

Dry Hopping And Its Effects On Beer Bitterness, The IBU Test Beer Foam, and pH

**By Dr. John Paul Maye
Technical Director**


Hopsteiner®

Hop Acids In Hops

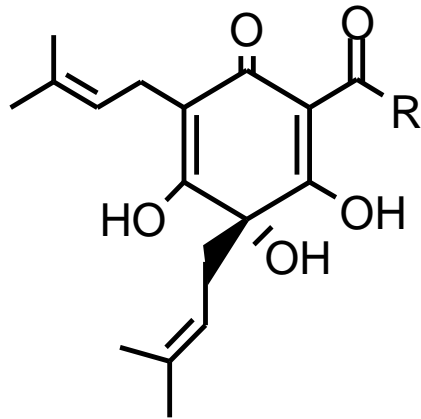
**UV Spectrophotometric Analysis (UV Spectro) &
High Performance Liquid Chromatography (HPLC)**

**Humulinones & Their Concentrations In Hops and Hop
Pellets and Dry Hopped Beers**

**Dry Hopping Experiments: Effects on Beer Bitterness,
the IBU Test, Beer Foam, and pH**

Conclusion

The Two Major Hop Acids In Hops

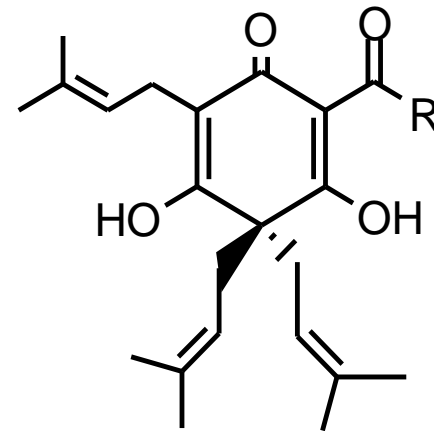


Alpha Acids

(Humulone)

2 – 20 %w/w

Variety Dependent



Beta Acids

(Lupulone)

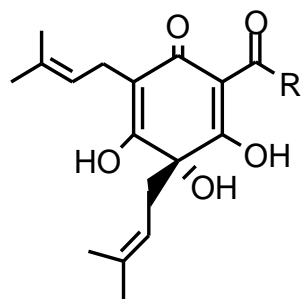
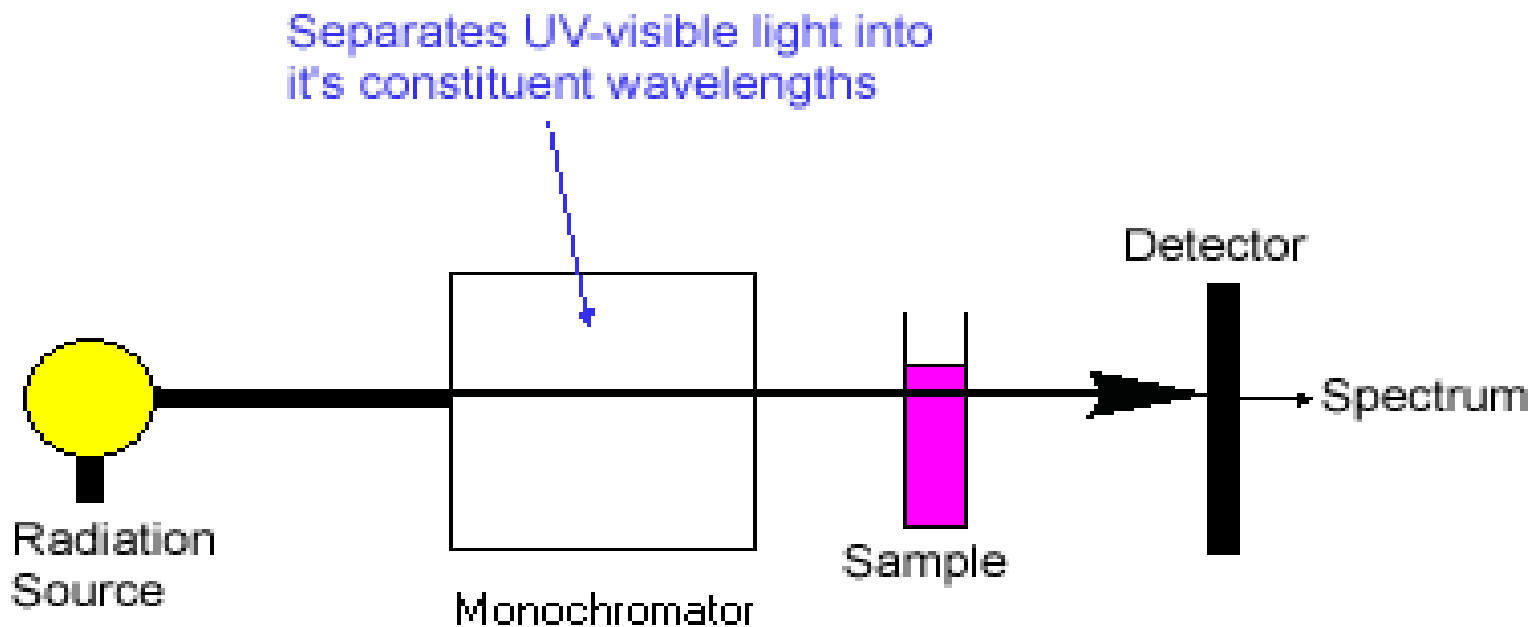
2 – 8 % w/w

