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

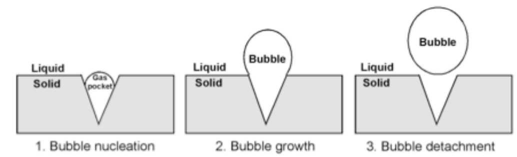


Figure 2: Schematic representation of the bubble formation sequence, after Ronteltap et al., (*1991*).

- 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)

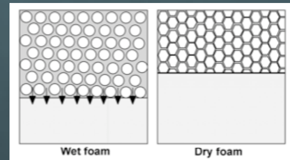


Figure 3: Schematic representation of drainage. In dry foam the bubbles take on a honeycombe-like polyhedric structure showing well defined plateau borders between bubbles, after Prins and Marle, (*1999*).

- Coalescence – bubble coarsening via rupture
    - good surface viscosity & elasticity properties
    - hydrophobic interactions (foam positive)
    - lipids and detergents (foam negative)

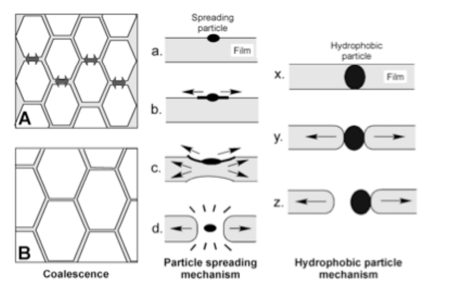
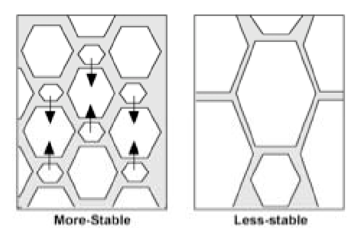


Figure 5: Schematic representation of coalescence (A, B), the particle spreading mechanism (a, b, c, d), and hydrophobic particle mechanism (x, y, z), after Ronteltap et al., (*1991*)

- Disproportionation – bubble coarsening via gas diffusio
    - gas composition (N2 can't diffuse as well as CO2)

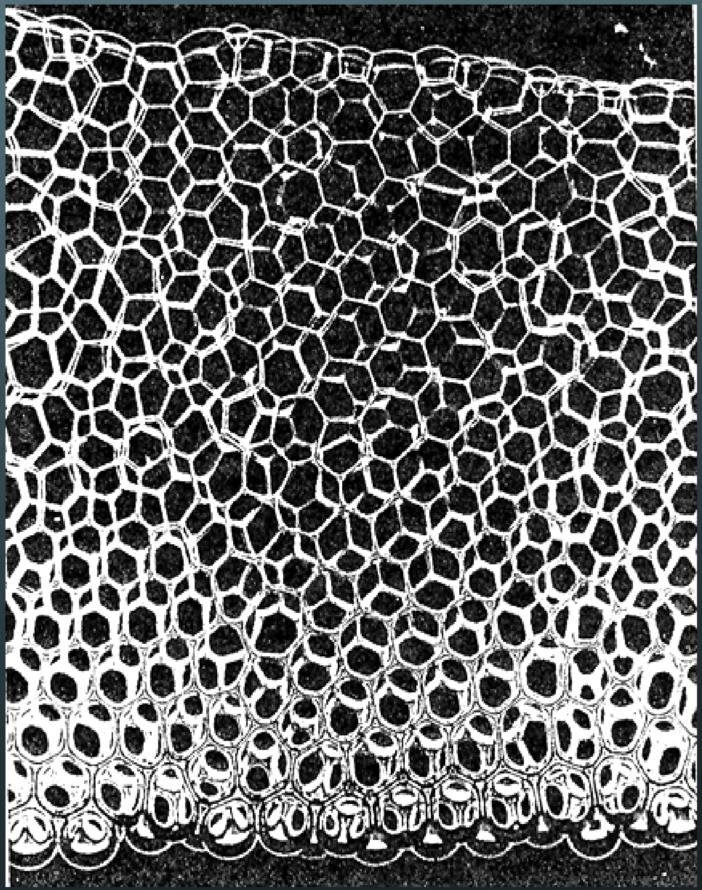


Schematic representation of bubble disproportionation.

