

# Beer Haze and Colloidal Stabilization

Karl J. Siebert

Food Science  
& Technology

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CORNELL UNIVERSITY • GENEVA

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## The Phenomenon of Haze

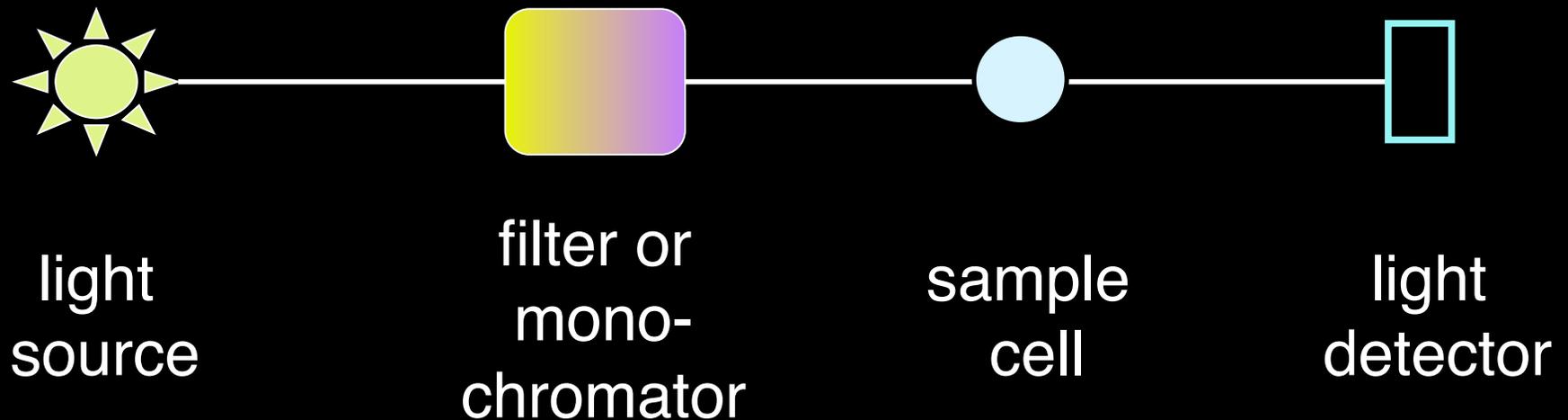
When insoluble particles are suspended in a liquid they scatter light, which makes a sample appear turbid or hazy.

Colloidal particle suspensions are indefinitely stable because collisions with the solvent molecules (caused by Brownian motion) keep the particles in suspension.

Larger or less buoyant particles tend to settle out.

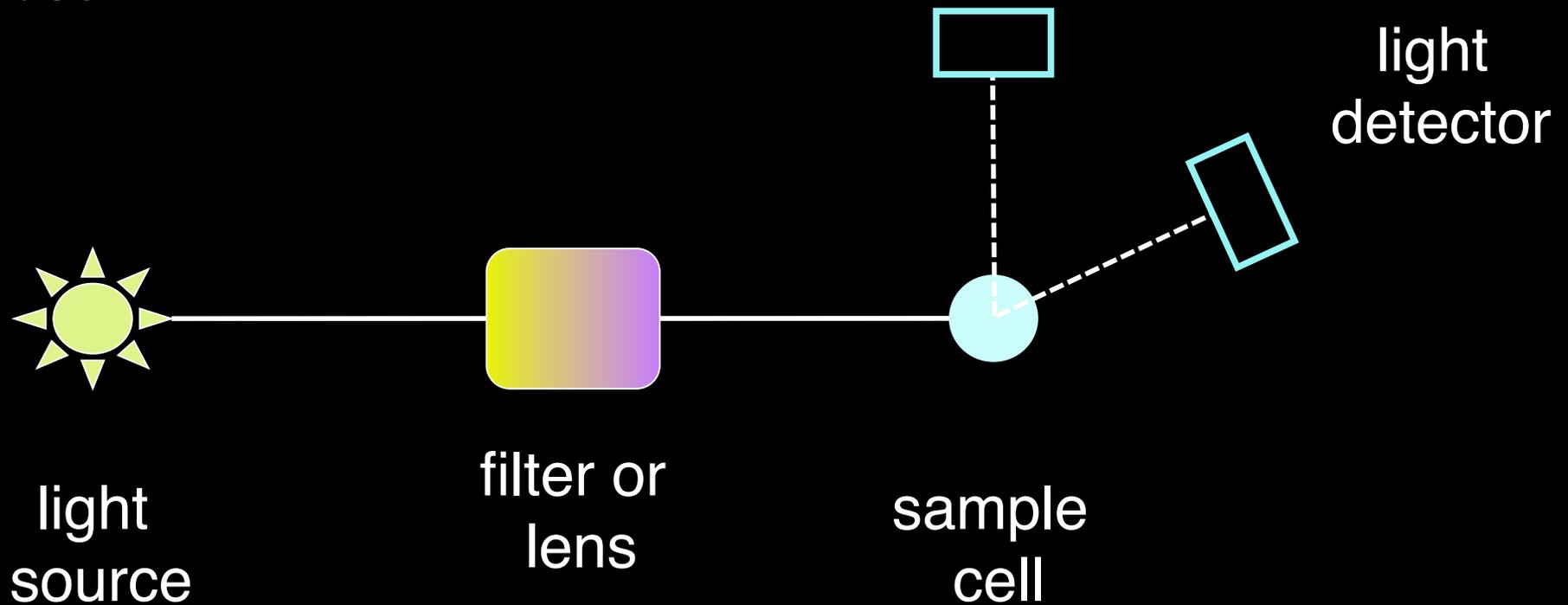
# Photometer

With a photometer, some light that is scattered (as well as that absorbed) fails to reach the detector and results in reduced transmission.



# Turbidimeter

A **turbidimeter** responds to light that is scattered or deflected by interaction with particles. Turbidimeter detectors often are placed either at a narrow angle (typically  $11 - 25^\circ$ ) or at a  $90^\circ$  angle to the incident light beam.



Light absorption (in the visible range) makes samples appear colored.

Light scattering makes samples appear cloudy or turbid.

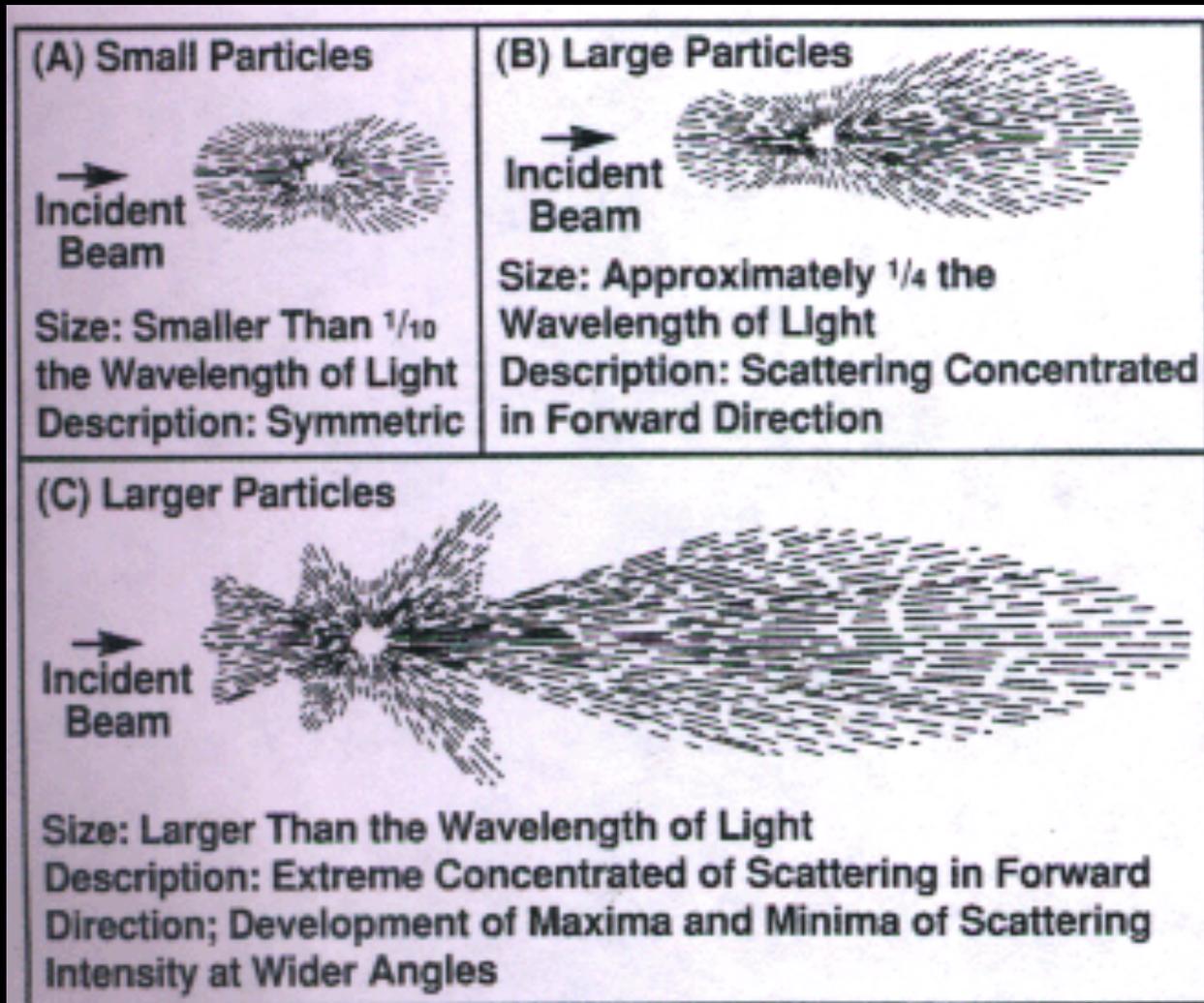


## Haze Perception

The unaided human eye cannot actually see colloidal size particles, but can readily detect scattered light.

Particles scatter light at various angles depending on the wavelength of the light and the size and shape of the particle.

# Theory for spherical particles



There are quite a few reports in the brewing literature stating that narrow angle scattering is more sensitive for large particles (such as yeast, which has a mean particle diameter on the order of  $10\ \mu\text{m}$ ) and that  $90^\circ$  scattering is more sensitive for small particles, such as chill haze (generally thought to be on the order of  $0.25\ \mu\text{m}$  or smaller).

Gales (*JASBC* 2000) pointed out that this is actually not correct, and is an artifact due to size differences between particles used for calibration and those actually present.

# Sources of Haze and Sediment in Beer

Foreign matter

- adsorbents

- filter aids

Crystalline material

- oxalates

Microbes

- yeast

- bacteria

Polysaccharides

- starch, pentosan,  $\beta$ -glucan

Protein-Polyphenol complexes

Foreign matter is normally only seen when process failures occur.

That is also true for oxalate hazes (assuming calcium addition is done normally).

Hazes produced by bacterial contamination (either by direct scattering or through formation of haze as a result of bacterial metabolism) are typically seen only if filtration or pasteurization fails for some reason.

**Yeast cell wall fragments** arise when yeast is subjected to a **shearing** force (in a disc centrifuge or during agitation).

Disc centrifugation was shown to produce yeast cell wall fragments under some conditions; these were particularly resistant to sedimentation and harmful to filterability (Siebert et al., 1987).

Haze material was released from agitated yeast; this occurred to a greater extent at pH 2 or 8 than at pH 4 (Lewis & Poerwantaro, 1991).

Both the haze and the pH of a yeast suspension in beer increased slightly with increasing sheer severity (Stoupis & Stewart, 2003).

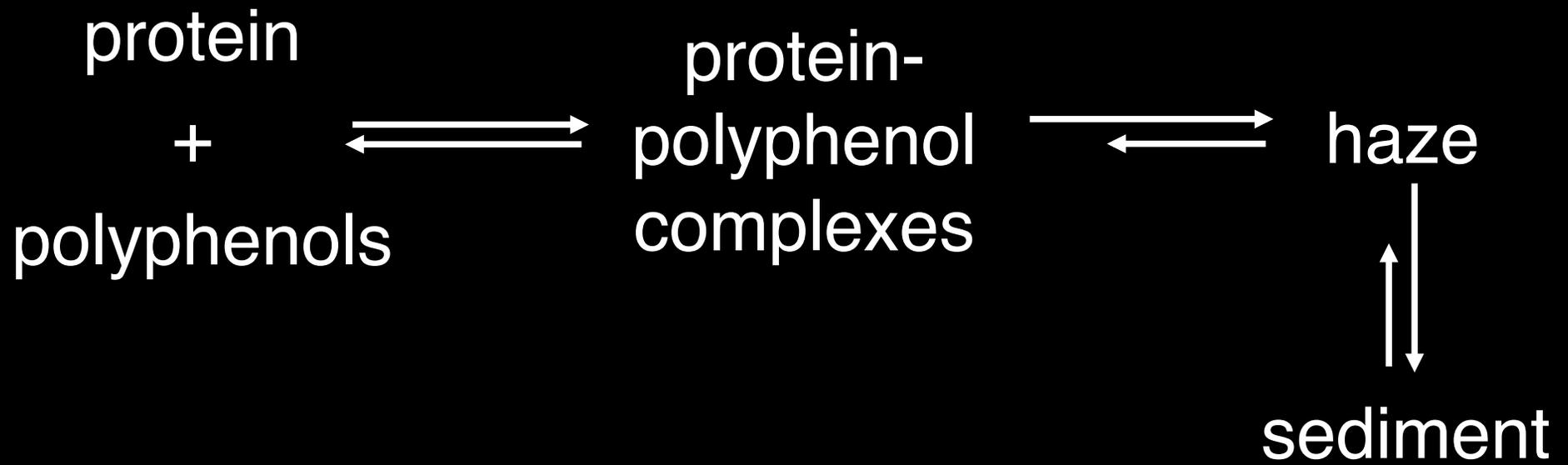
## Carbohydrate Hazes

Relatively pure carbohydrate hazes in beer are known (starch crystals, pentosans, etc.) but infrequently encountered.

Hazes isolated from beer are typically found to contain 70% - 80% carbohydrate, about 20% protein and a small amount of polyphenol (Belleau & Dadic, 1980, 1981; Siebert et al. 1981). However, it is well known that stabilization can be achieved by removing polyphenol or protein or both. So the carbohydrate must simply be entrained when the protein-polyphenol haze particles form.

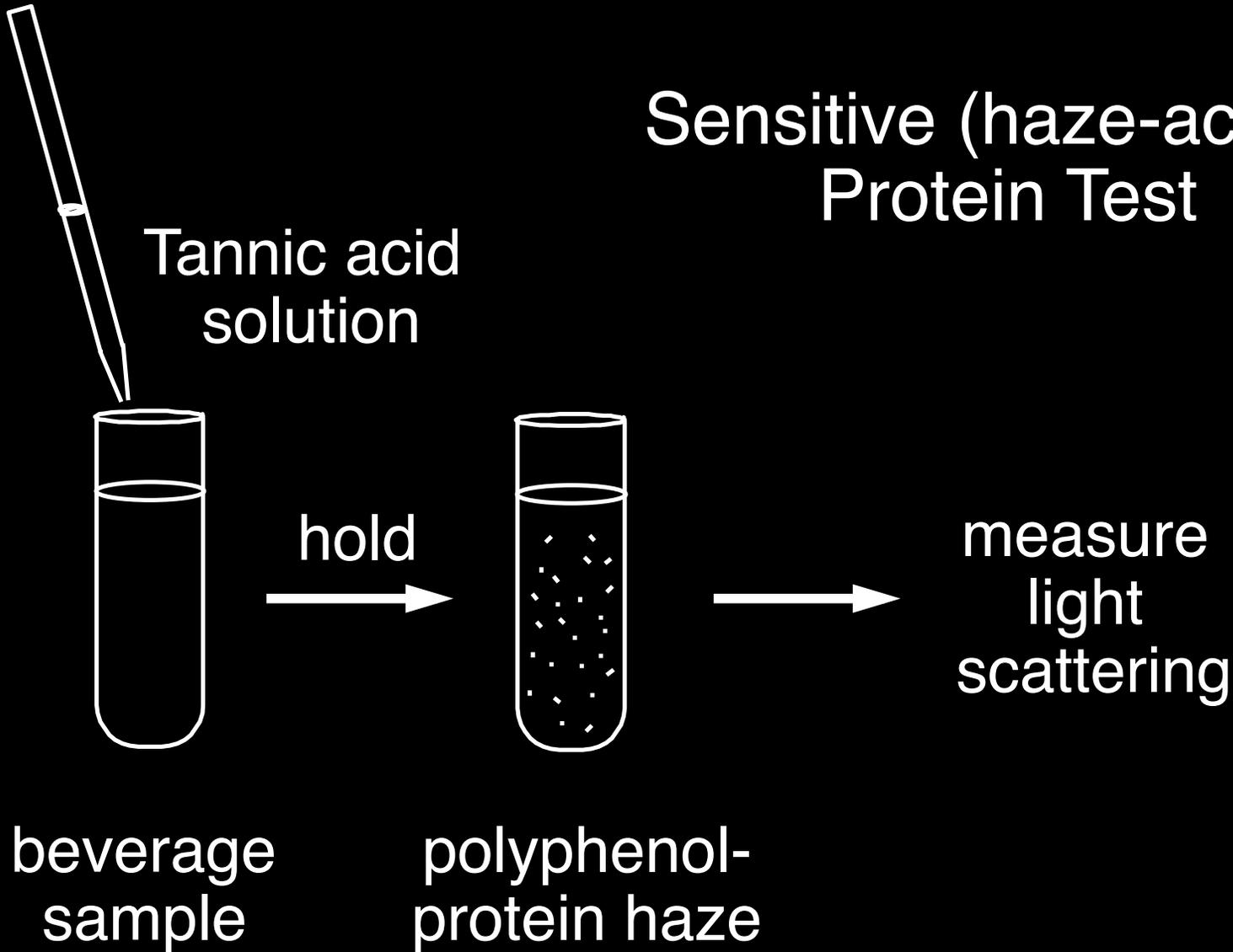
# Main Haze Formation Mechanism

Hazes in beer can occur from a number of causes, but most often arise from protein-polyphenol interaction.