Variety Dependent

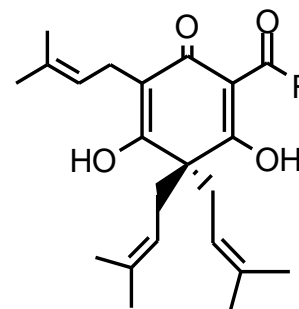
In North America Alpha Acids And Beta Acids In Hops Are Analyzed By Spectrophotometric Analysis (UV Spectro)



5.0 g freshly ground hops, 100 mL toluene, shake 30 min.
 1 mL diluted to 500 mL with alkaline methanol.

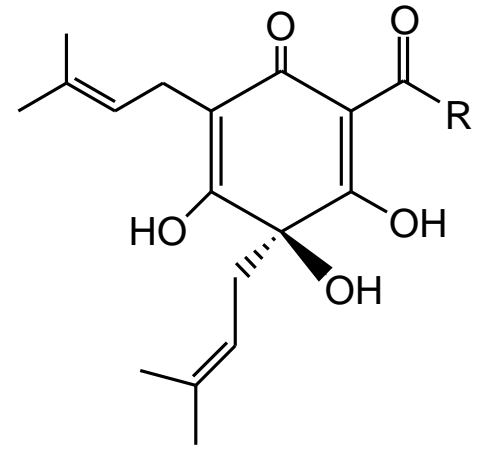
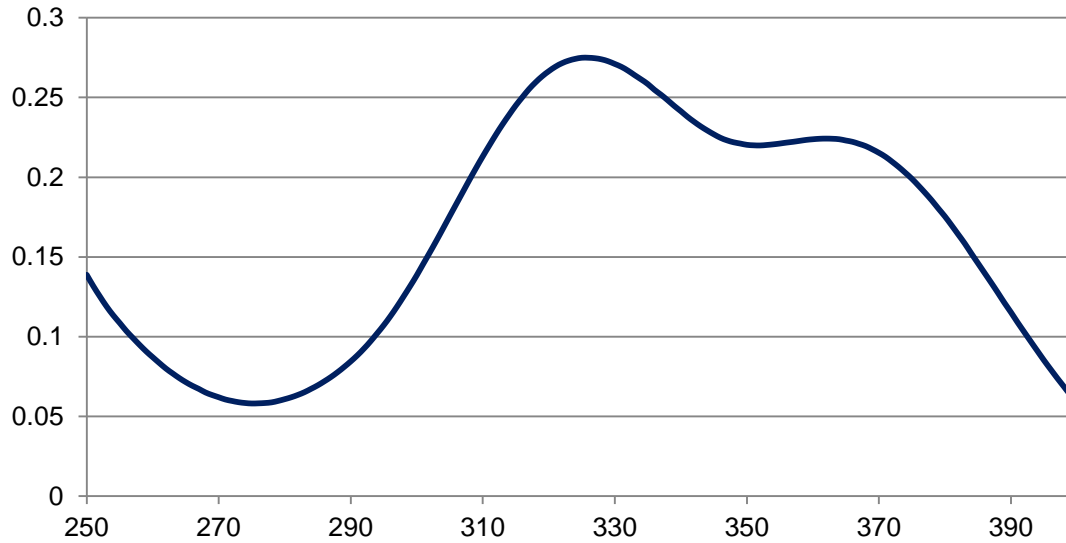