From *Beer: A Quality Perspective, Handbook of Alcoholic Beverages*, Bamforth, Russell and Stewart, Elsevier 2009.

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.



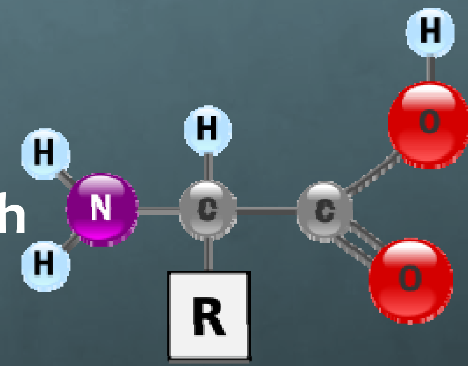
Barley Does Not Want To Make Beer

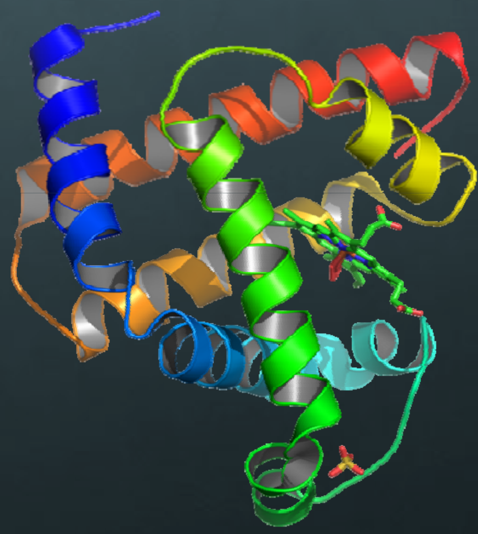
- It wants to be pollinated. (Don’t we all)
- In order to be pollinated, it needs to grow from a seed into a plant.
- The seed is equipped with a protein carbohydrate matrix called the *endosperm*, and enzymes (proteases) that will break that endosperm down into useful growth building blocks called amino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



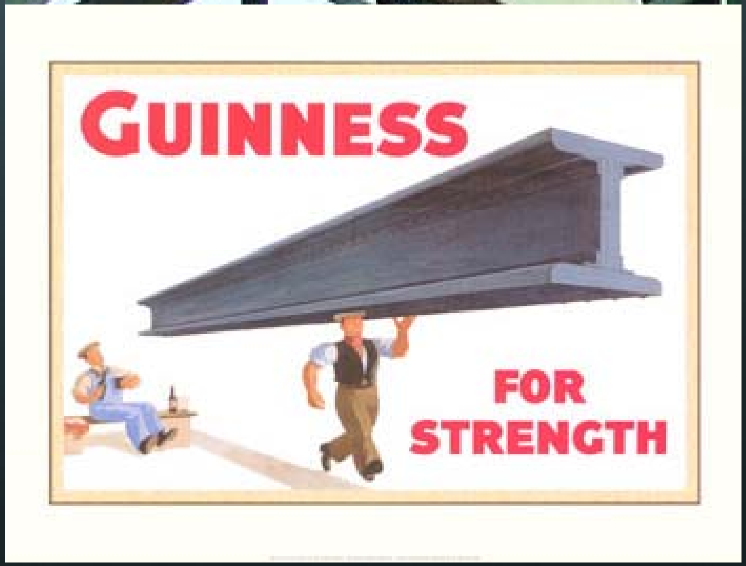


Players

- 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).