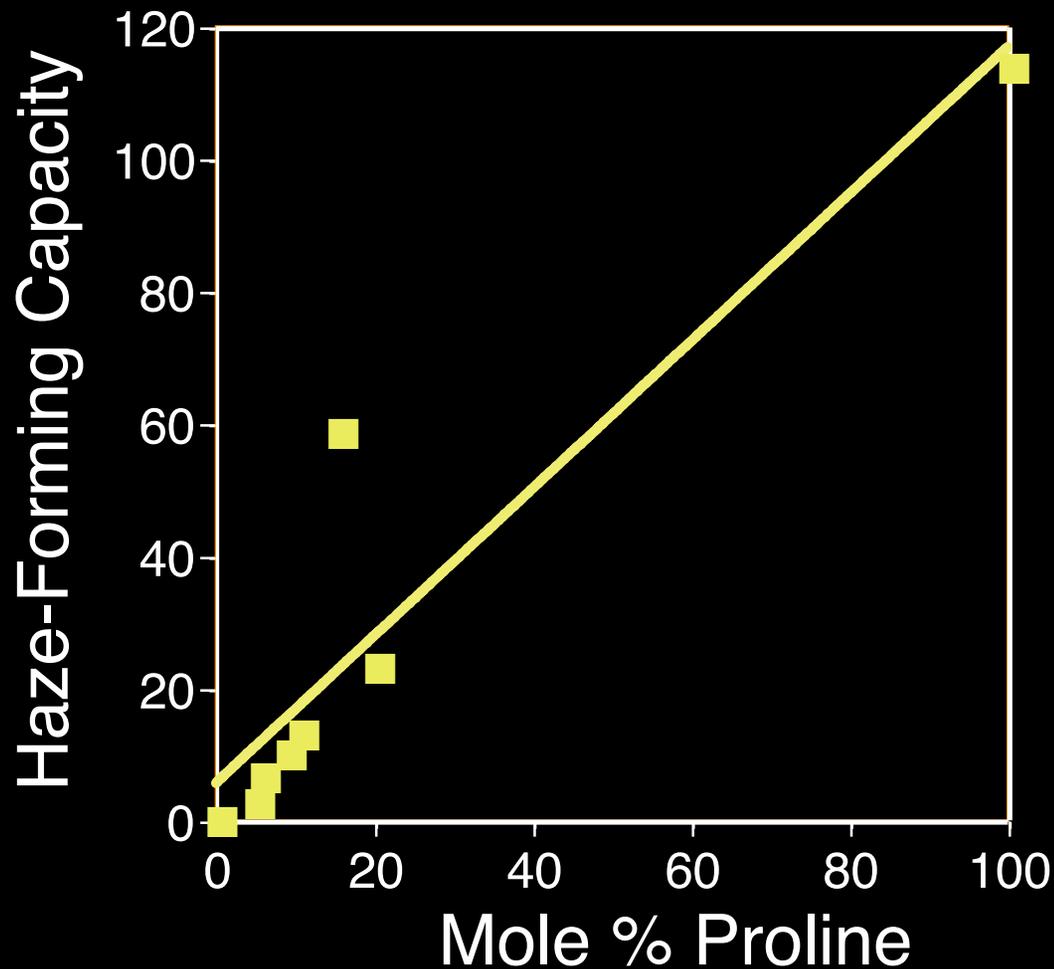
# The Nature of Beer Haze-Active Protein

# Sensitive (haze-active) Protein Test

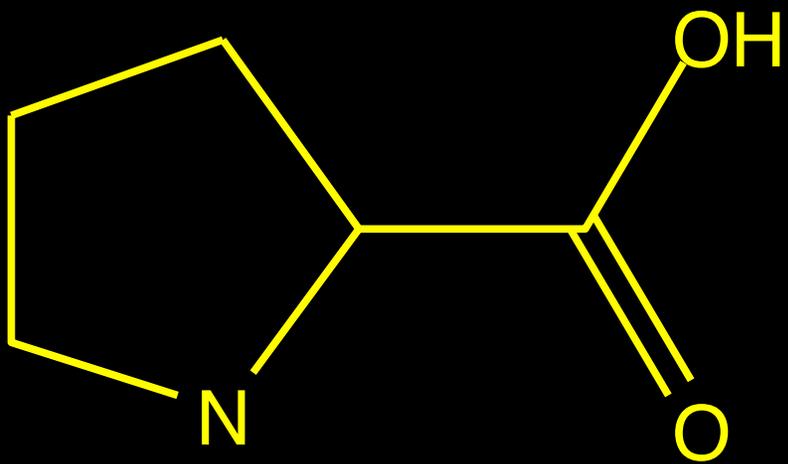


Thompson & Forward, *J. Inst. Brew.* 75: 37-42, 1969.

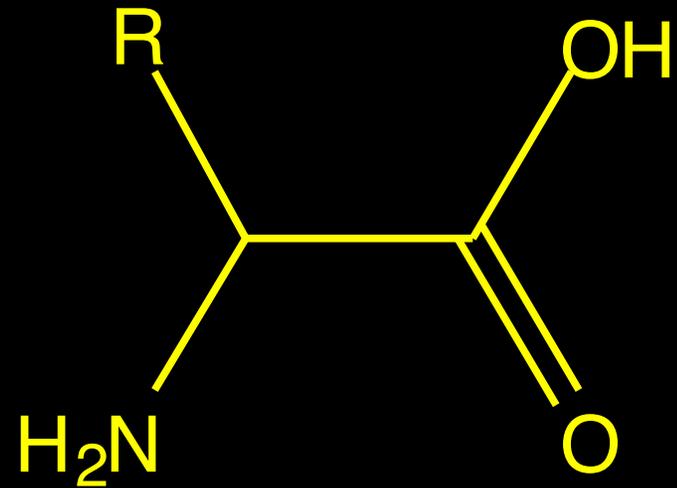
# Haze Forming Capacity of Various Proteins and Peptides Combined with Catechin and Heated



Data from: Asano et al. *J. Amer. Soc. Brew. Chem.* 40: 147-154, 1982.



Proline



Other Coded  
Amino Acids

We know from Asano's work (*JASBC* 1982) that the **beer haze-active protein** is derived from **barley hordein**.

Gliadin is the wheat protein that is analogous to barley hordein.

Both gliadin and hordein are prolamins (alcohol-soluble, proline-rich proteins).

Both have high contents of the amino acids proline and glutamine.

Gliadin is commercially available.

# Amino Acid Sequence of Barley Hordein (haze active protein in beer)

## Partial Sequence of Barley Hordein

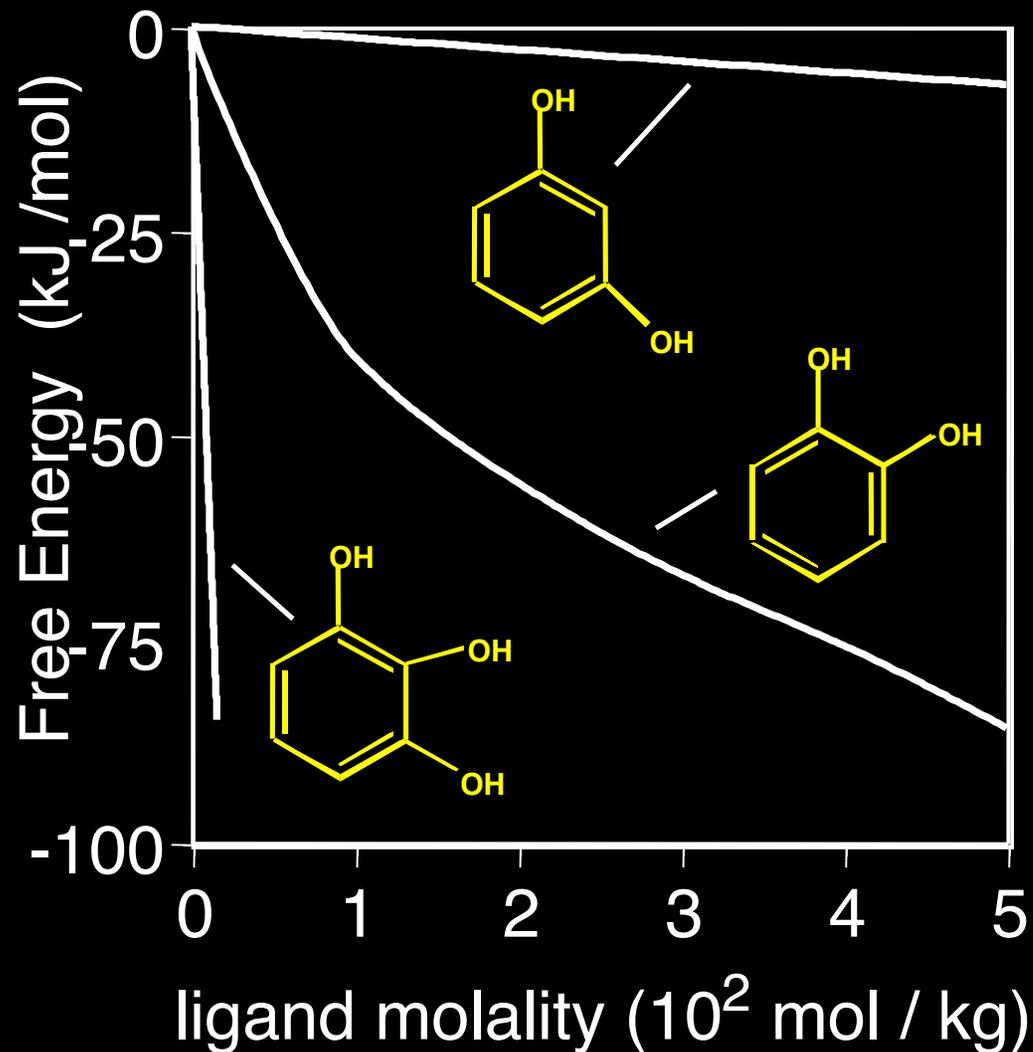
Q Q Q P F P Q Q P I P Q Q P Q P Y P Q -  
Q P Q P Y P Q Q P F P P Q Q P F P Q Q -  
P V P Q Q P Q P Y P Q Q P F P P Q Q P -  
F P Q Q P P F W Q Q K P F P Q Q P P F -  
G L Q Q P I L S Q Q Q P C T P Q Q T P L -  
P Q -

**P** = proline

**Q** = glutamine

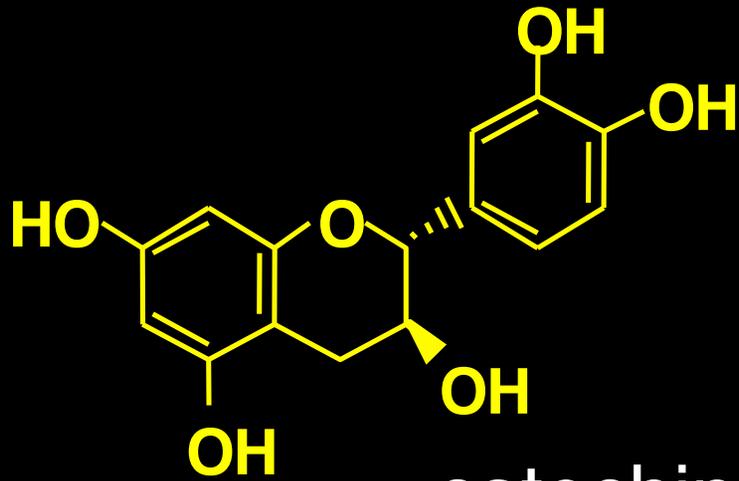
# The Nature of Haze-Active Polyphenols

# Polyphenol Association with BSA at pH 6.5

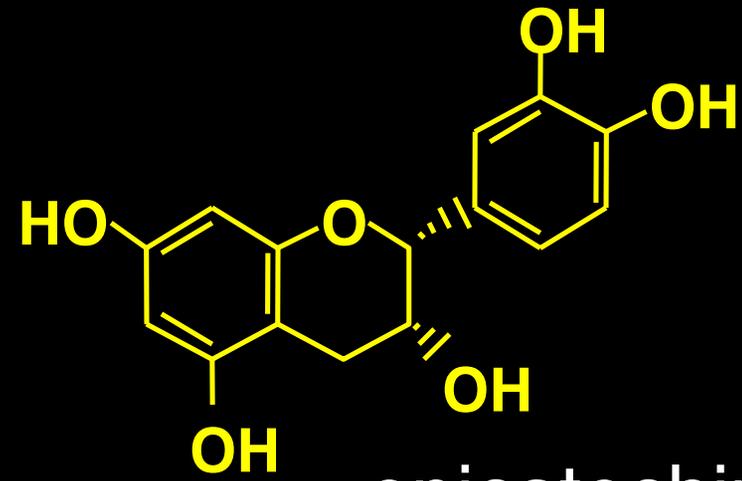


McManus et al., *J. Chem. Soc. Perkin Trans. II*: 1429-1438, 1985.

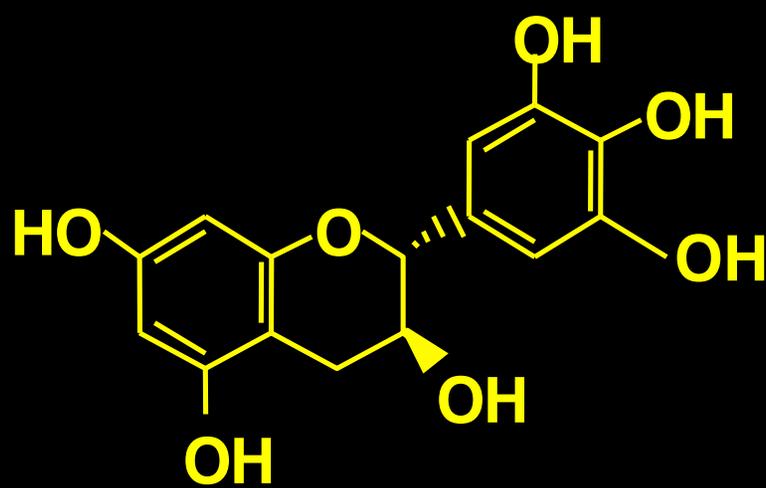
# Proanthocyanidin Building Blocks



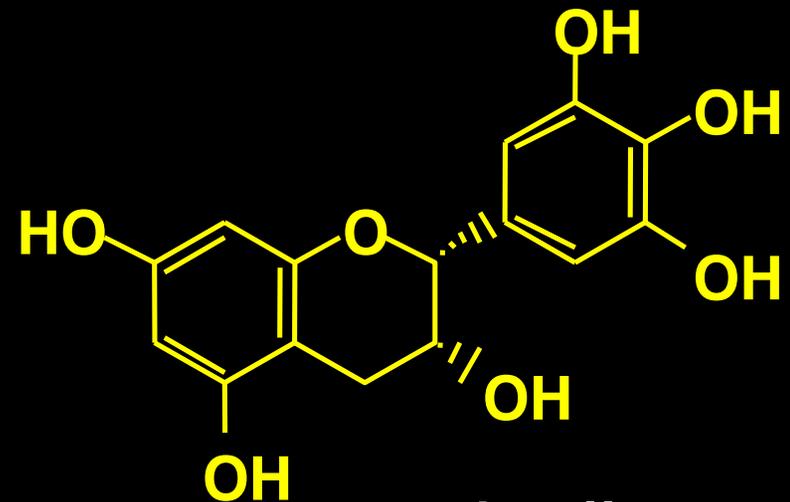
catechin



epicatechin

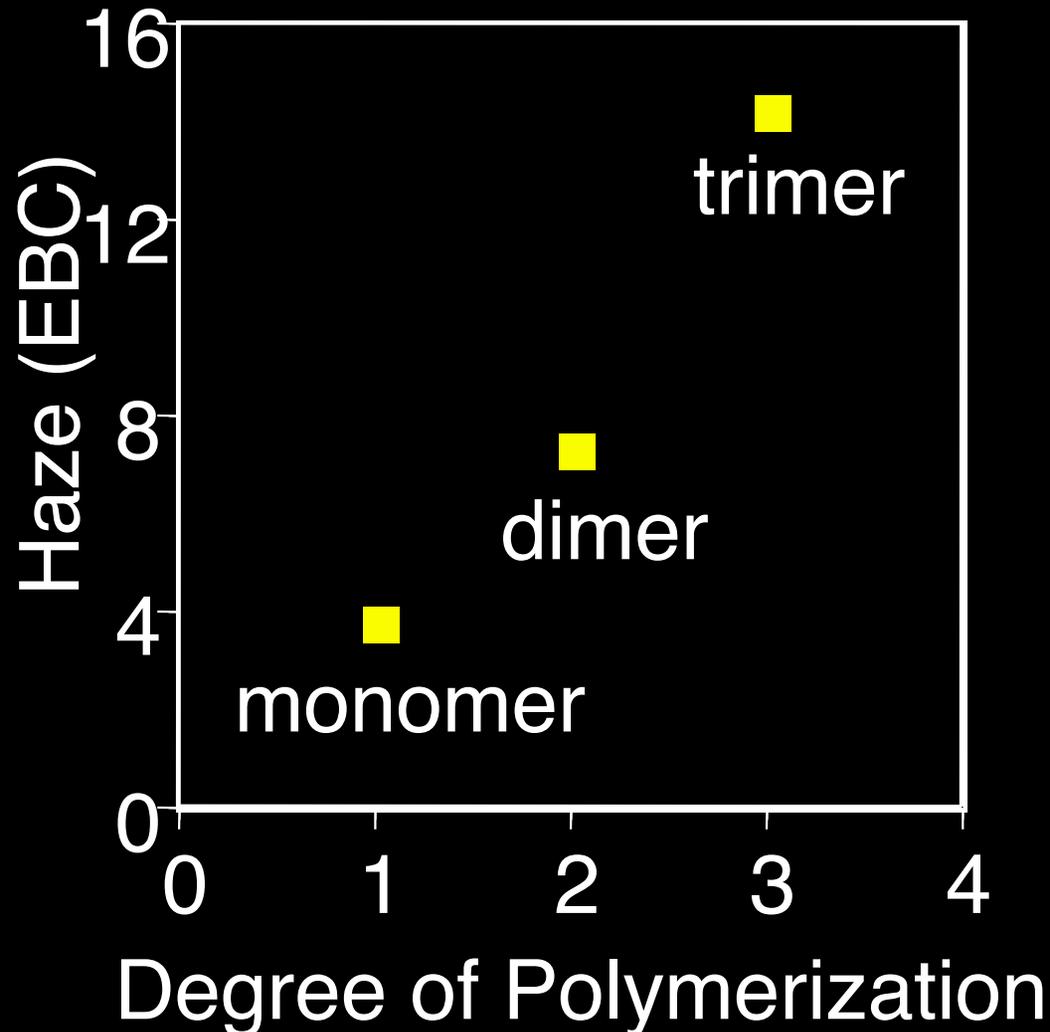


gallocatechin



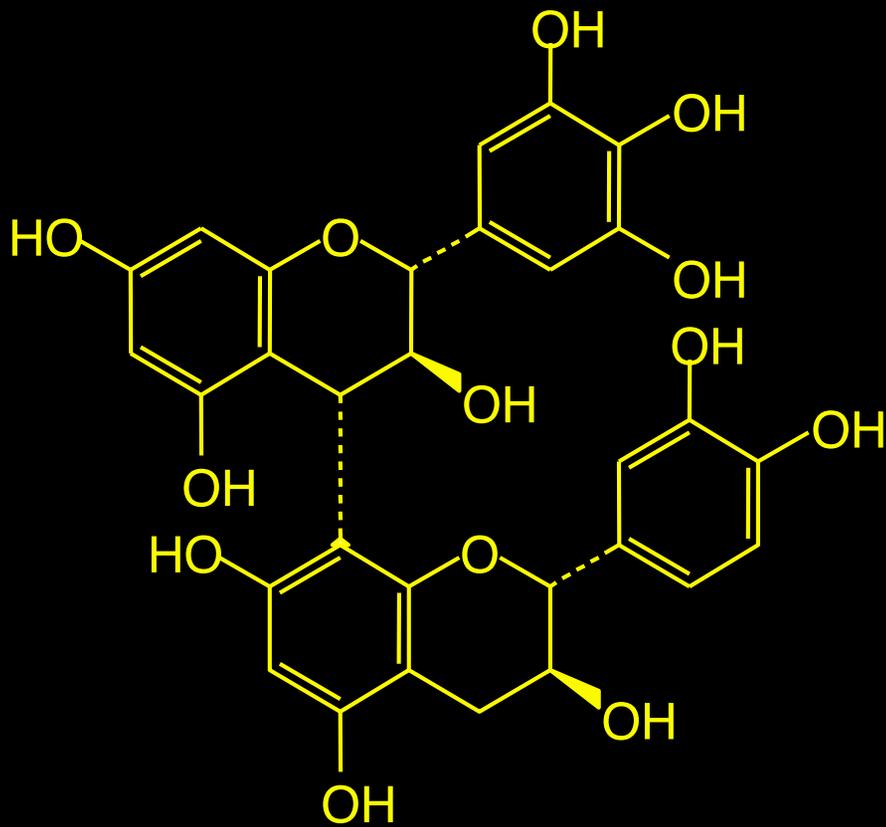
epigallocatechin

# Effect of Proanthocyanidin (Catechin) Polymerization

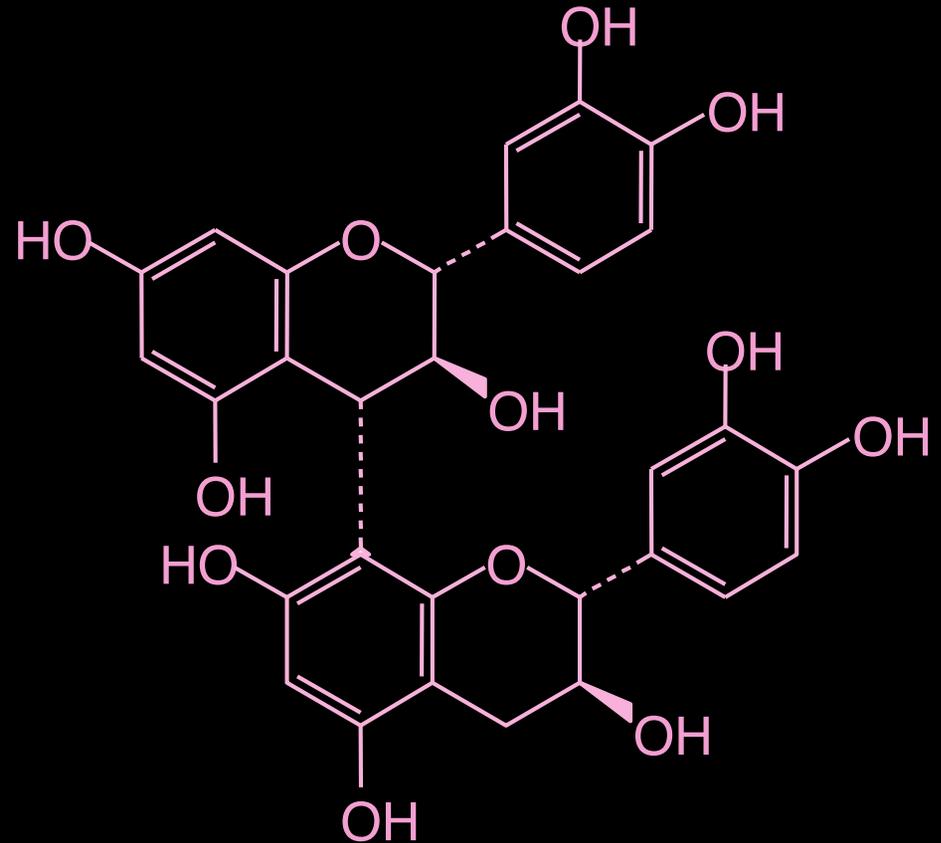


Mulkay & Jerumanis, *Cerevisia* 8: 29-35, 1983.