Alpha Acids

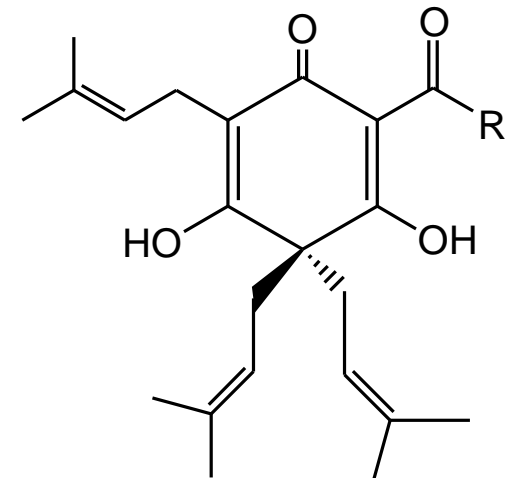
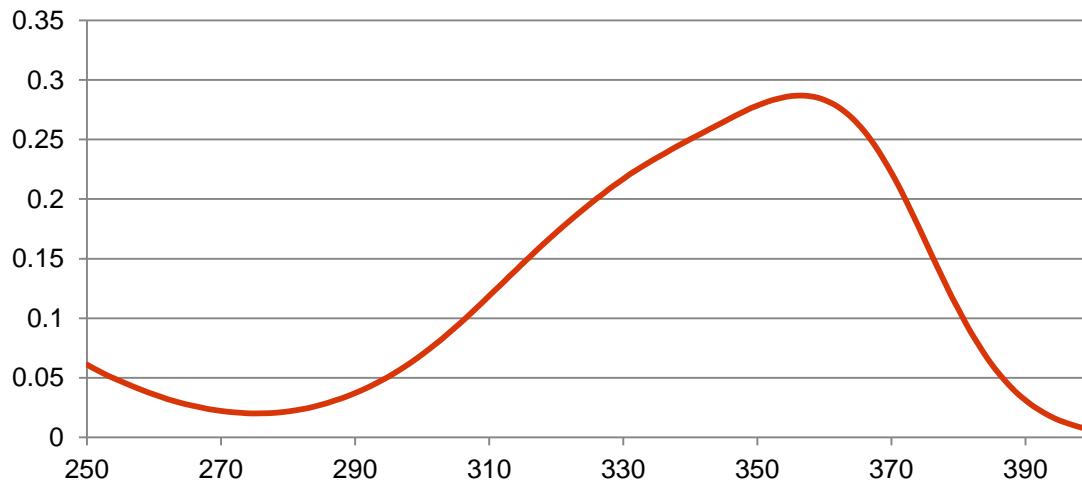


Beta Acids

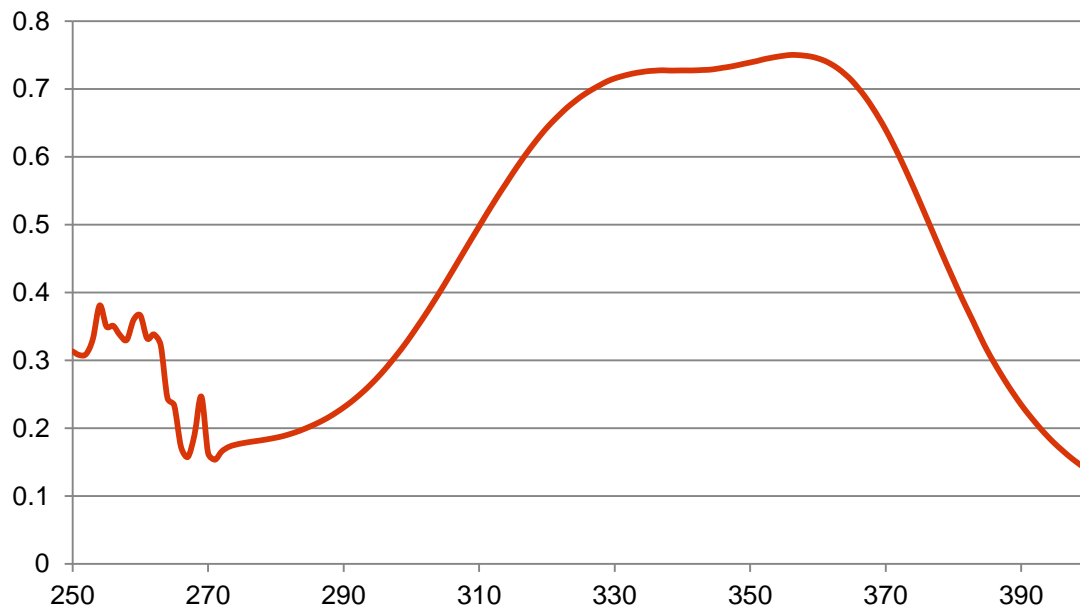
Alkaline Alpha Acids Spectra



Alkaline Beta Acids Spectra



Alkaline Cascade Hops



Using the absorbance data from the UV Spectrometer and the below formulas one can determine the concentration of alpha acids and beta acids in hops.