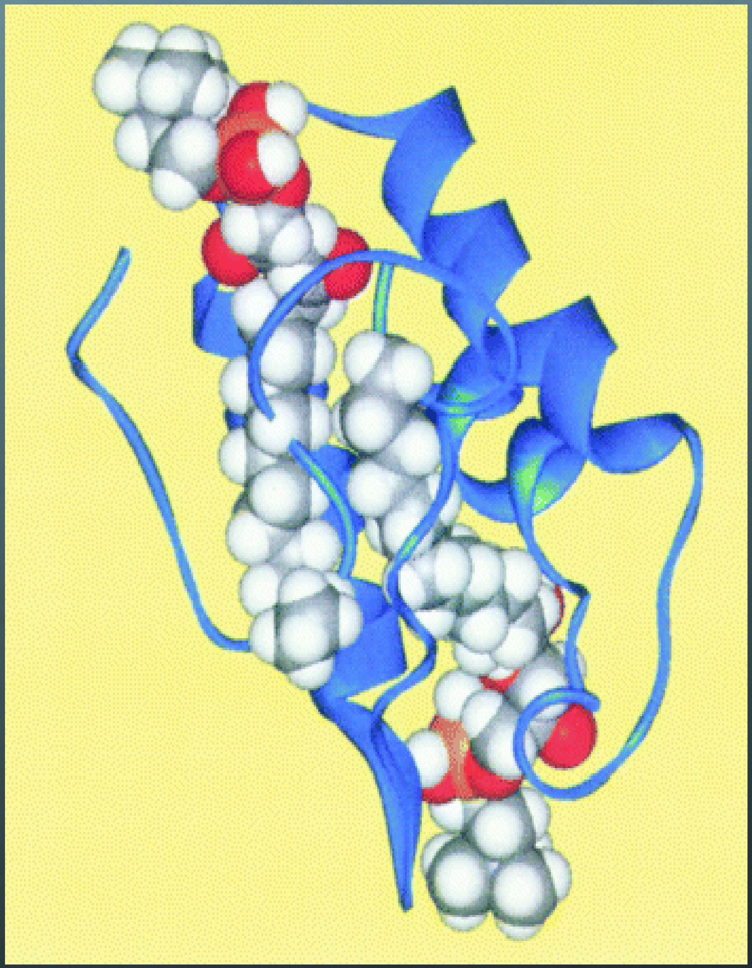
The Struts – Protein LPT1

- ~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

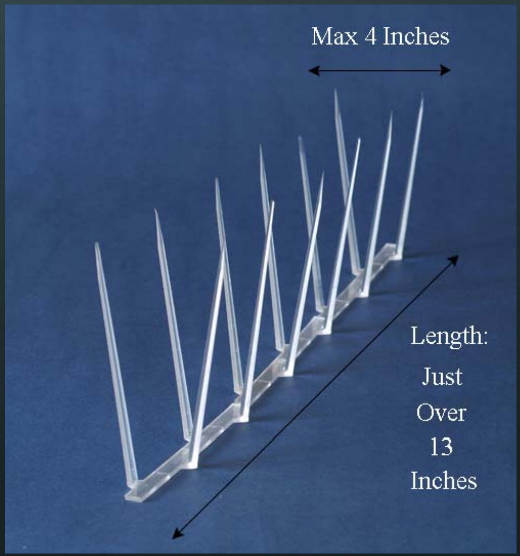
Lipids in Action

- 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.