# Prominent Beer Proanthocyanidin “Dimers”

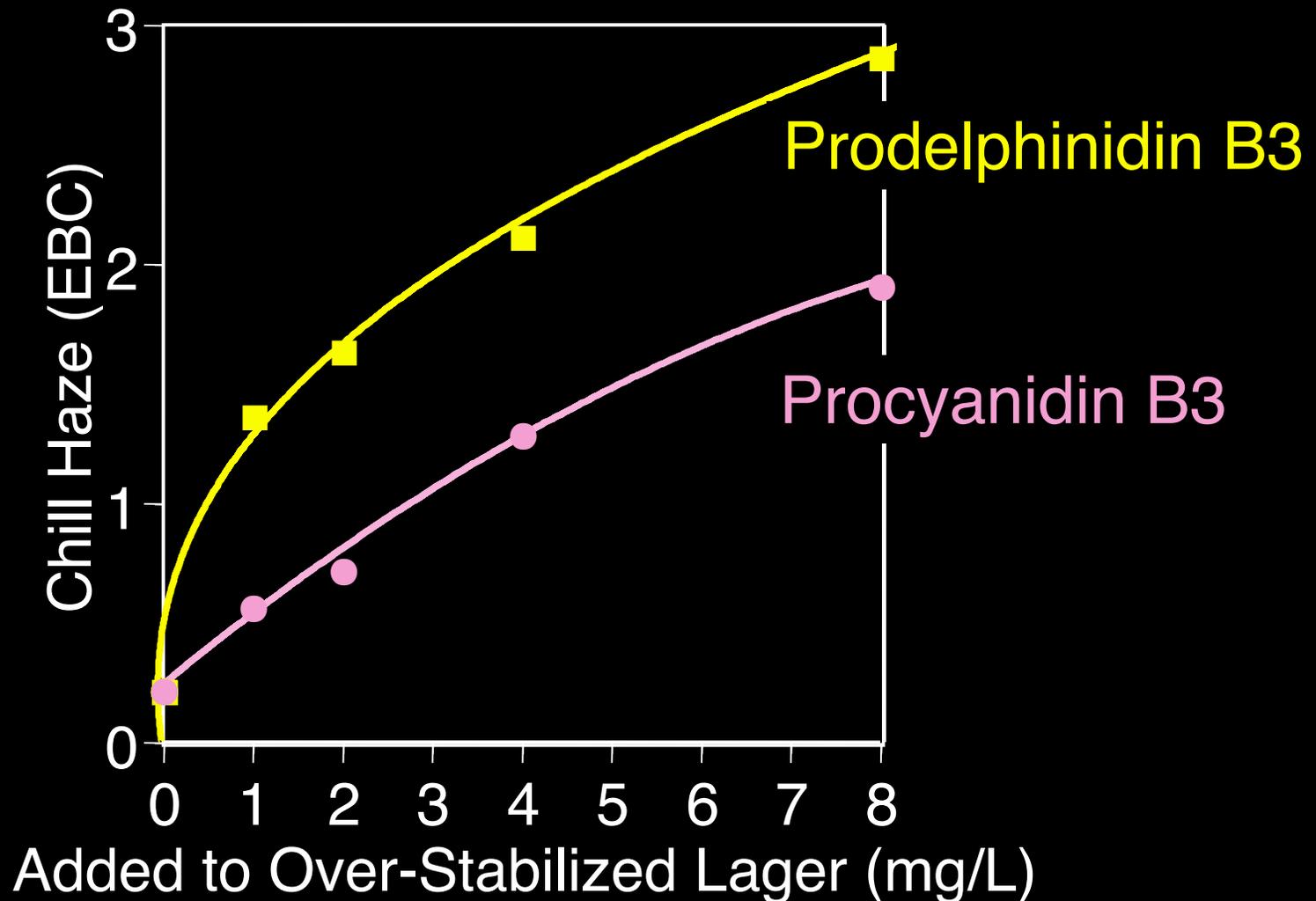


Prodelphinidin B3



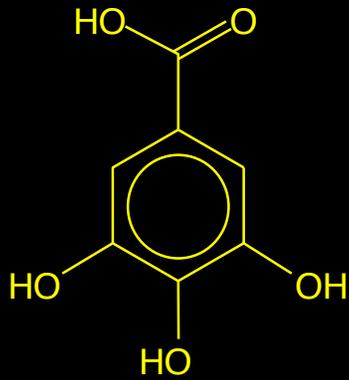
Procyanidin B3

# Effect of Dimeric Proanthocyanidins on Beer Haze

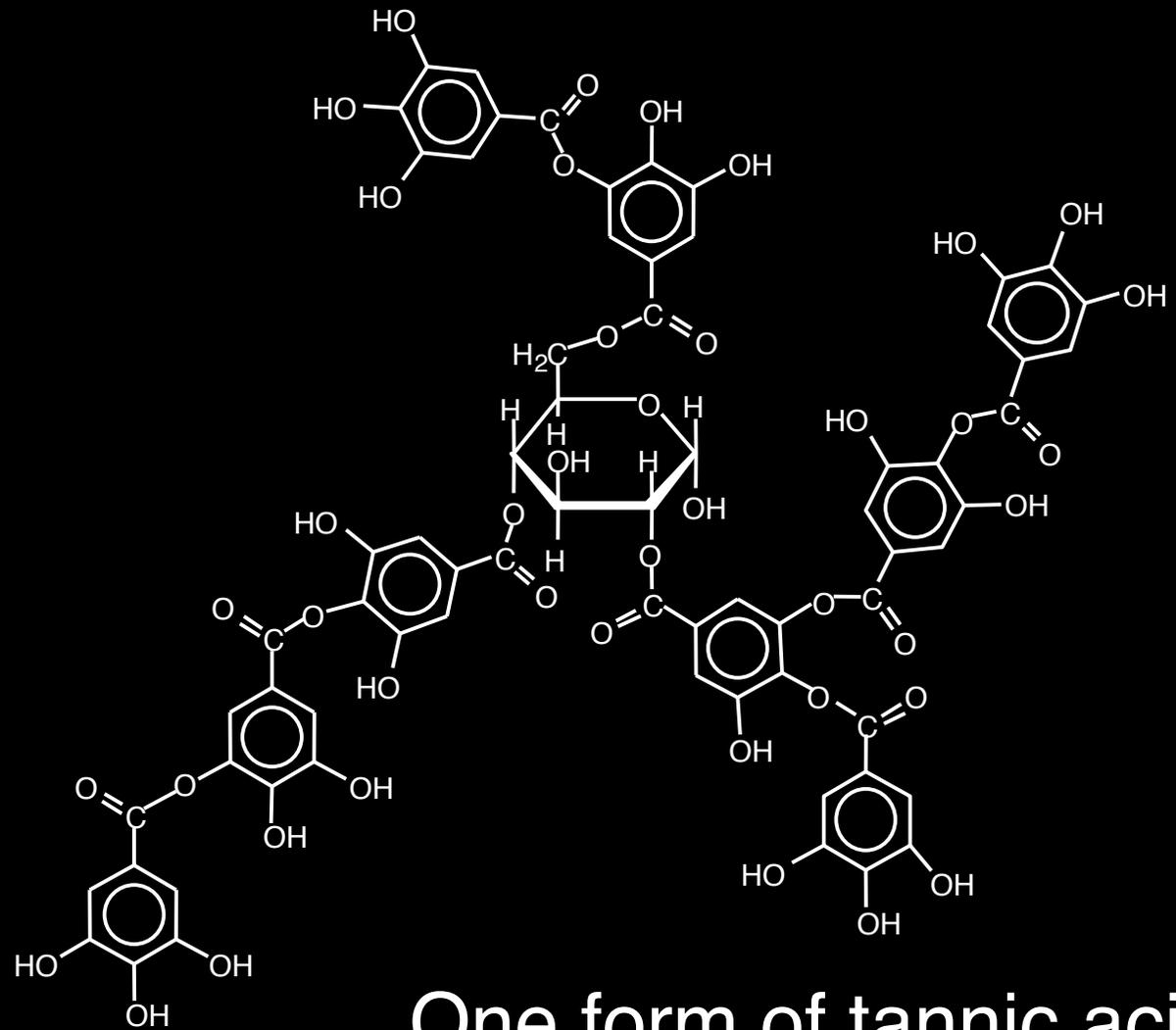


McMurrough et al., *J. Am. Soc. Brew. Chem.* 54: 141-148, 1996

# Gallotannins



Gallic acid



One form of tannic acid

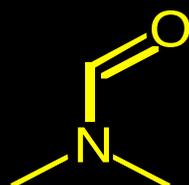
# The Nature of Protein-Polyphenol Interaction

Because protein-polyphenol haze can be induced by cooling and dispelled by warming we know it is not caused by covalent bonding.

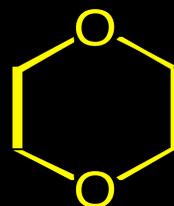
That leaves non-covalent interactions, which could be due to hydrogen bonding, hydrophobic bonding or ionic bonding.

## Expected Effects of Added Substances

N,N-dimethyl formamide (DMF) - a hydrogen bond acceptor; this should compete with and reduce hydrogen bonding between proteins and polyphenols.



Dioxane - a non-polar, but water miscible solvent; this should compete with and reduce hydrophobic bonding between proteins and polyphenols.



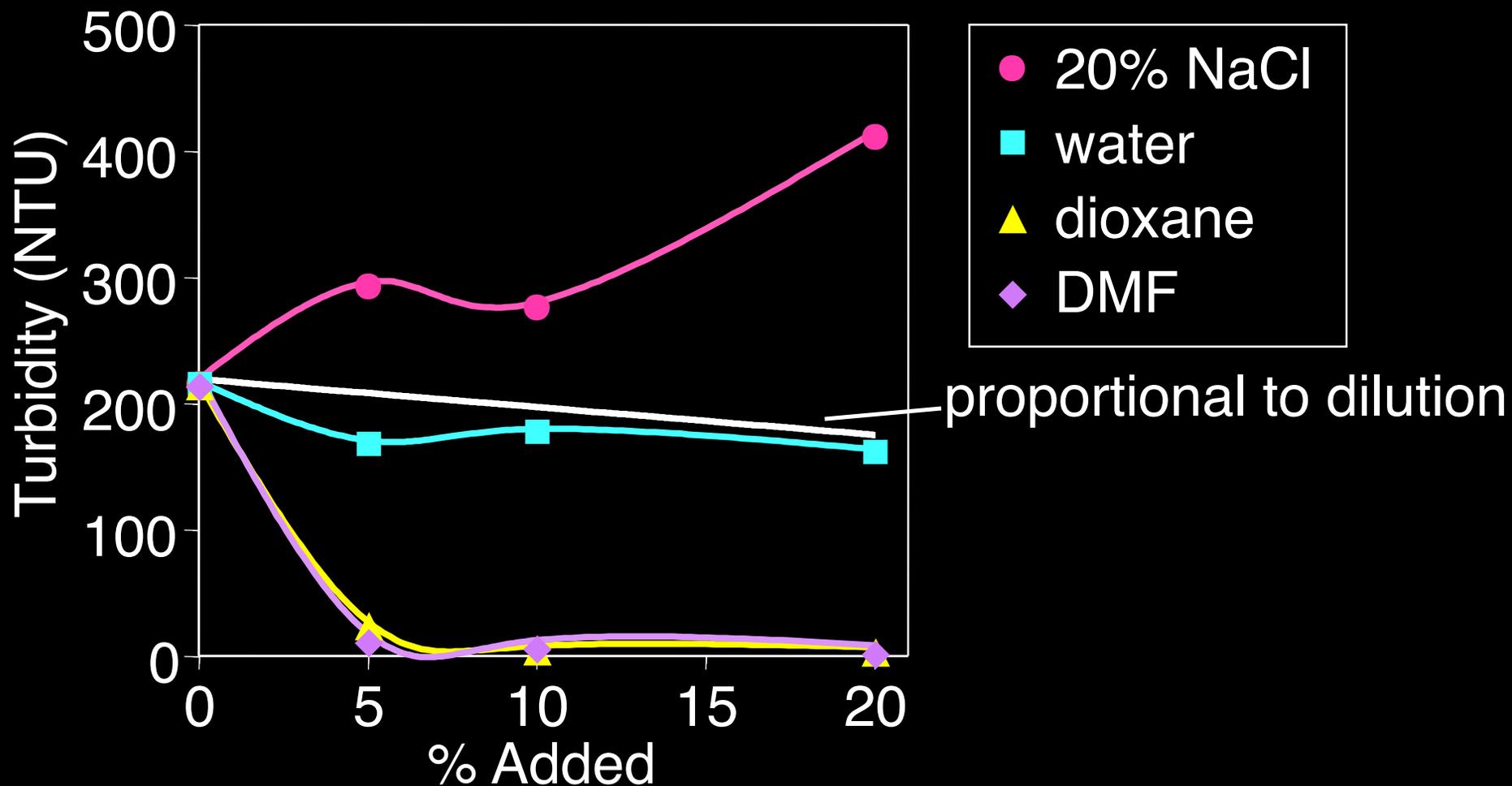
NaCl solution - highly ionic; this should compete with and reduce ionic bonding between proteins and polyphenols.

Asano et al. *JASBC* 40: 147-154, 1982.

<u>Added:</u>	<u>Haze Inhibition</u>
No addition	-
25% DMF	+
25% Dioxane	+
5% NaCl	-

Conclusion: protein-polyphenol interaction involves hydrogen and hydrophobic bonding, but not ionic bonding.

The indicated substance was added to pre-formed gliadin-catechin in haze in buffer



Data from Siebert et al. *J. Agric. Food Chem.* 44: 80-85, 1996.

Oh et al. (*J. Agric. Food Chem.* 28: 394-398, 1980) carried out model system experiments in which tannins were combined with either gelatin or polyproline.

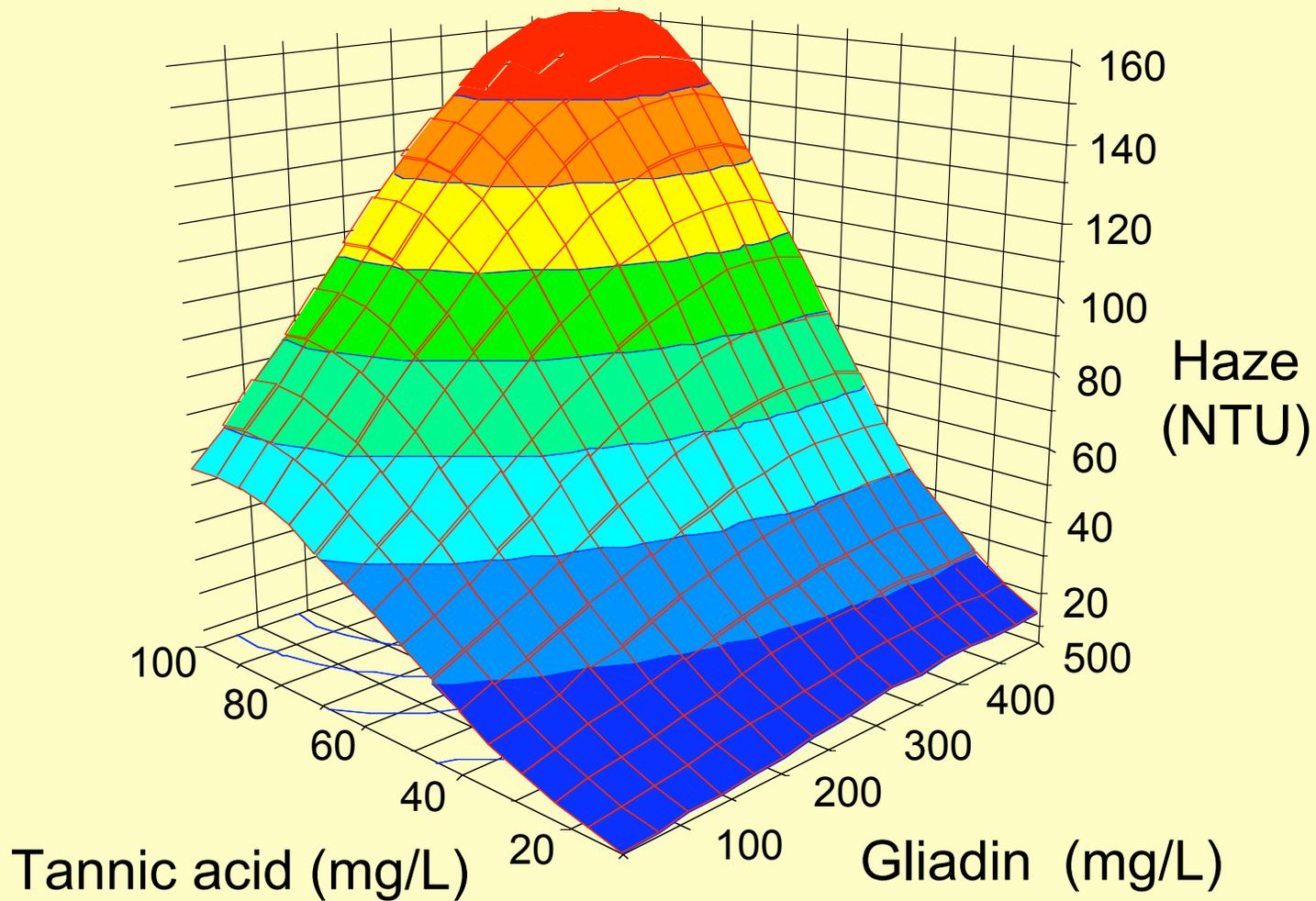
They found that if either ionic strength or temperature increased, the haze increased. They pointed out that both phenomena are characteristic features of hydrophobic bonding.

