks called amino acids.
- The sole purpose of malt proteases is to make large proteins into amino acids.
The Nature of Proteins

From Wikipedia:

- An amino acid is a molecule containing both amine and carboxyl functional groups.
- A protein (aka. Polypeptide) is a polymeric chain of amino acids joined together by peptide bonds between the amino and carboxyl groups of adjacent amino acids.
- A peptide bond is a chemical bond formed between two molecules when the carboxyl group of one molecule reacts with the amine group of the other molecule, thereby releasing a water molecule.
Players

- A peptide is a short chain of amino acids
- A polypeptide is a long chain of amino acids
- A “Protein” is a stable polypeptide that has a defined structure and nature.
- Lipids are organic building blocks including fats, waxes, oils, gums, fatty acids, glycerides, sterols, and sterol esters.
Building Foam Structure

- **Girders**
  - Malt proteins: Z and Hordeins

- **Struts (cross-bracing)**
  - Malt protein LPT1

- **Gusset Plates**
  - Iso Alpha Acids
  - Melanoidins

- **Rivets**
  - Metal ions
Two Types of Girders

- Protein Z is thought to be the “better” girder, having longer stability.
- Hordeins are “faster” and “cheaper”, having less stability but are easier foam formers.
- Hydrolyzed hordein is thought to exclude Protein Z in foam.
- Similar to the difference between building with steel versus wood.
Protein Z

- Protein Z is thought to be the primary foam former.
- Protein Z is a water soluble protein (ie. albumin), with a typical molecular weight of about 40,000 mass units.
- Protein Z is often 30% glycosylated (bound to a sugar molecule) due to Maillard reactions in the boil.
- Protein Z has the highest surface viscosity and elasticity of the malt proteins.
Protein Z

- The amount of Z depends on the barley variety, but modern malting varieties with high diastatic power typically have high levels.

- The amount of Z in the wort is not significantly affected by proteolytic enzymes in the mash.

- Z is affected by Kohlbach Index (modification)
  - KI < 40 typically have low amounts of Z
  - KI > 40 typically have high amounts of Z
Hordein

- Hordein is the major storage protein of barley, and are generally insoluble until hydrolyzed during malting or mashing.
- Hordein is a glycoprotein, like gluten, that is typically glycosylated.
- Hordeins ARE affected by proteolytic enzymes.
- Protein rests make smaller Hordein pieces, all the way down to amino acids.
- Hordeins are most common in less-modified malts (KI < 40).
The Struts – Protein LPT1

- ~9.7 kDa hydrophobic protein.
  - Is concentrated in beer foam.
  - LPT1 plays two roles in foam
    - In its native foam, it acts to scavenge lipids that strongly destabilize foam.
    - In its denatured form (after the boil) it is a very effective foam stabilizer in conjunction with Protein Z and Hordein.
Lipids in Action

- Lipids are a double-edged sword – good for yeast nutrition, bad for foam.

- One of the most damaging types of lipids are the hydroperoxides formed by the malt enzyme lipoxygenase acting on linolenic acid (common lipid).

- Consider them to be pigeon poop on the girders.
LTP1 binding of lipids

- LPT 1 in its native form acts to bind up lipids and prevent them from destabilizing the foam.
- They’re pigeon guards.

3D structure of LTP1 binding two lyso-phosphatidylcholine residues in hydrophobic fold. From Douliez et al., 2001 Eur. J. Biochem. 384-388
LTP levels during wort boiling
(from Robinson et al., 2008 IBD conference Auckland, NZ)
Increase in LTP levels

(from Robinson et al., 2008 IBD conference Auckland, NZ)
The Gussets – Iso-alpha and Melanoidins

Both iso-alpha acids and melanoidins have been demonstrated to be foam stabilizers.

The mechanism is thought to be hydrogen bonding of the foam proteins, similar in action to a gusset plate where girders and struts join.

Melanoidins (glycosylated proteins) from Maillard reactions are polar molecules and are thought to improve foam stability the same way as Iso-alpha acids.
Comparison of Lacing Index

Pictures of the lacing index test glass at the completion of the test using commercial beers hopped in a variety of ways.

A. "Tetra" hopped beer (11 BU "tetra"), Lacing index = 5.66
B. Iso - "tetra" hopped (23 BU ~5 BU "tetra"), Lacing index = 4.65
C. Iso-hopped beer (41 BU), Lacing index = 2.49
Do you like your Foam **Natural** or **Enhanced**?