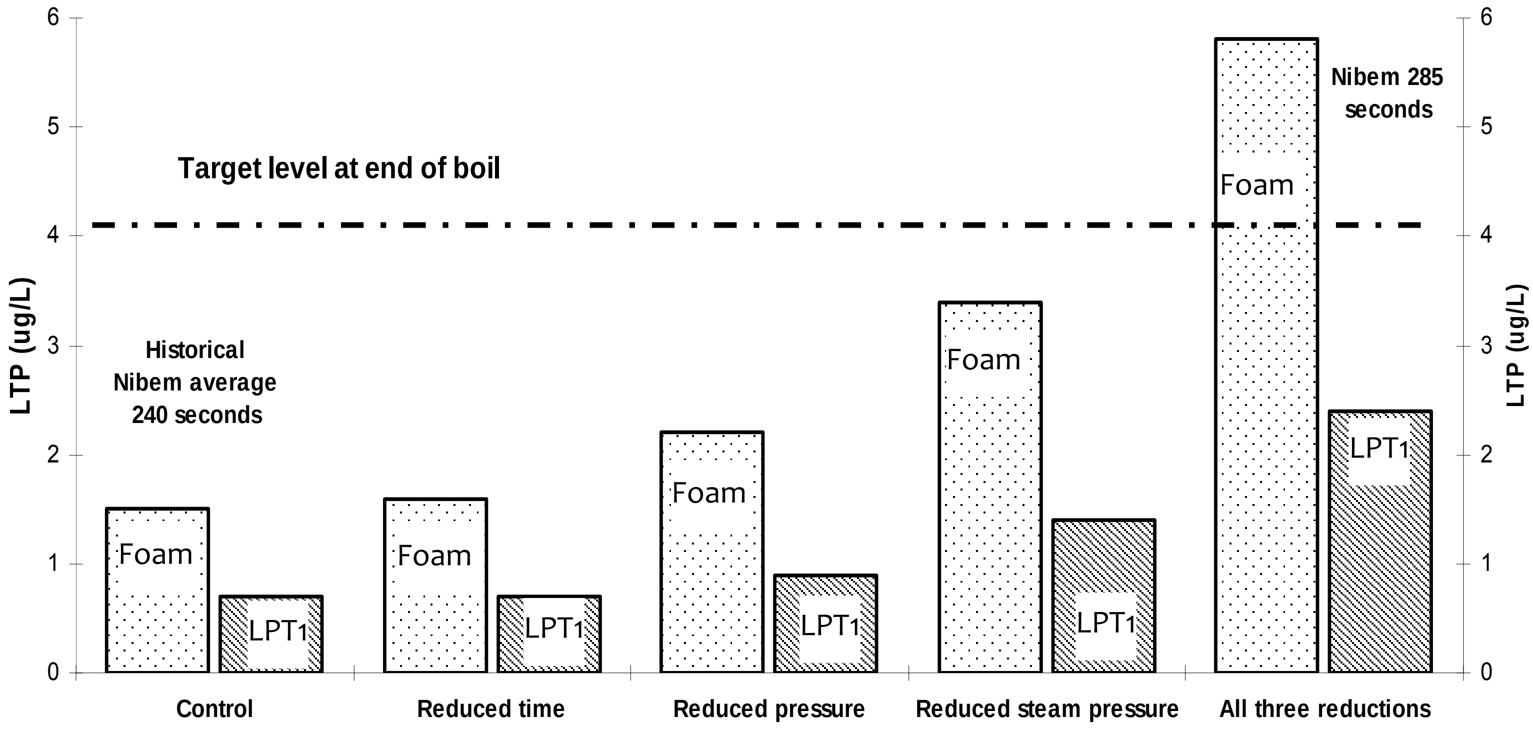
3D structure of LTP1 binding two lyso-phosphatidylcholine residues in hydrophobic fold. From Douliez et al., 2001 Eur. J. Biochem. 384-388

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)