Work with buffer model systems showed that protein concentration (gliadin), polyphenol concentration (tannic acid), pH, and alcohol all affect haze intensity.

A statistical experiment design was used to collect data that was used to construct a response surface model.

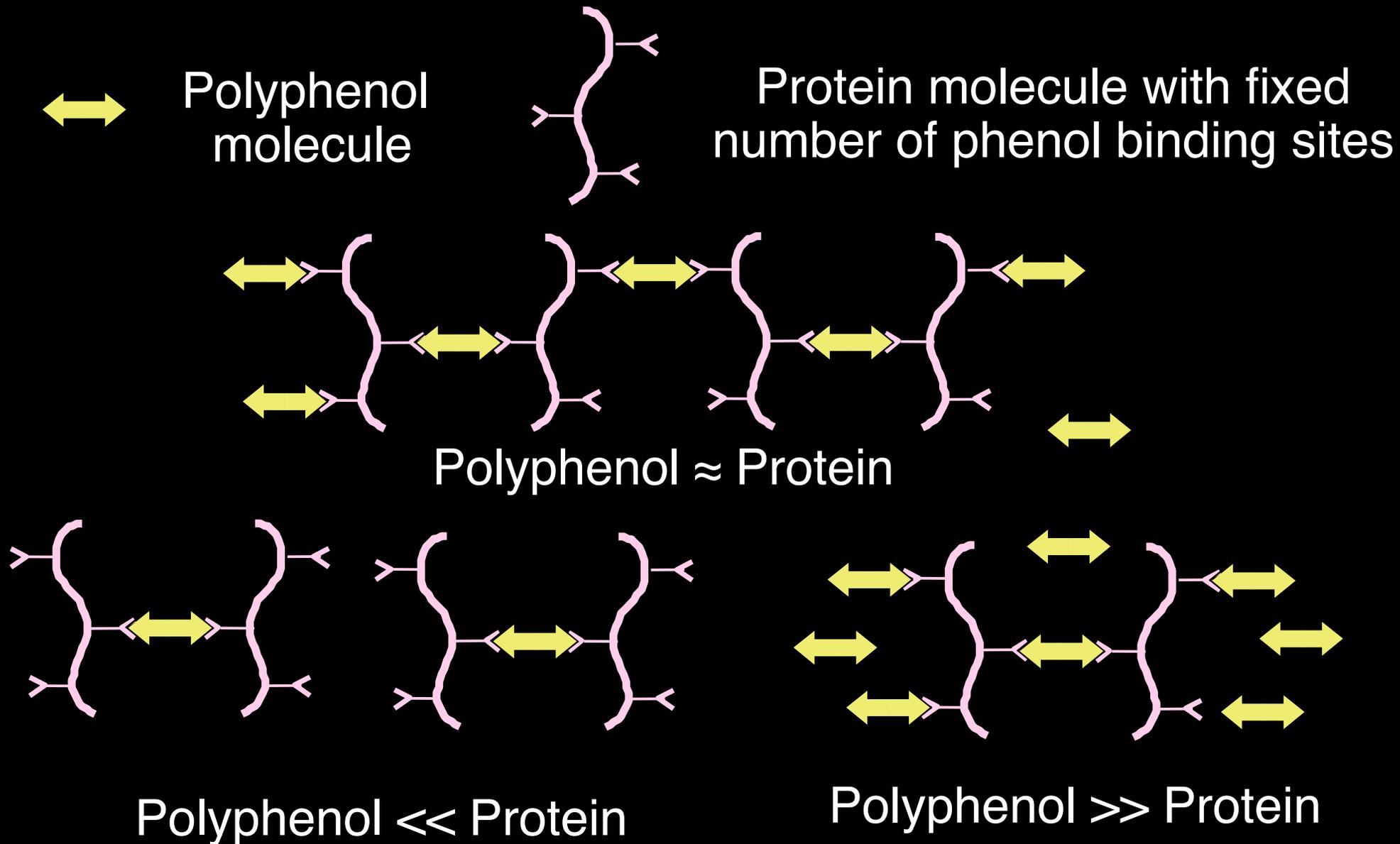
Siebert et al., *J. Agric. Food Chem.* 44: 1997-2005, 1996

# Behavior of Gliadin - Tannic Acid Model System



Siebert et al., *J. Agric. Food Chem.* 44: 1997-2005, 1996

# Concept of Protein-Polyphenol Interactions

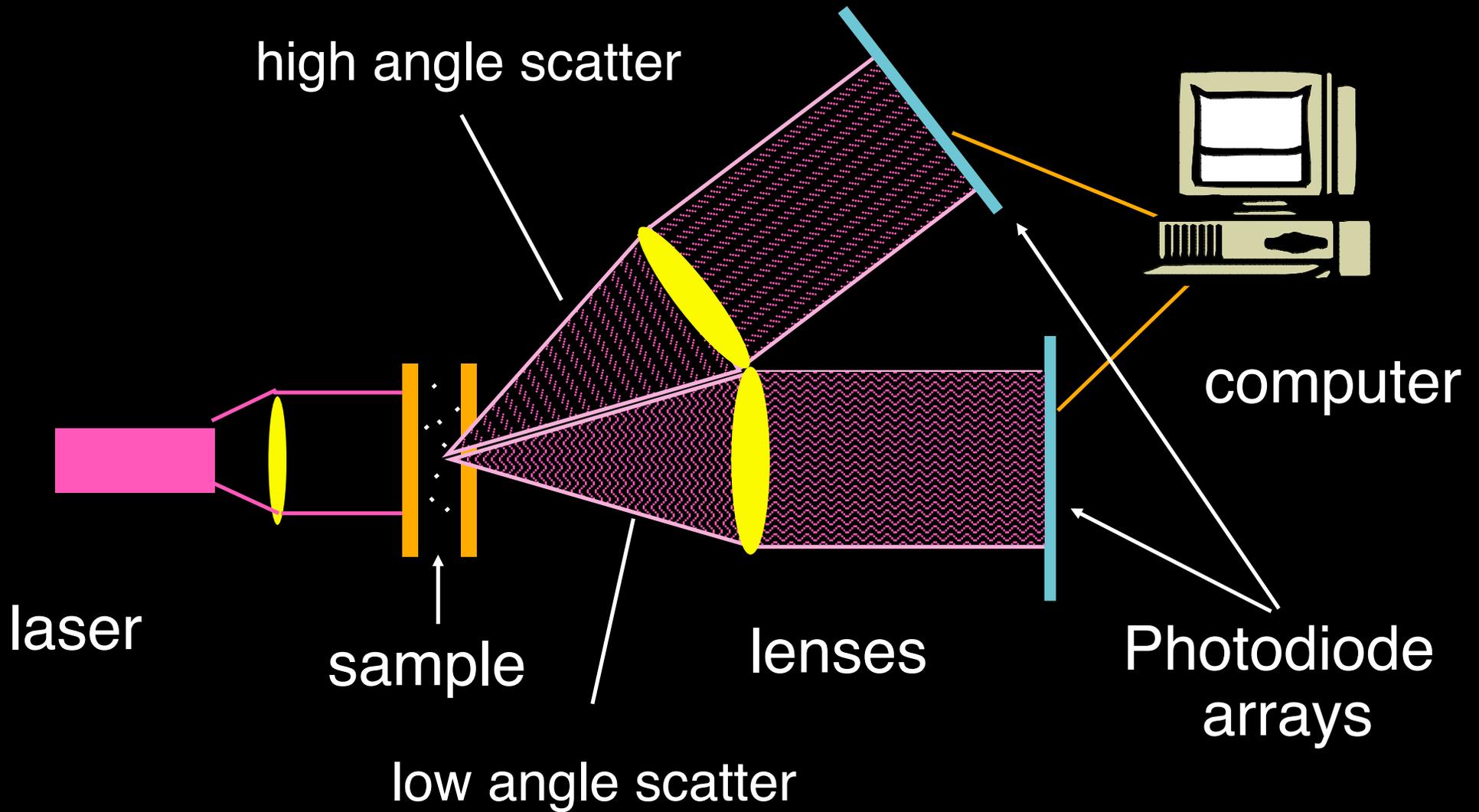


Siebert et al., *J. Agric. Food Chem.* 44: 80-85, 1996.

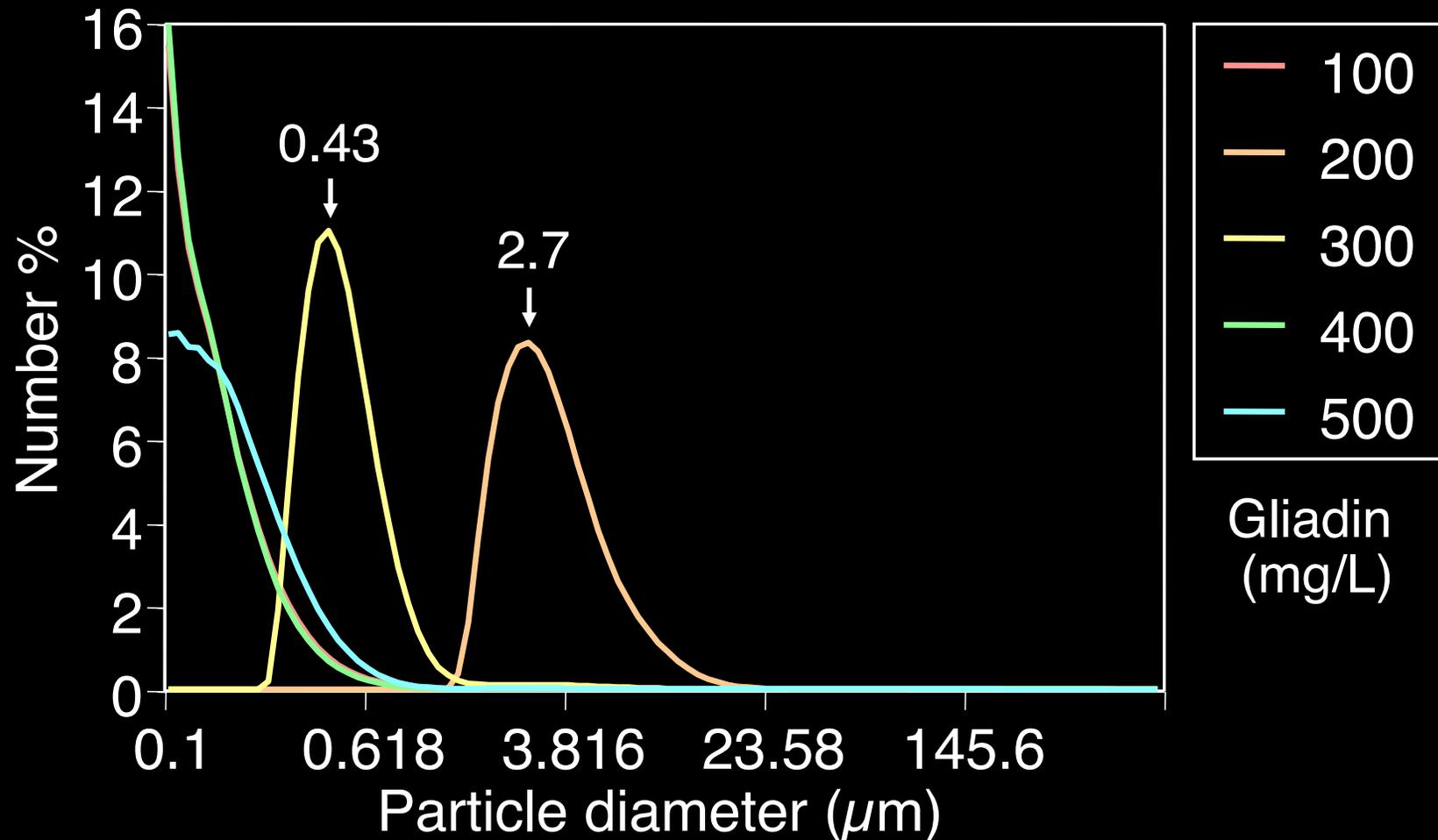
# Laser Diffraction Particle Size Analyzer



# Laser Diffraction Particle Size Analyzer

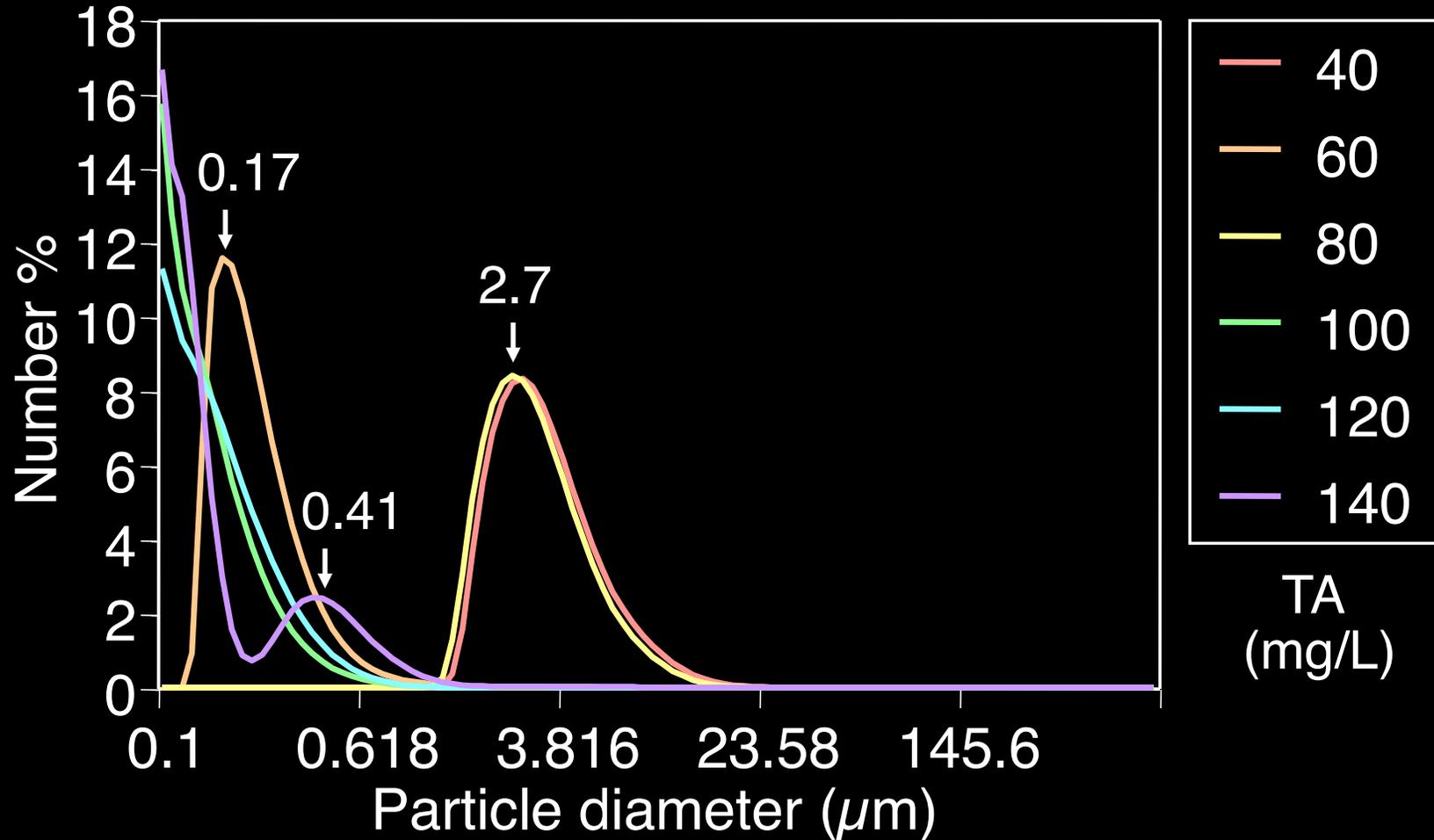


# Particle size distribution plots for 40 mg/L TA at pH 4.5



Siebert & Lynn, *J. Amer. Soc. Brew. Chem.* 58: 117-123, 2000.

# Particle size distribution plots for 200 mg/L Gliadin at pH 4.5

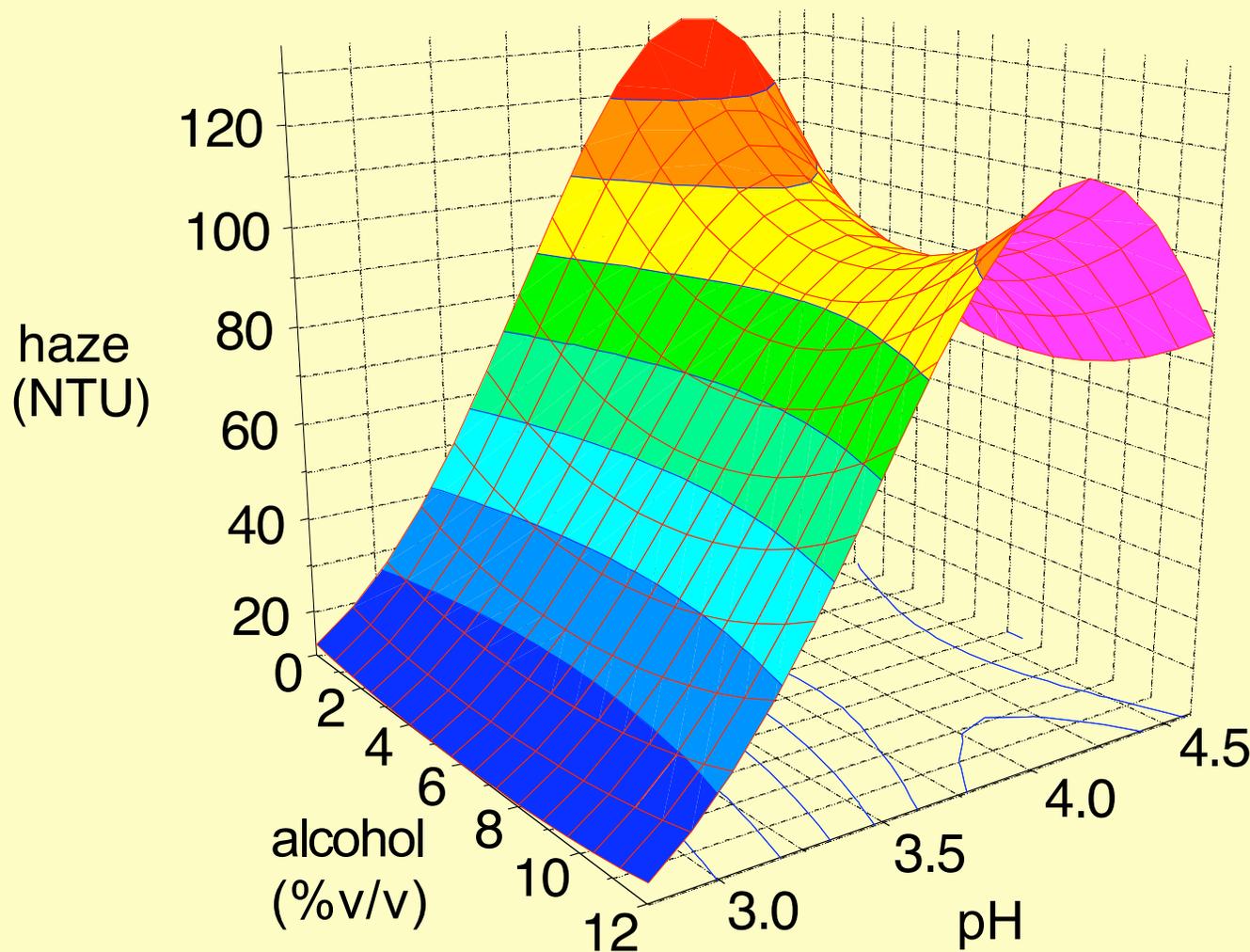


Siebert & Lynn, *J. Amer. Soc. Brew. Chem.* 58: 117-123, 2000.