The use of Tetra-hop extract can be compared to the use of silicone.

Sure it looks great at first, but....
The Rivets – Metal Ions

1. strongest affinity: Mn$^{2+}$, Al$^{3+}$, Ni$^{2+}$, Sn$^{2+}$
2. weaker affinity: Mg$^{2+}$, Zn$^{2+}$, Ca$^{2+}$ and Ba$^{2+}$
3. no affinity: Li$^+$, Na$^+$, K$^+$
   (Hughes and Simpson, 1995)

Zinc additions of as little as 2ppm have been shown to significantly increase foam stability.

Zinc and Aluminum are very hard to get into solution.

Calcium is your best bet.
Brewing Processes and Protein

- Malts and Adjuncts
- Mashing
- Boiling
- Fermentation
Malts and Adjuncts

Barley, wheat, and rye malts and adjuncts contain lots of the necessary proteins. There is nothing special about adding wheat to a recipe for head retention.

Maize, Rice and (semi-)refined sugars do not.

The kilning of malt denatures Protein Z and Hordein, so high-kilned malt (>10 SRM) don’t contribute much foam-active protein.

But melanoidins from roasted malts have been shown to produce stable foams.
The Ideal Foam Malt?

• High protein content with good extract.
• Moderate KI (40-45) with good NSP modification.
• High levels of:
  - protein Z
  - LTP1
  - foam promoting hordeins
Mashing

To recap: Hordeins are insoluble proteins that are predominant in less-modified malts.
Therefore you need a protein rest to make them soluble.
But, a protein rest makes the soluble portion become smaller, which is fine up to a point because the useful range is 10-30 kDa for foam-active hordein.
Too long in the protein rest and you will produce FAN (foam-negative, like lipids).
Producing too much soluble hordein can displace the more stable protein Z in the foam.
Haze Active Proteins

- Derived from barley hordeins
- Rich in proline
- Range in molecular weight

Hordeins are:
- Polymorphic
- Two major groups (B & C), two minor groups (D & γ)
- B and C (MW 35 kDa–50 kDa), D (MW ~90 kDa)
- 35-55% of the total protein fraction in barley

Sticks are proteins, blobs are polyphenols
Boiling

- Boiling puts proteins thru the wringer.
- Large protein composition differences in worts before the boil become small protein differences after the boil.
- The concentration of all protein species is reduced by an order of magnitude.
- For this reason, boiling high gravity worts and diluting in the fermenter to achieve larger volumes of lighter beer will result in lower protein levels and poor foam.
- As seen on an earlier slide, over-boiling can denature protein. Do not over-boil! (too hot, too hard, too long >2 hours)
Fermentation

- Do not stress the yeast.
  - Stressed yeast excrete more proteinase A, which particularly affects proteins around 10 kDa, like LPT1.
  - Yeast excrete proteinase A even after fermentation is finished.

- Autolyzing yeast excrete lots of lipids.
  - You may want to cold condition or filter the beer to reduce the yeast after packaging for better long term foam performance.
Different Beers, Different Foams

American Pale Ale
- Well modified malt
- Lots of protein Z
- Single infusion mash
- Reasonable boils, good LPT1 levels
- Lots of iso-alpha acids
- Often calcium additions
- Good Foam

Old World Pilsner
- Less Modified malt
- Hordein is dominant
- Decoction/multi rest mash
- Typically longer boils to reduce DMS
- Moderate iso-alpha acids
- Often calcium additions
- Good Foam
## Different Beers, Different Foams

### American Stout
- Well modified malt plus roasted malts
  - Overall, less protein Z
  - Single infusion mash
- Reasonable boils, good LPT1 levels but less overall
- Lots of iso-alpha acids
- Lots of melanoidins
- Often calcium additions

### Dobbelbock
- Mostly Munich malt (a Less Modified malt)
  - Hordein is dominant
  - Decoction/multi rest mash
- Typically longer boils to encourage high melanoidins.
- Low iso-alpha acids
- Often calcium additions

### Good Foam
Avoid protein rests on well-modified malts

More hordein will cause the beer to foam more readily, but the foam will be less stable due to displacement of protein Z.

There MIGHT be no difference between haze-active hordeins and foam-active hordeins – more research is needed.

A little bit of tetra-hop extract will do wonders for your foam, but too much looks odd.
References