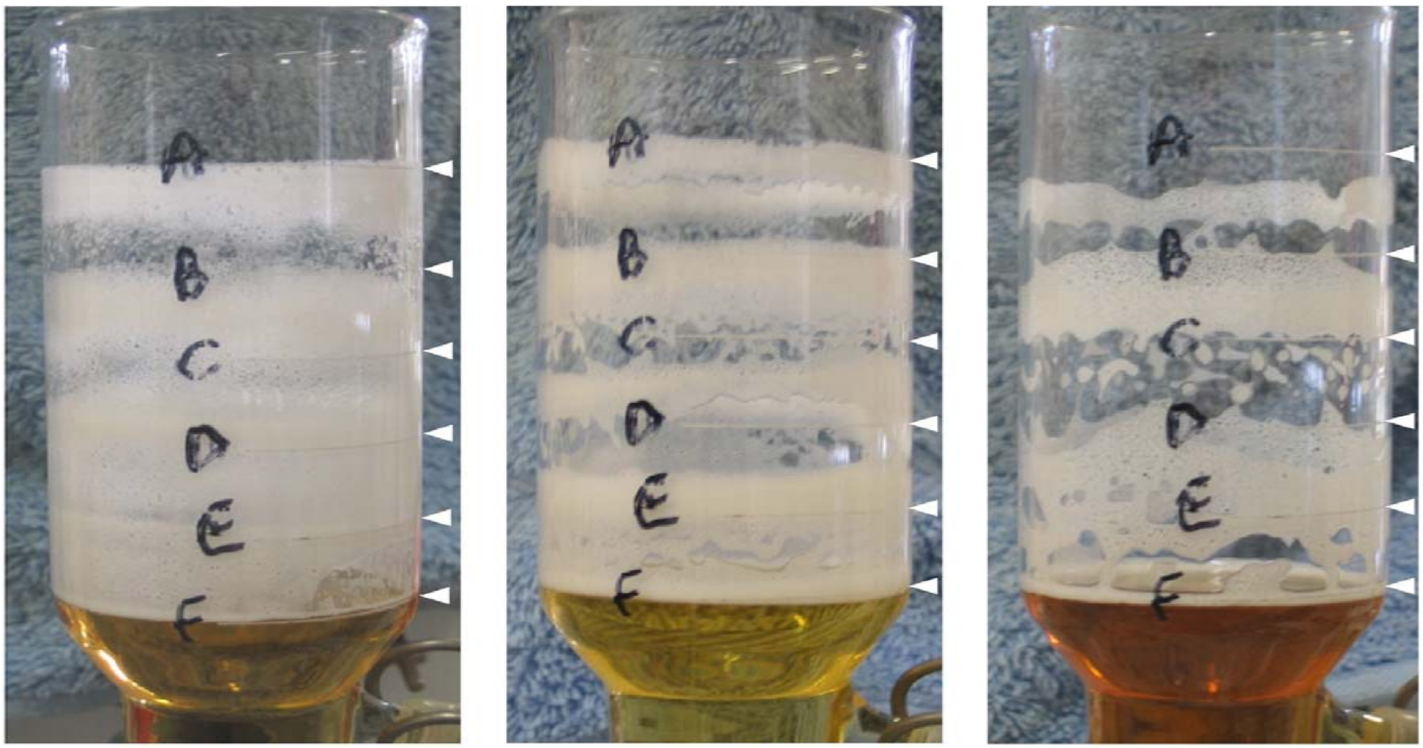


The Gussets – Iso-Alpha and Melanoidins

- 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

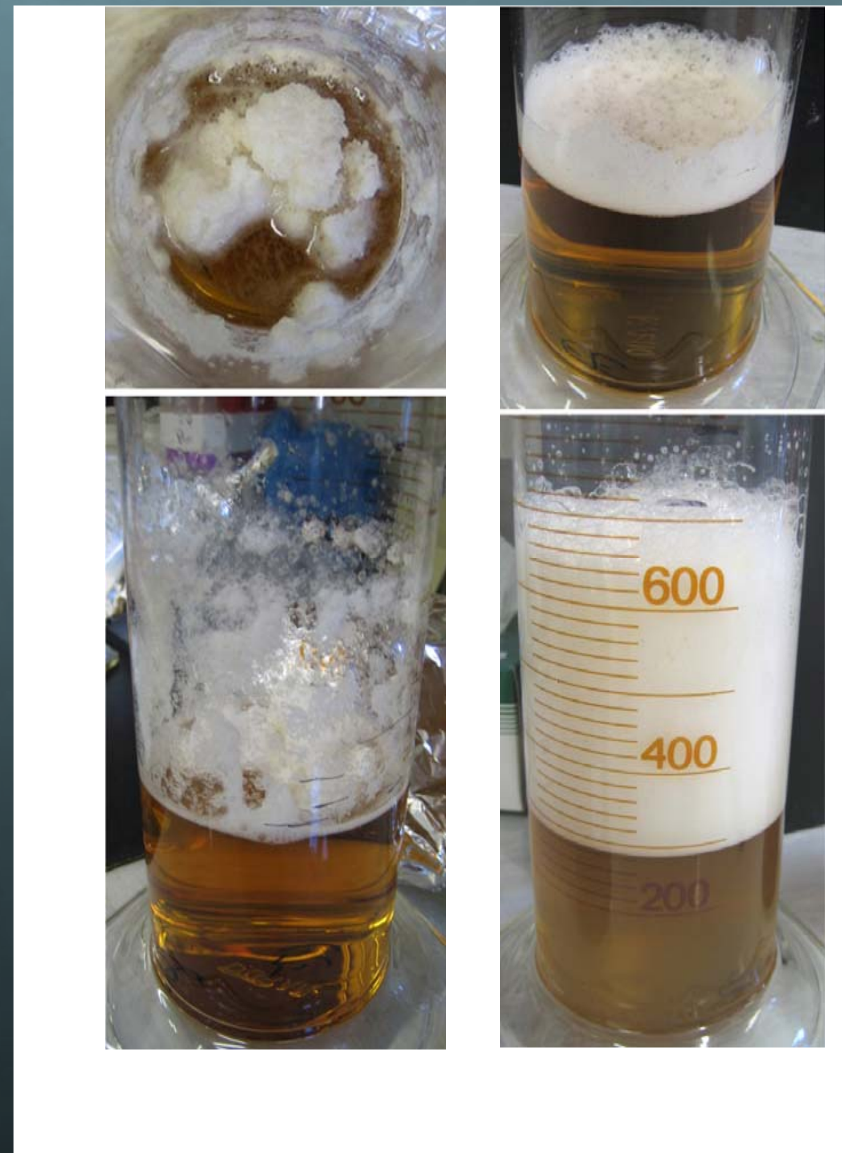
Pictures of the lacing index test glass at the completion of the test using commercial beers hopped in a variety of ways.