Basically the results confirmed the mechanistic concept proposed earlier. The results showed differences in particle size with different gliadin/TA proportions. Larger particles were seen with intermediate ratios of protein/polyphenol than with either lower or higher ratios.

The particle sizes are amazingly quantized. Instead of gradual shifts of a monomodal distribution, particles of distinctly different particle size were seen under different conditions.

# Behavior of Gliadin - Tannic Acid Model System



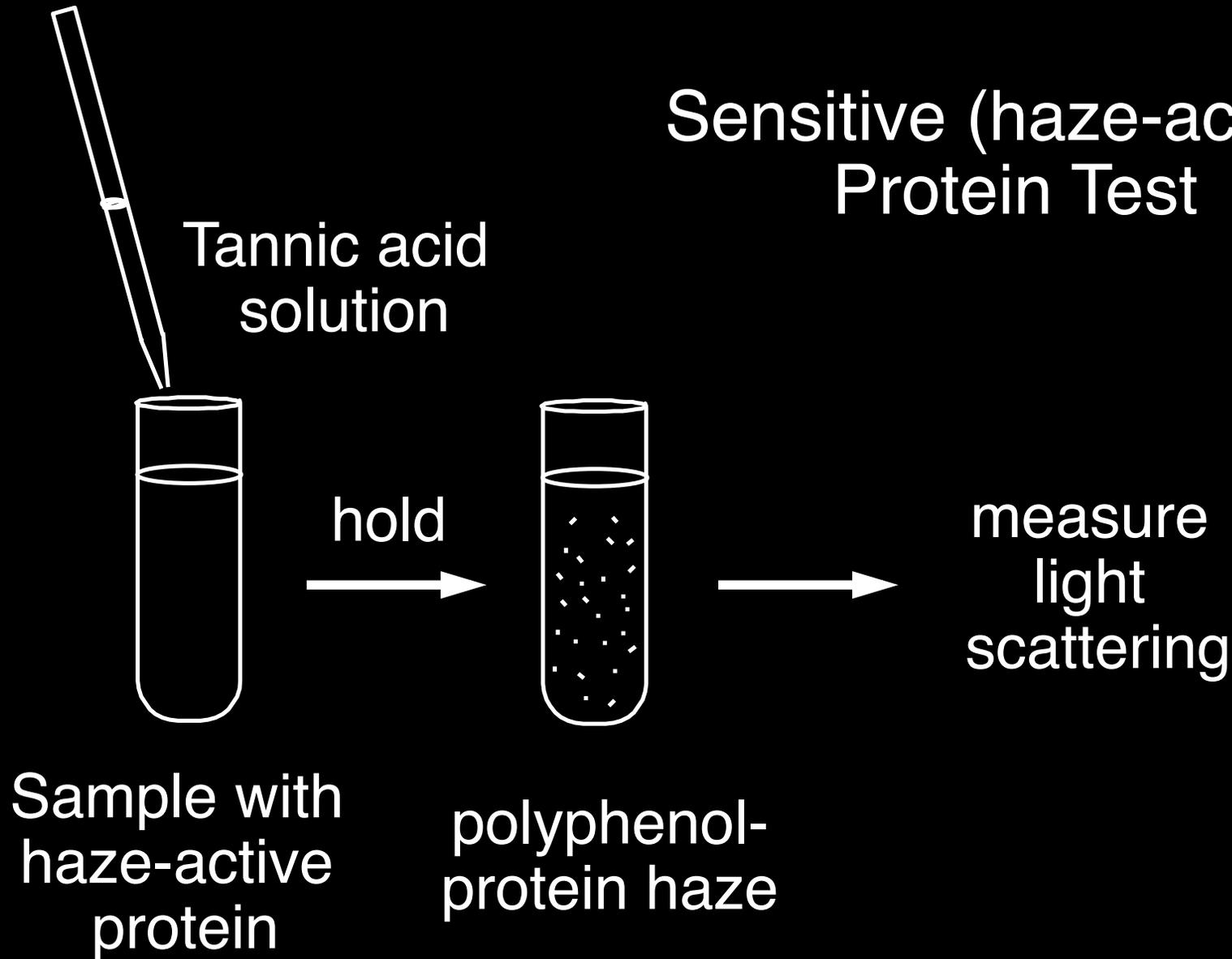
Siebert et al., *J. Agric. Food Chem.* 44: 1997-2005, 1996

The effect of pH on haze intensity is striking. About 7 times as much haze was produced with the same amounts of protein and polyphenol when pH increased from 3 to slightly above 4. Further increases in pH resulted in declines in haze intensity.

Additional studies showed the decline continued until at least pH 6.5.

# Beverage Haze

# Sensitive (haze-active) Protein Test



## Haze (NTU) Produced by Addition of Various Amounts of Tannic Acid (TA) to Beverages

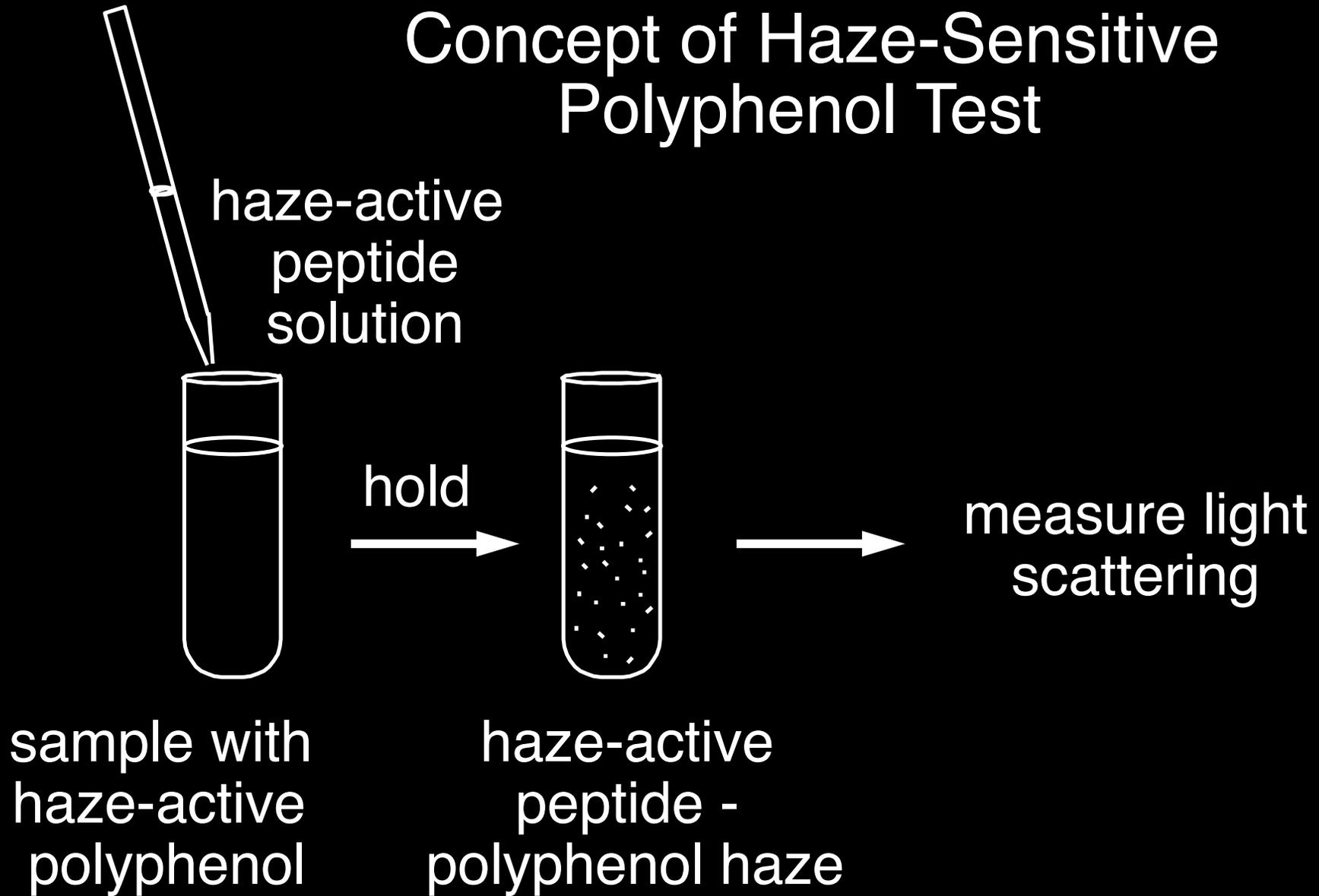
<u>TA (g/L)</u>	<u>Apple Juice 1</u>	<u>Apple Juice 2</u>	<u>Unstab. Juice</u>	<u>Beer 1</u>	<u>Beer 2</u>
0.00	16	0	56	4	2
0.50	18	2	62	2357	1811
1.25	19	2	68	4174	3393
2.50	23	3	71	5302	4244

Siebert et al., *J. Agric. Food Chem.* 44: 1997-2005, 1996

So beer has a lot of haze-active (HA) protein while apple (and most other fruit) juice has very little.

Grape juice and wine also have very little.

# Concept of Haze-Sensitive Polyphenol Test



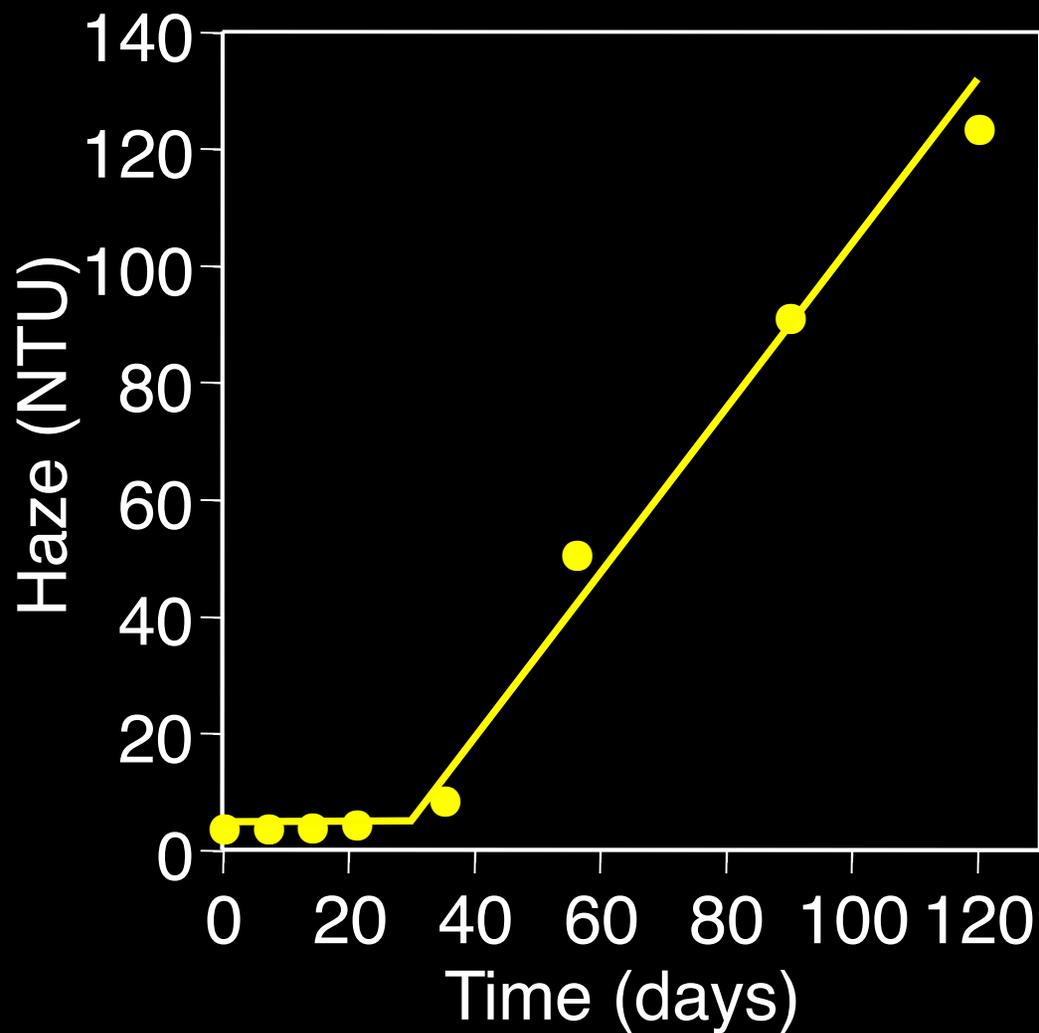
## Haze (NTU) Produced by Addition of Various Amounts of Gelatin to Beverages

<u>gelatin</u> <u>(mg/L)</u>	<u>Apple</u> <u>Juice 1</u>	<u>Apple</u> <u>Juice 2</u>	<u>Unstab.</u> <u>Juice</u>	<u>Beer 1</u>	<u>Beer 2</u>
0	17	2	93	4	2
100	254	62	168	5	2
200	307	24	243	5	2
400	329	11	289	5	3

Siebert et al., *J. Agric. Food Chem.* 44: 1997-2005, 1996

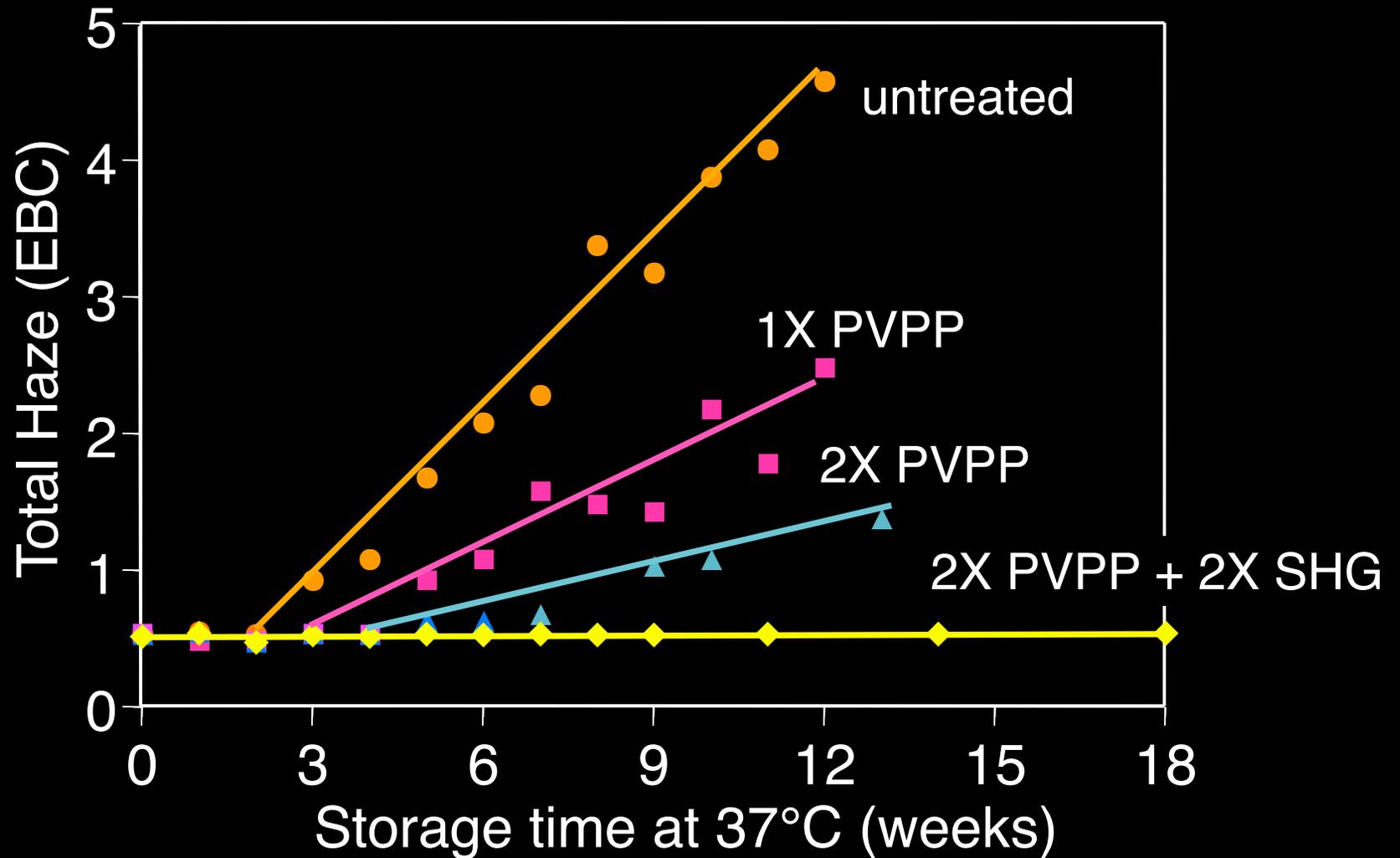
# Time Course of Haze Development

# Time Course of Protein-Polyphenol Haze Development



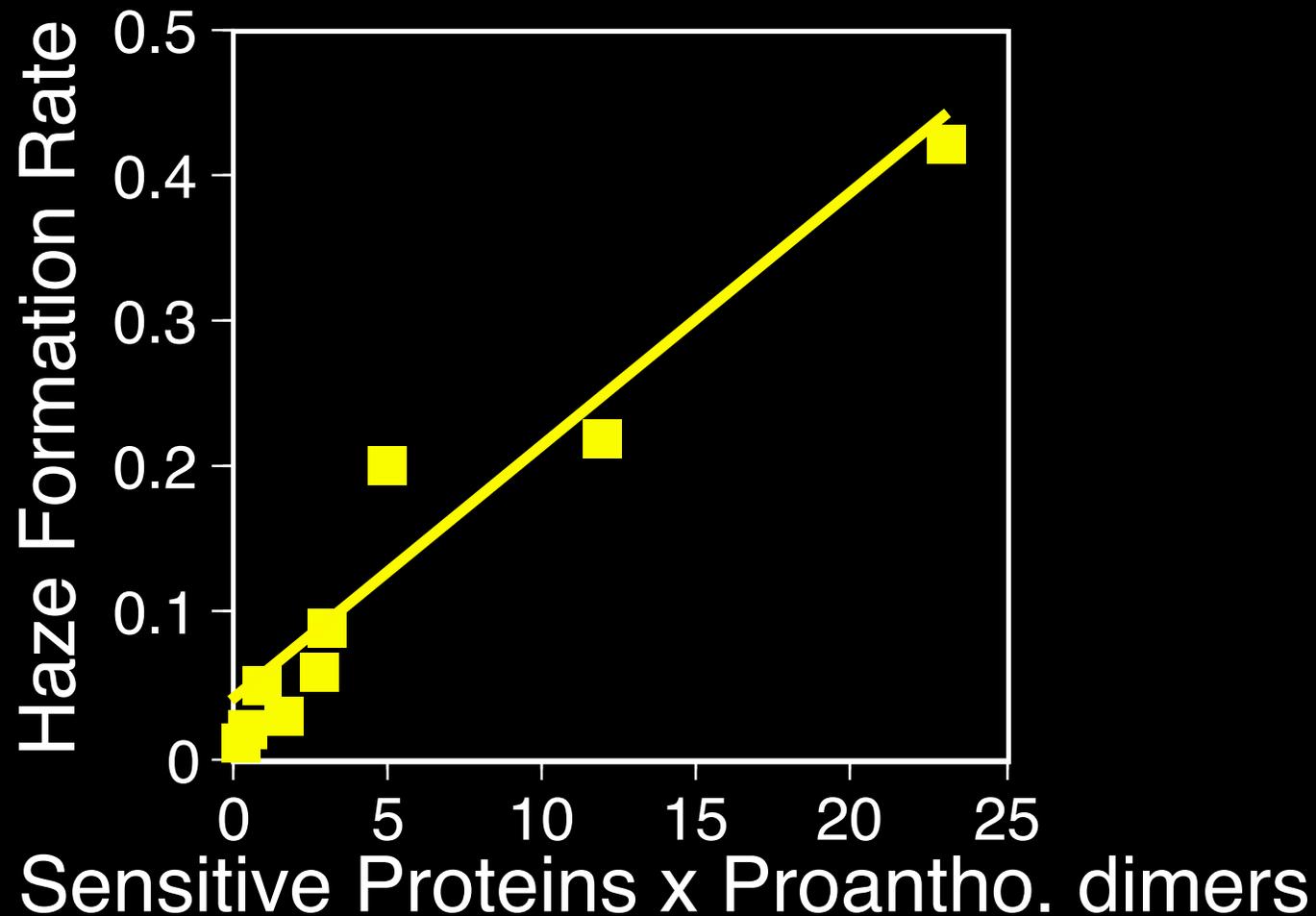
Siebert, *J. Agric. Food Chem.* 47: 353-362, 1999

# Haze Development in Beer Treated With PVPP/SHG



McMurrough et al., *J. Amer. Soc. Brew. Chem.* 50: 67-76, 1992.