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… .



A. Cylinder pour test of tetra hopped beer showing: "whipped egg-white, ice-bergs" of powdery lace and foam.

B. Cylinder pour test of an iso-hopped beer: at the beginning and completion of test.

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

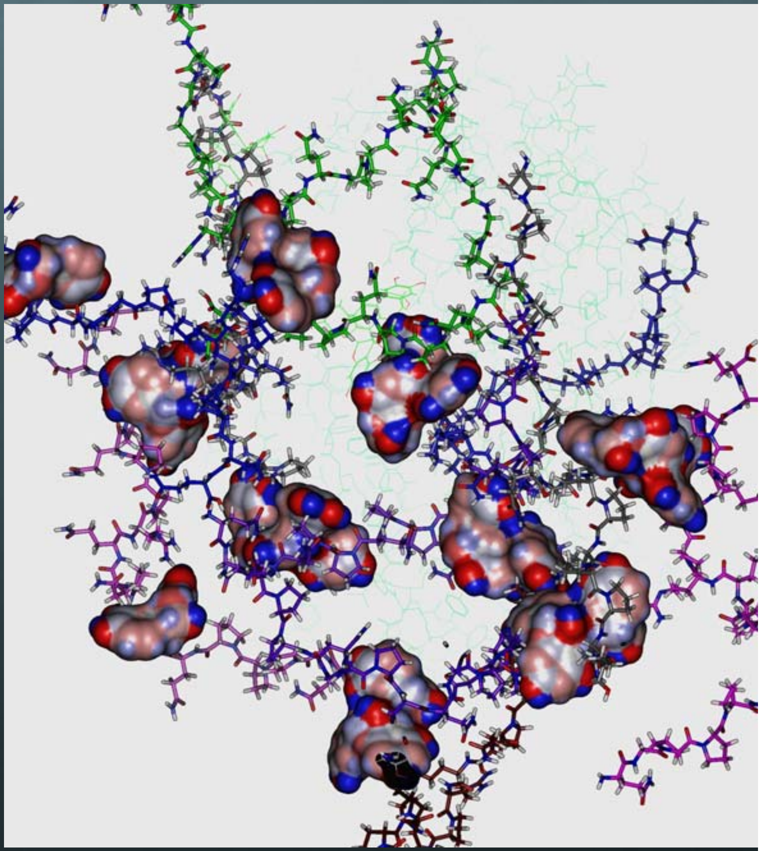
Mashing

- 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
- **Good Foam**

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**

Summary

- 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.

Refereneces

Evans, D.E., and Bamforth, C.W. (2008) Beer foam: achieving a suitable head, in “Handbook of alcoholic beverages: Beer, a quality perspective” edited by Bamforth, C.W., Russell, I., and Stewart, G.G., Elsevier New York, 2009.

Bamforth, CW, Beer Haze, J.Am.Soc.Brew.Chem. 57(3):81-90, 1999.

Evans, D.E., Surrel, A., Sheehy, M., Stewart, D., and Robinson, L.H. (2008) Comparison of foam quality and the influence of hop a-acids and proteins by five foam analysis methods. Journal American Society of Brewing Chemists, *65*:1-10.