# Haze Development Model for Beer



McMurrough et al. *J. Amer. Soc. Brew. Chem.* 50: 67-76, 1992.

## Implications of relationship

$$\Delta(\text{haze})/\text{time} = [\text{HA protein}] \cdot [\text{dimeric proanthos}]$$

Stabilization can be achieved by reducing HA protein, HA polyphenol (dimeric proanthocyanidins), or both.

Reducing HA protein by 50% should be equal in effect to reducing HA polyphenol by an equal percentage.

# Beverage Stabilization

Beer is usually stabilized in some way to delay the onset of protein-polyphenol haze formation beyond the intended product shelf-life.

# Methods of Colloidal Stabilization

## Proteolytic Enzymes

Papain, bromelain, etc.

Proline specific protease

## Cold Sedimentation

## Fining agents

Proteins (gelatin, isinglass)

Tannins (tannic acid)

## Adsorbents

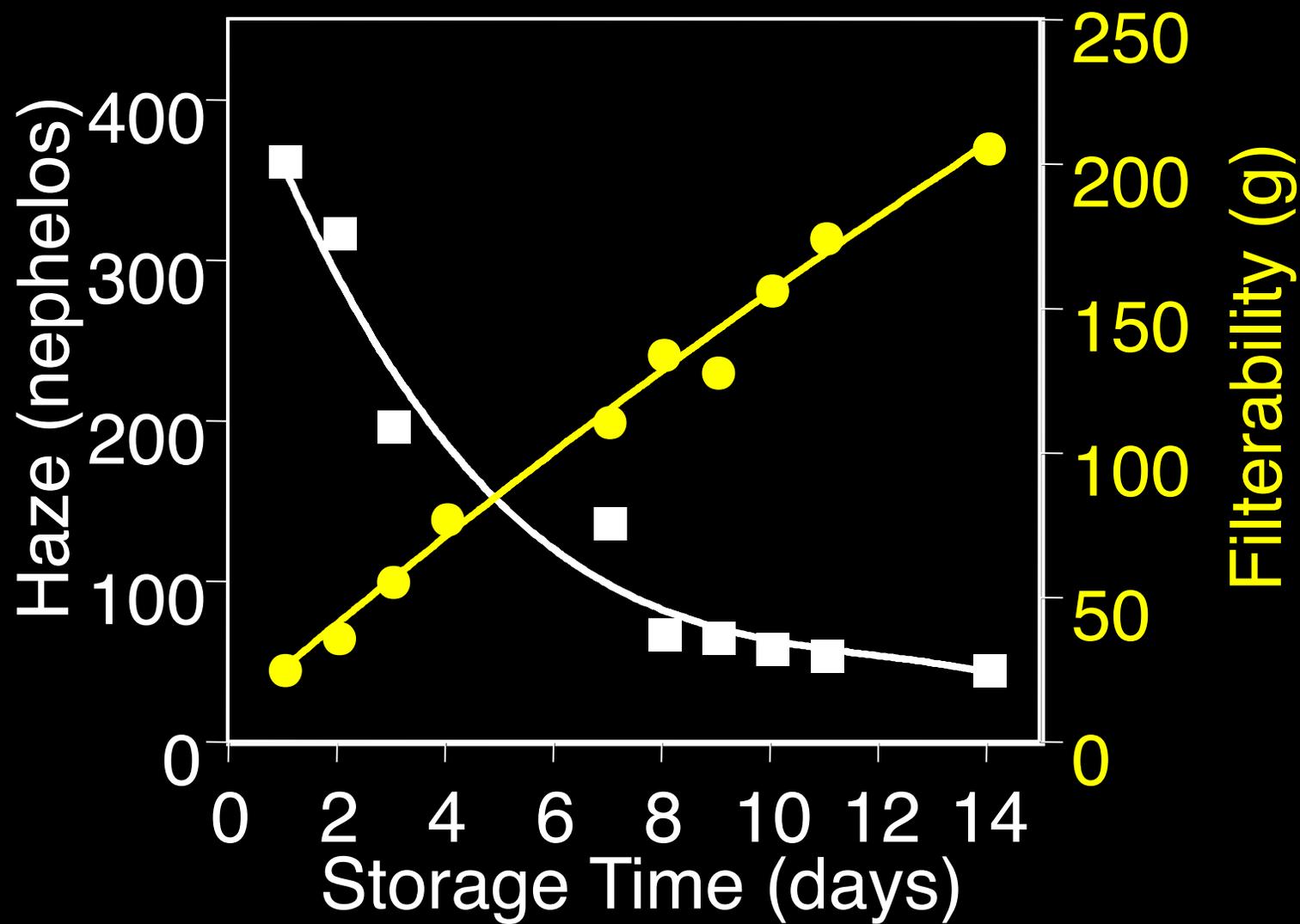
Protein adsorbents (bentonite, silica gels)

Polyphenol adsorbents (polyvinylpolypyrrolidone)

Withdrawing energy from a system by chilling, either during maturation or in the package, can result in greater precipitation of larger and denser particles.

It can also result in loss of solubility of some marginally soluble material, forming more colloidal matter.

During maturation the prolonged chilling tends to favor precipitation of particles. This leads to turbidity decreases and filterability increases. Both are related to removal of colloidal particles through sedimentation.



Siebert et al., *MBAA TQ 24*: 1-8, 1987.

Ideally, brewers would like to settle colloidal material out during cold maturation.

This spares the load on filters, leads to more efficient operation and longer filter runs.

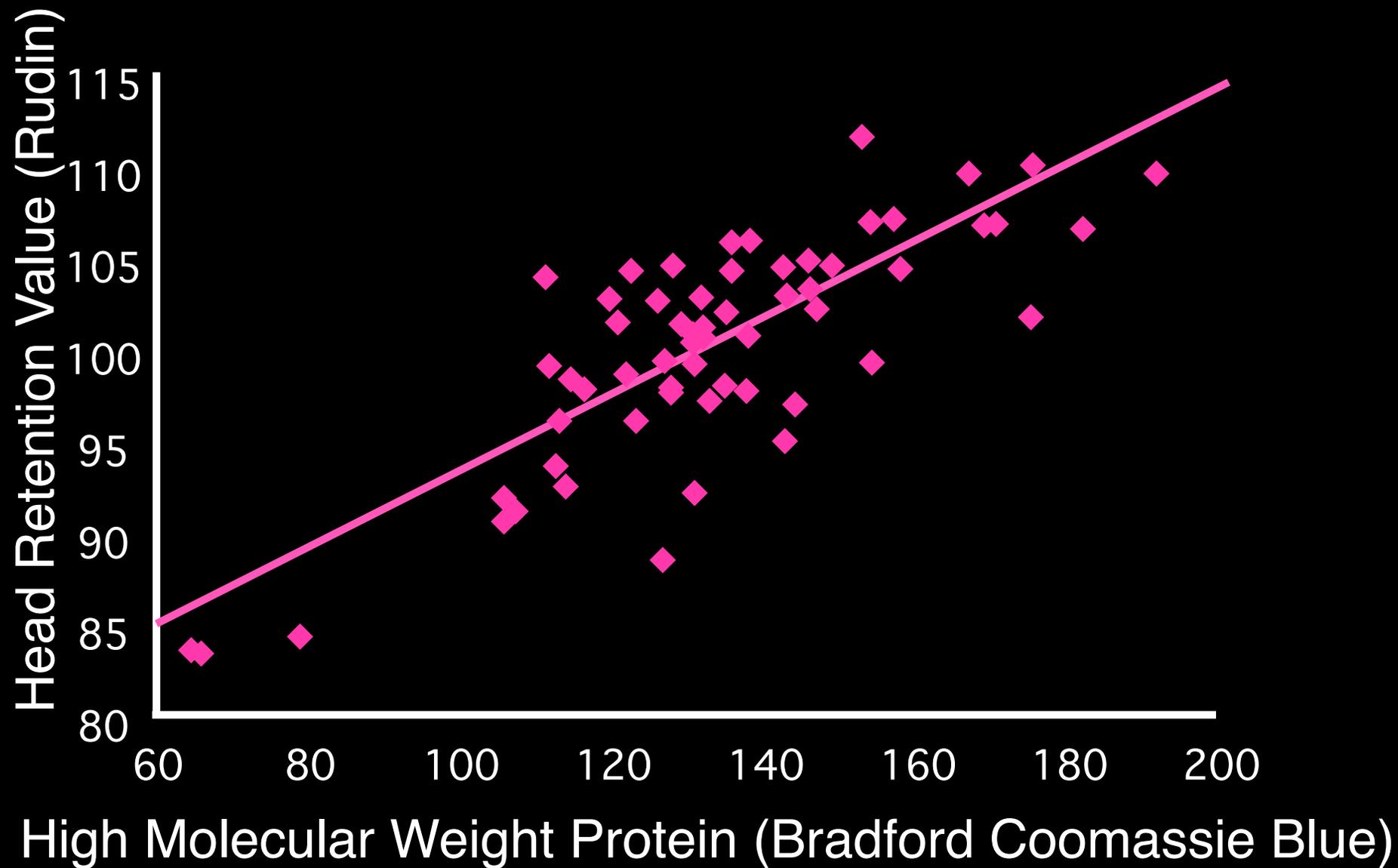
However, this requires long times at low temperatures, which is uneconomical.

Fining agents can speed up this process. Fining agents are generally either haze-active (HA) proteins (gelatin, isinglass) or HA polyphenols (tannic acid).

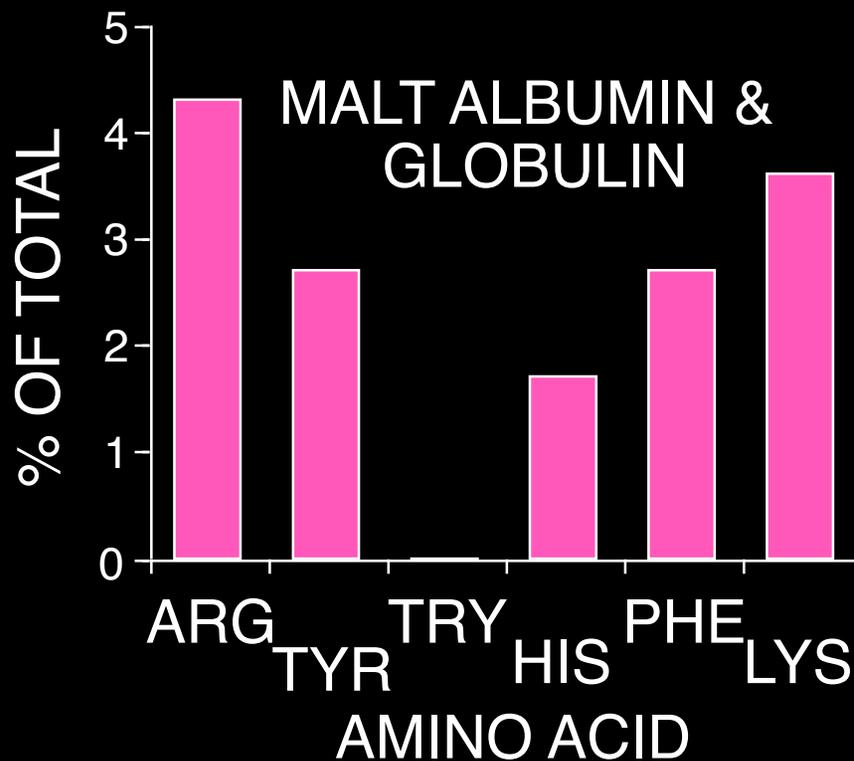
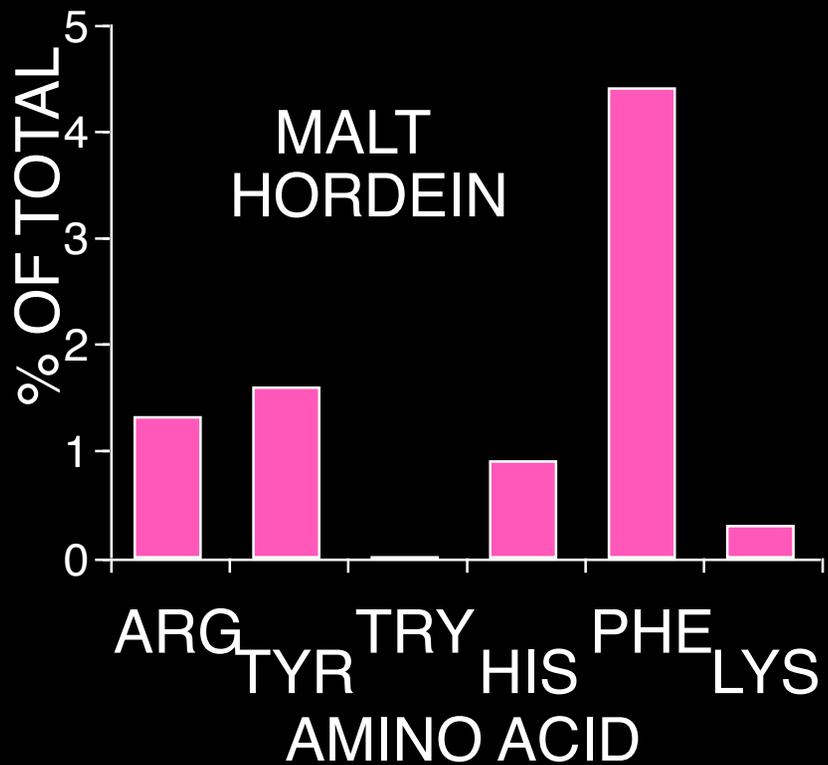
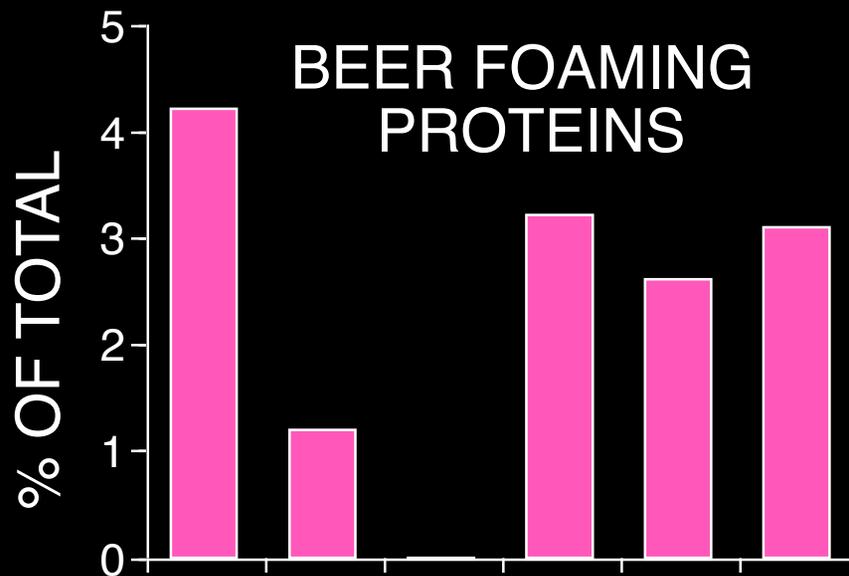
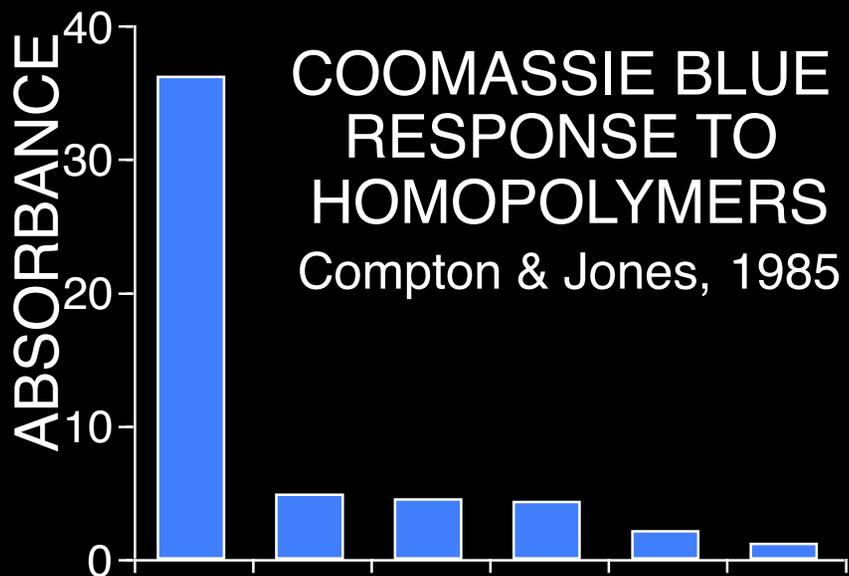
Foam is an important property of beer that, like haze, involves protein. The protein involved in foam has a different composition than that involved in haze.

However, methods of stabilization that non-selectively remove protein are destructive to foam and so unacceptable.

It has been observed that the response of a commonly used protein assay (Bradford Method, based on Coomassie blue dye binding) gives a response in beer that correlates with foam performance.



Siebert & Knudson, *MBAA Tech. Quart.* 26: 139-146, 1989.



In beer, Coomassie blue dye binding (as used in the Bradford method for protein measurement) virtually ignores the large amount of haze-active hordein and responds to the smaller amount of foam-active albumins and globulins.

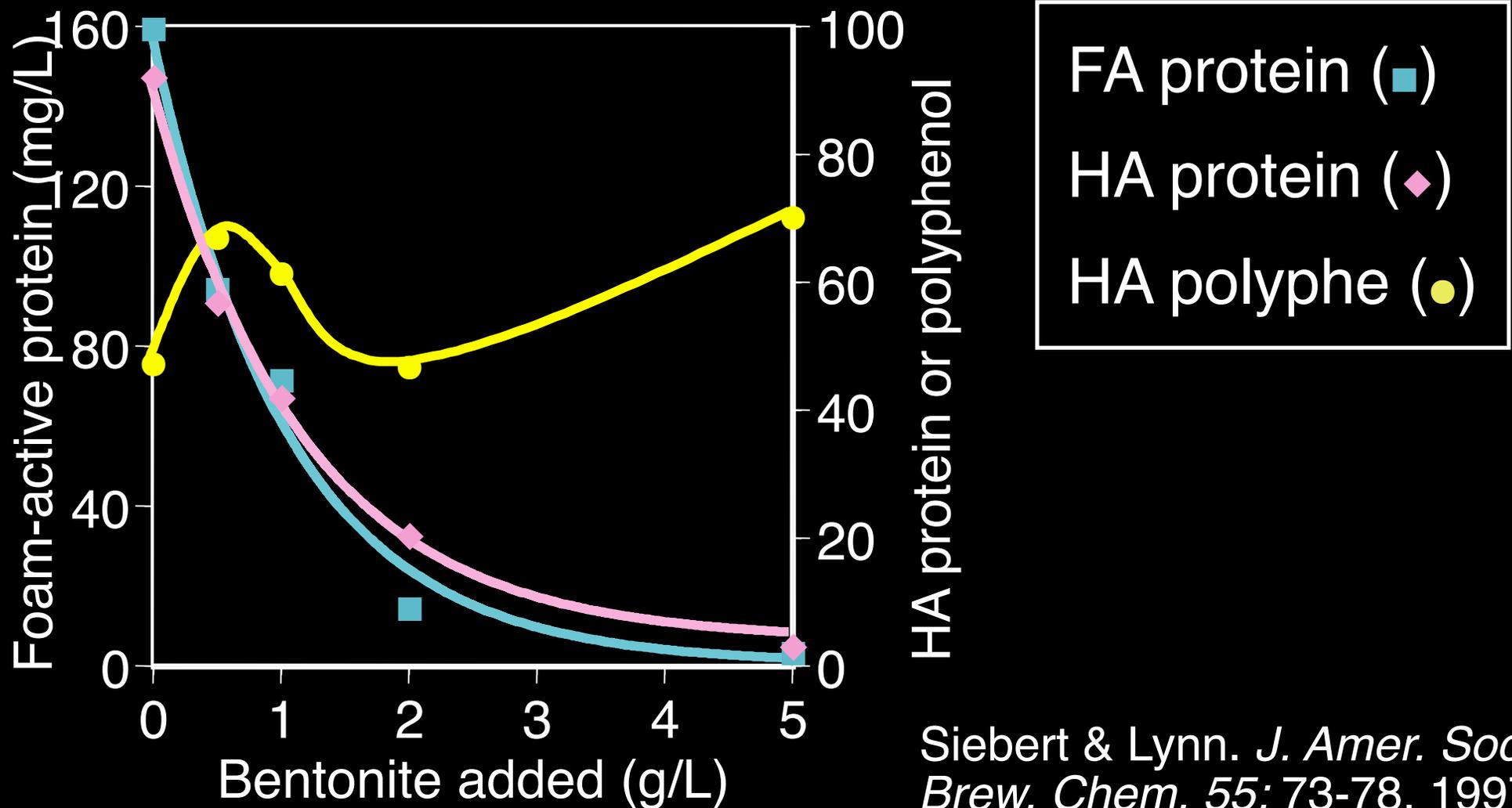
Coomassie blue can then be used as a direct assay of foam-active protein in beer.

Siebert & Knudson, The relationship of beer high molecular weight protein and foam, *MBAA Tech. Quart.* 26: 139-146, 1989.

Unchillproofed beer was treated with different amounts of each of several adsorbents (bentonite, silica, PVPP).

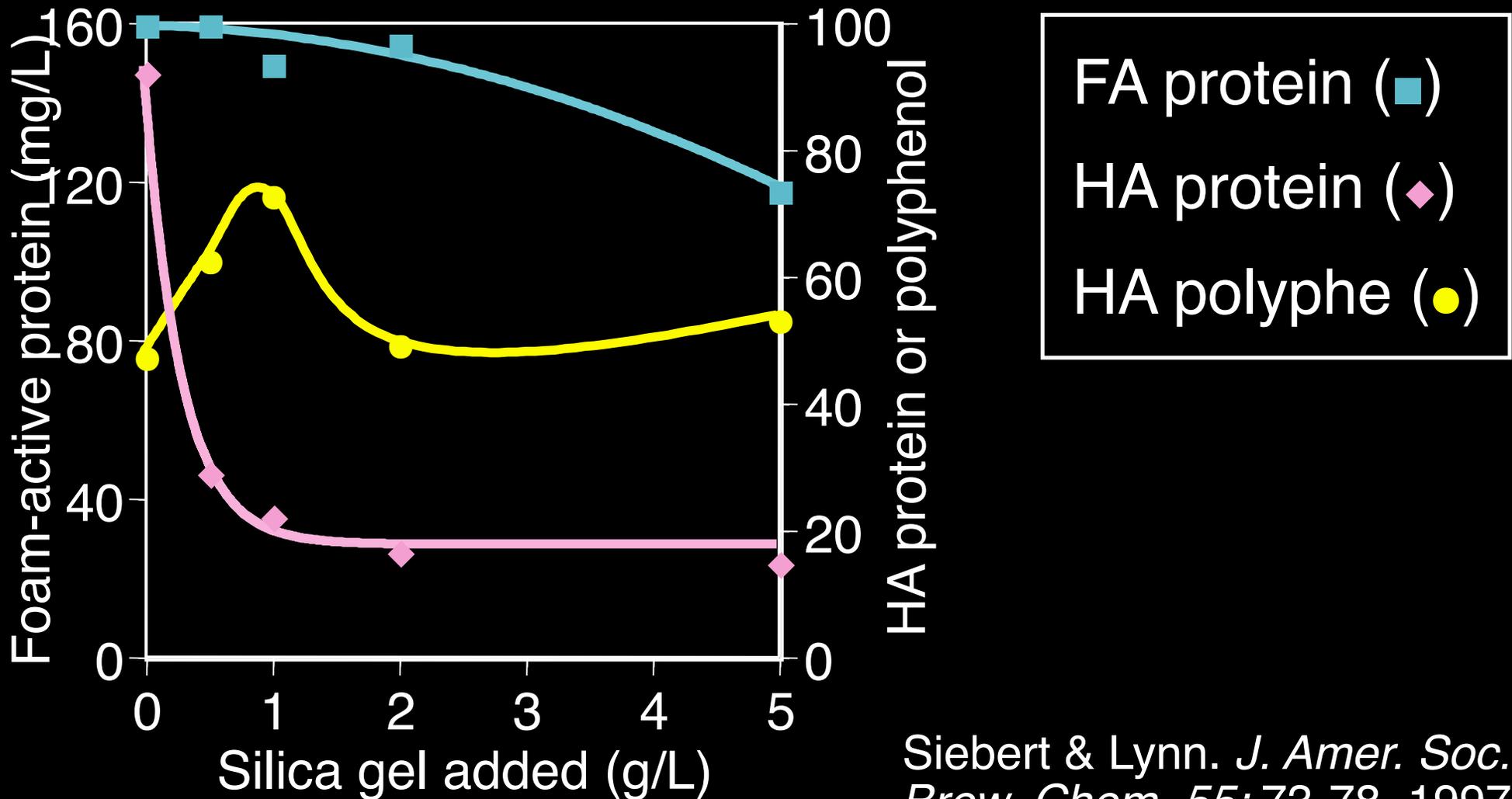
The haze-active protein, foam-active protein (by Coomassie blue dye binding) and haze-active polyphenol levels were estimated.

# The Effects of Bentonite on Haze-Active (HA) and Foam Active (FA) Beer Constituents



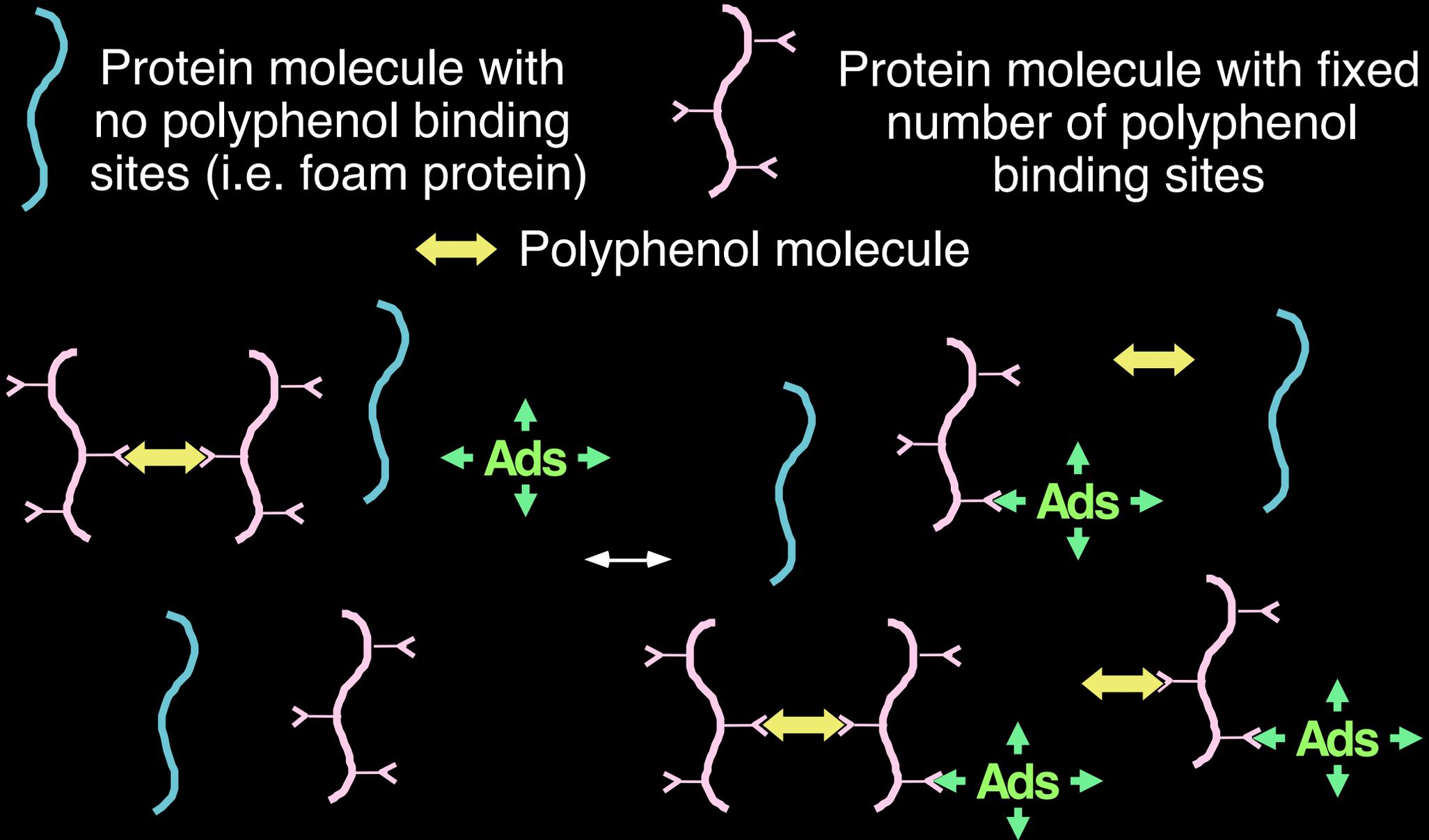
Siebert & Lynn. *J. Amer. Soc. Brew. Chem.* 55: 73-78, 1997.

# The Effects of Silica Gel on Haze-Active (HA) and Foam Active (FA) Beer Constituents

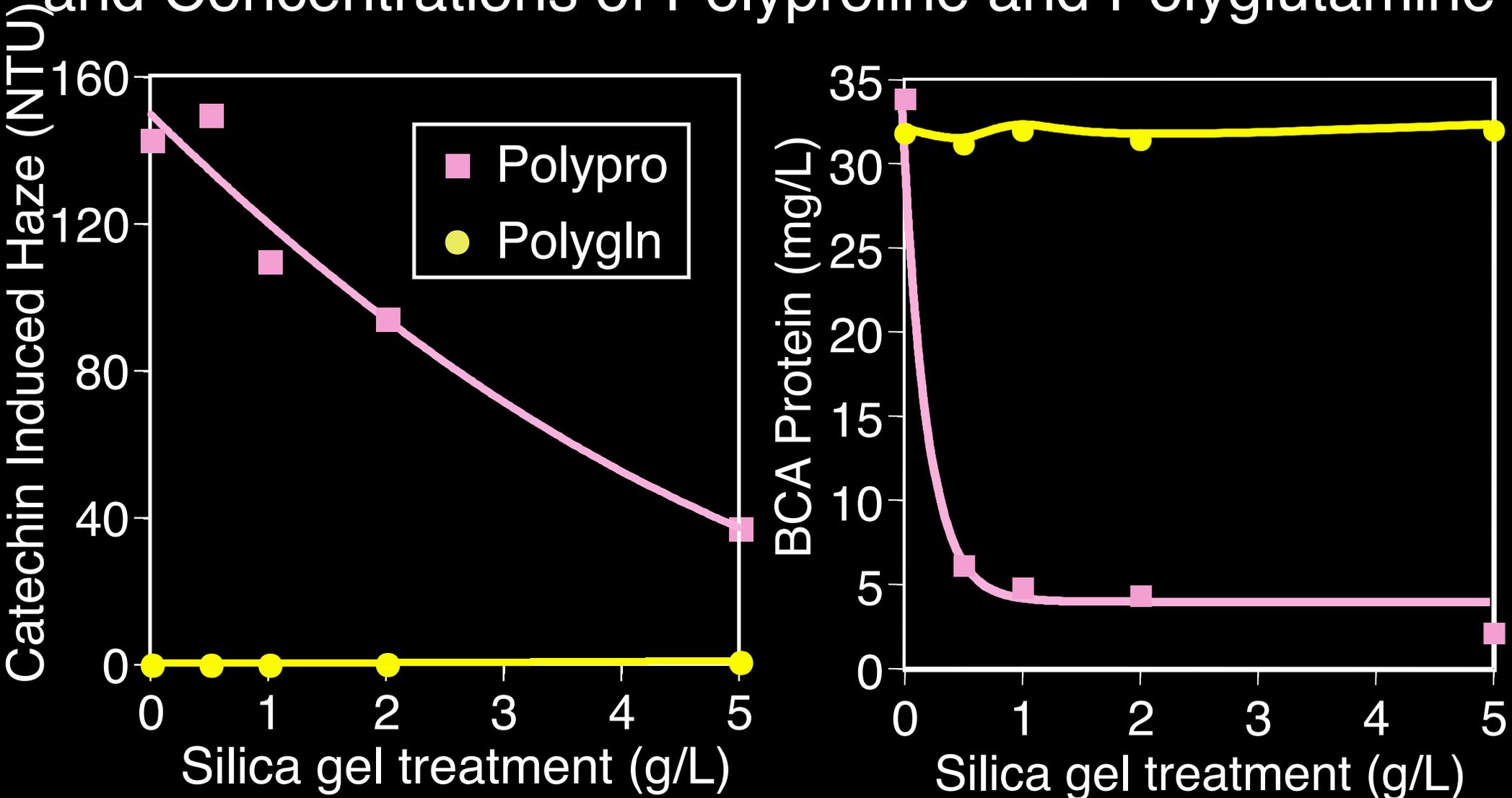


Siebert & Lynn. *J. Amer. Soc. Brew. Chem.* 55: 73-78, 1997.

# Possible Protein Adsorbent Action



# Effect of Silica Gel on the Haze Forming Activities and Concentrations of Polyproline and Polyglutamine



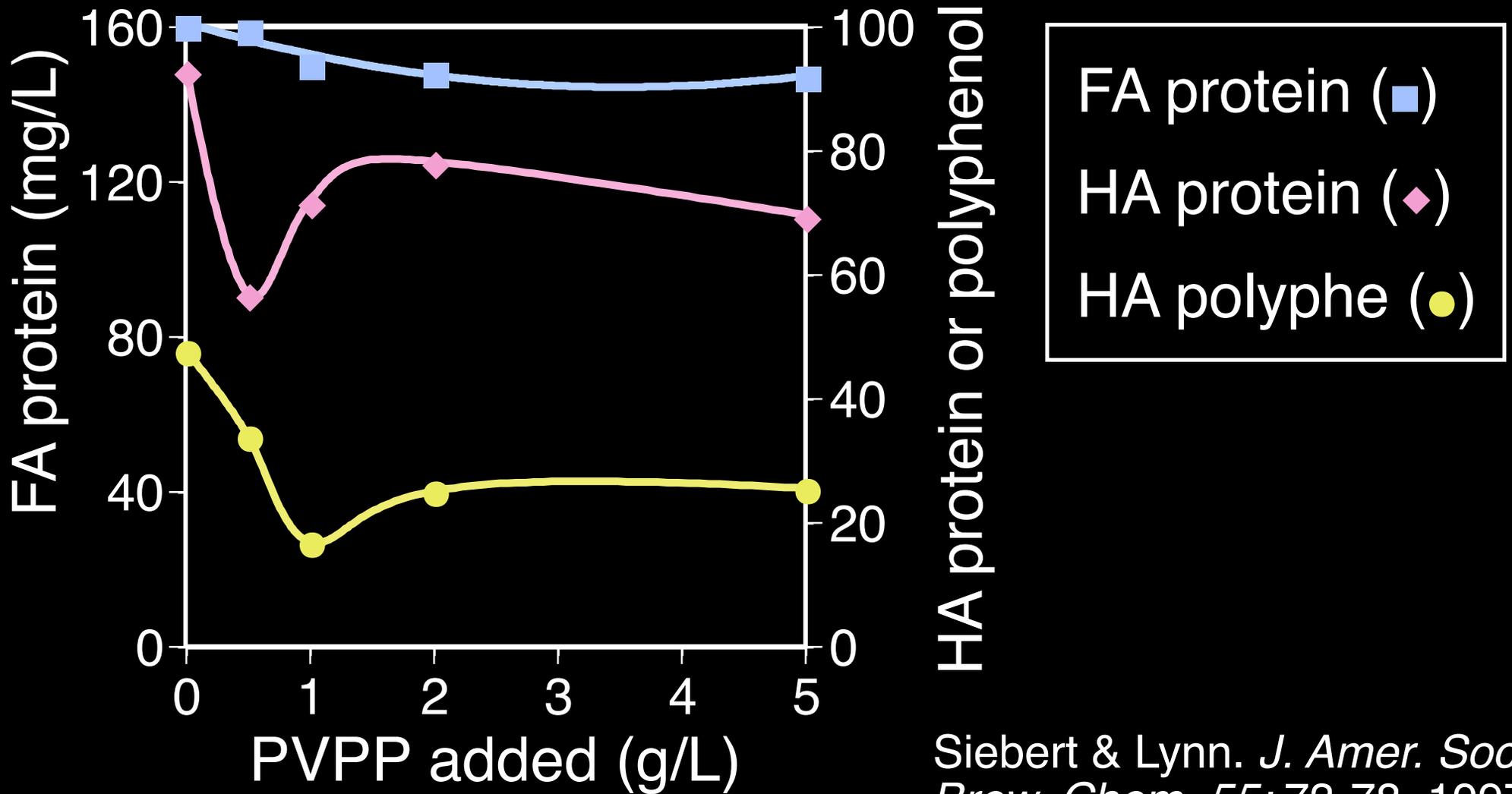
Siebert & Lynn. *J. Amer. Soc. Brew. Chem.* 55: 73-78, 1997.

Bentonite indiscriminately removes protein including both haze-active and foam-active protein.

Silica specifically removes most of the beer haze-active protein with little effect on foam-active protein at typical treatment levels.

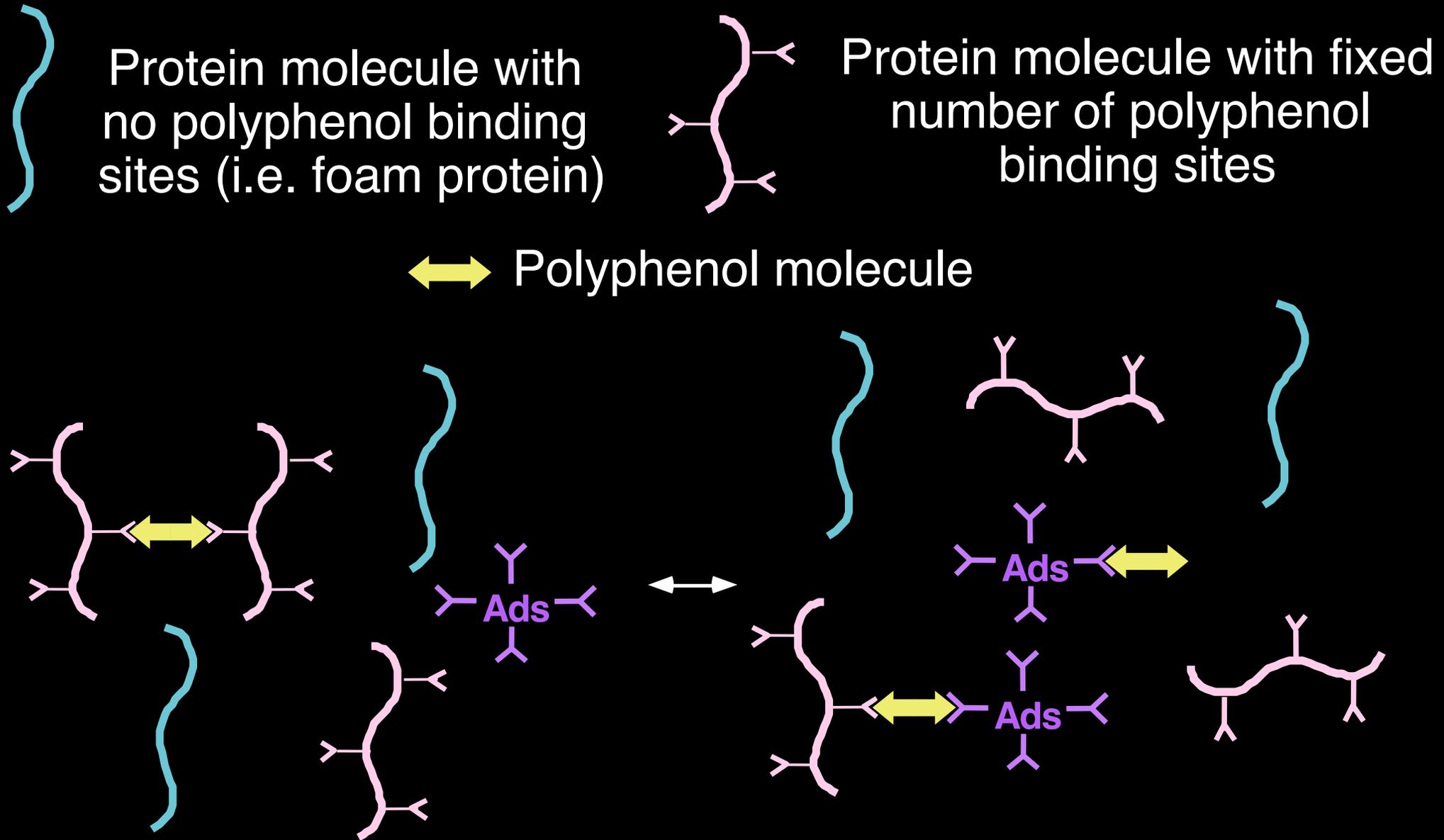
Silica selectivity is due to chemical interaction, and not pore or particle sizes other than their effects on accessible surface area.

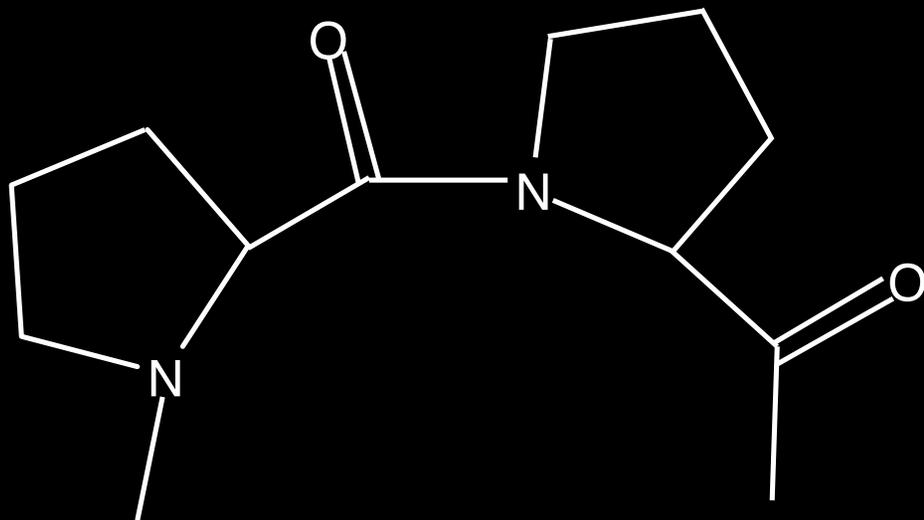
# The Effect of PVPP on Haze-Active (HA) and Foam-Active (FA) Beer Constituents



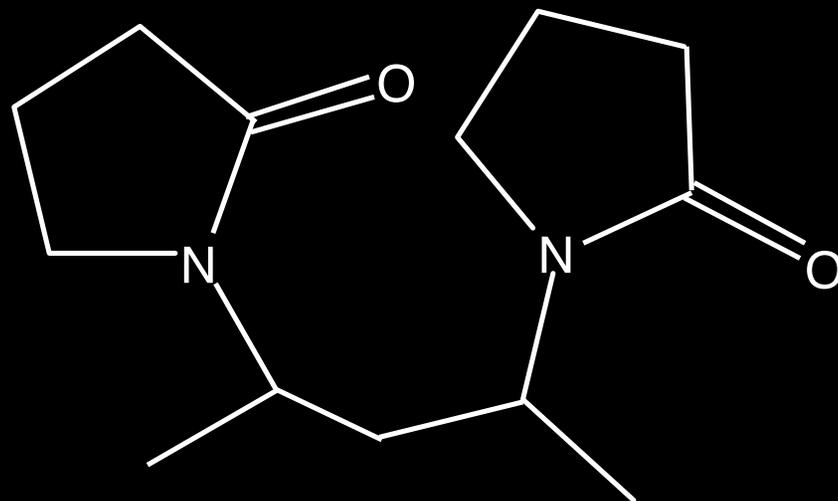
Siebert & Lynn. *J. Amer. Soc. Brew. Chem.* 55: 73-78, 1997.

# Possible Polyphenol Adsorbent Action





Polyproline  
segment



PVPP  
segment

# PVPP

In beer, PVPP removed about 50% of HA polyphenol and about 20% of HA protein.

# Factors Influencing Haze Particle Size

Haze particle size affects

Sedimentation

Cold storage

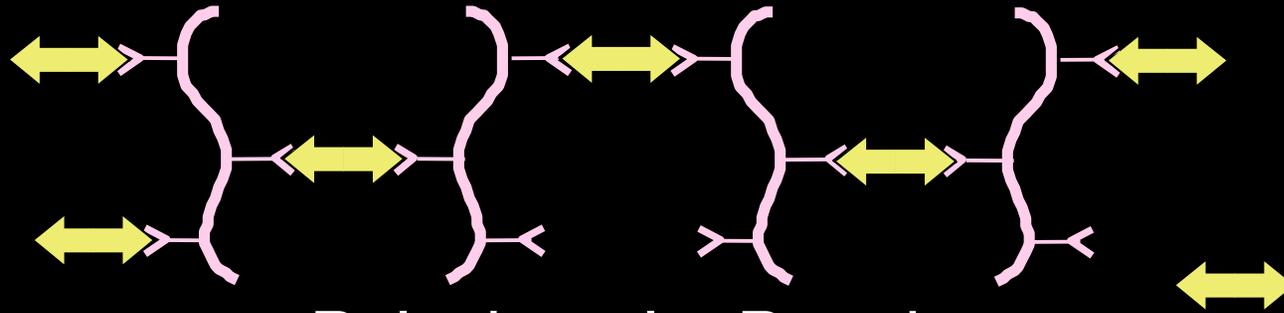
Centrifugation

Filtration

Mash separation

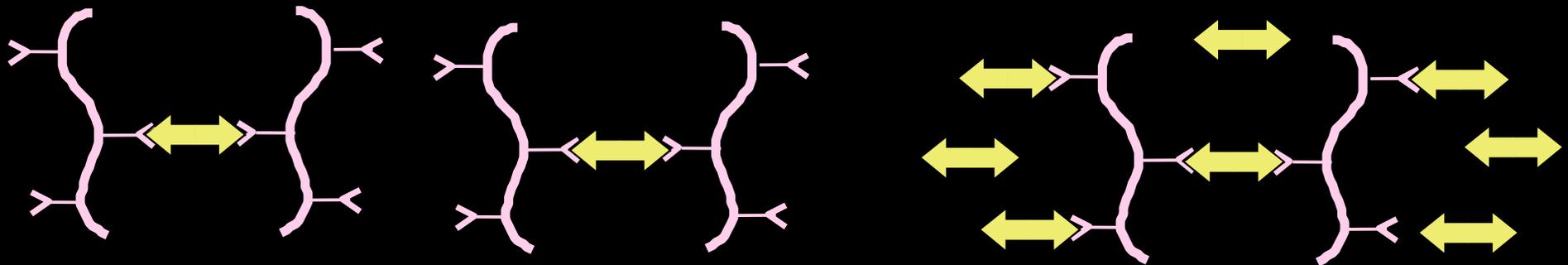
Final filtration

## Larger Particle Size



Polyphenol  $\approx$  Protein

## Smaller Particle Size



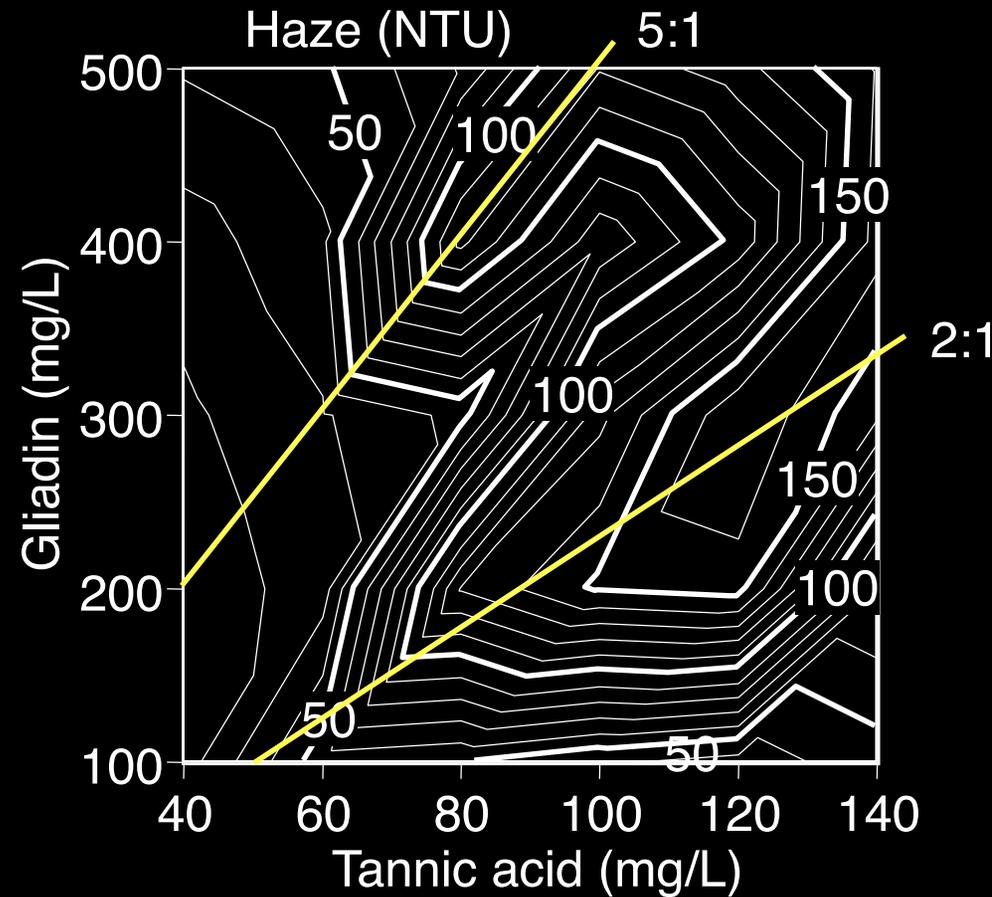
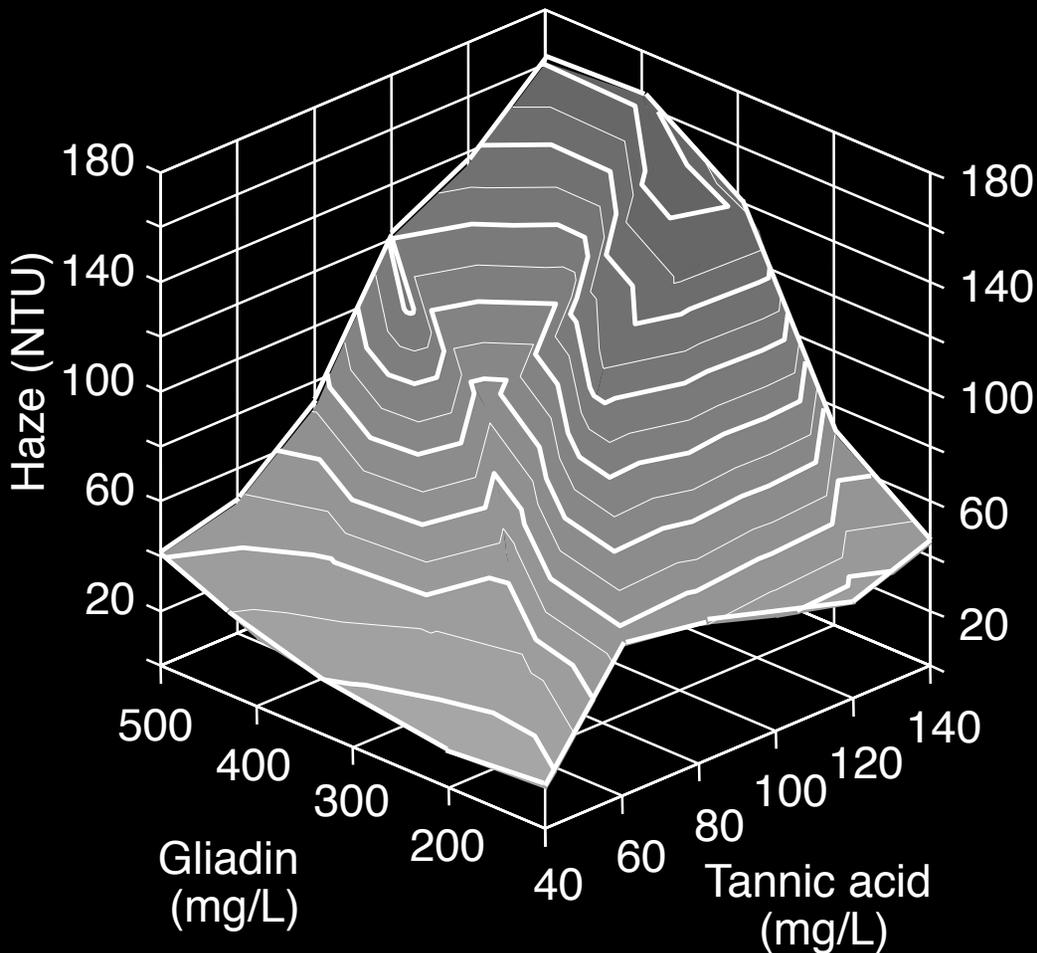
Polyphenol  $\ll$  Protein

Polyphenol  $\gg$  Protein

A more detailed study was carried out in pH 4.5 buffer model systems (0% ethanol) in which each of 5 concentrations of gliadin were combined with each of 6 levels of tannic acid. The haze intensities were measured and particle size distribution patterns were estimated with a laser diffraction particle size analyzer.

Siebert & Lynn, Effect of protein/polyphenol ratio on the size of haze particles, *J. Amer. Soc. Brew. Chem.* 58: 117-123, 2000.

# Haze intensity at various gliadin and TA levels at pH 4.5



Siebert & Lynn, *J. Amer. Soc. Brew. Chem.* 58: 117-123, 2000.

## Summary

Protein-polyphenol **haze intensity** is strongly affected by protein/polyphenol ratio and pH.

Haze **particle size** is also affected by protein/polyphenol ratio, with the largest size particles at intermediate ratios. Changes are more in the proportions of particles of discrete sizes than of gradual shifts of a monomodal population.

Particle size should impact sedimentation and filtration performance.

## Summary (continued)

Beer HA protein and HA polyphenol interact strongly (causing the greatest haze intensity with fixed amounts of protein and polyphenol) **near pH 4.2**. The amount of haze formed declines sharply at both higher and lower pH.

This appears to affect haze removal during beer maturation, fining activity and the effectiveness of silica and PVPP.

## Summary (continued)

The beer **proteins** that are involved in haze formation are rich in **proline** and derived from barley **hordein**.

The beer **polyphenols** involved in haze **bridge proteins together** to form complexes. These are primarily 'dimeric' proanthocyanidins. They originate mainly from malt but hops can also contribute.

## Summary (continued)

The **time course** of haze formation has an initial lag followed by an essentially linear increase. The latter is a function of the product of HA protein and HA polyphenol concentrations.

As a result, reducing either HA protein or HA polyphenol by a similar percentage should have a similar stabilizing effect.

## Summary (continued)

**Silica** attaches to the same sites in HA proteins to which polyphenols attach. This makes it specific for HA protein and largely spares the foam-active protein.

**PVPP** resembles the proline sites in HA proteins and competes for the HA polyphenol. It is at most able to remove about half of the beer HA polyphenol because most of that is already attached to beer HA proteins.

# Acknowledgments

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Karl J. Siebert  
Professor of Biochemistry  
Dept. of Food Science  
Cornell University  
Geneva, NY 14456  
kjs3@cornell.edu

<http://web.foodscience.cornell.edu/siebert>



## Review Articles on Haze

K.J. Siebert, Haze in beverages. In *Advances in Food and Nutrition Research* 57: 53-86, 2009.

K.J. Siebert, Haze formation in beverages. *LWT - Food Science and Technology*, 39: 987-994, 2006.

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