$$\% \text{ Alpha Acids} = d(-51.56A_{355} + 73.79A_{325} - 19.07A_{275})$$

$$\% \text{ Beta Acids} = d(55.57A_{355} - 47.59A_{325} + 5.10A_{275})$$

UV data can also be used to calculate the Hop Storage Index which gives the brewer an idea of the freshness of his hops.

$$\text{HSI (Hop Storage Index)} = A_{275}/A_{325}$$

HSI = 0.2 – 0.25 Very Fresh Hops

0.25 – 0.3 Fresh Hops

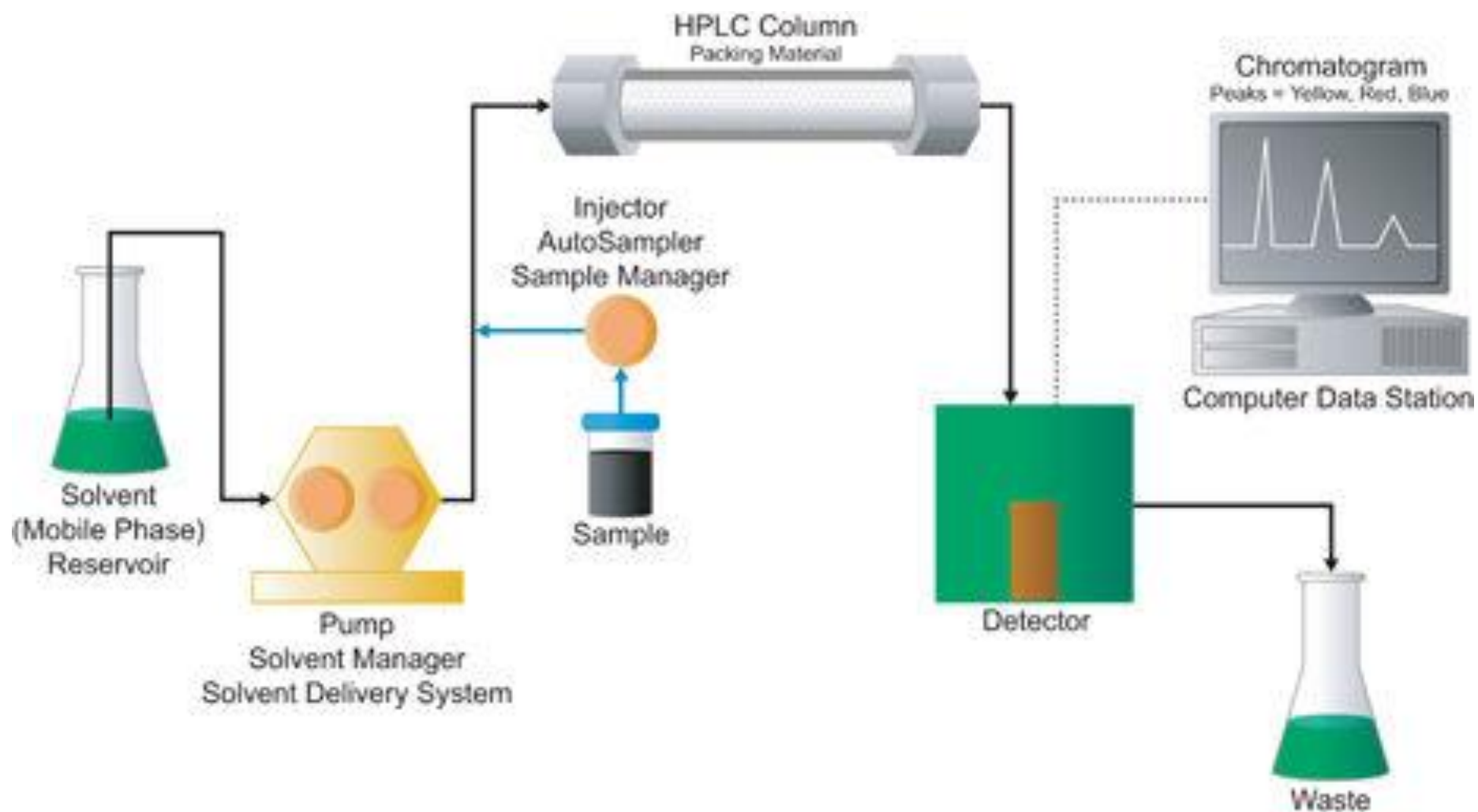
0.3 – 0.4 Slightly Oxidized (Good?)

>0.4 Oxidized

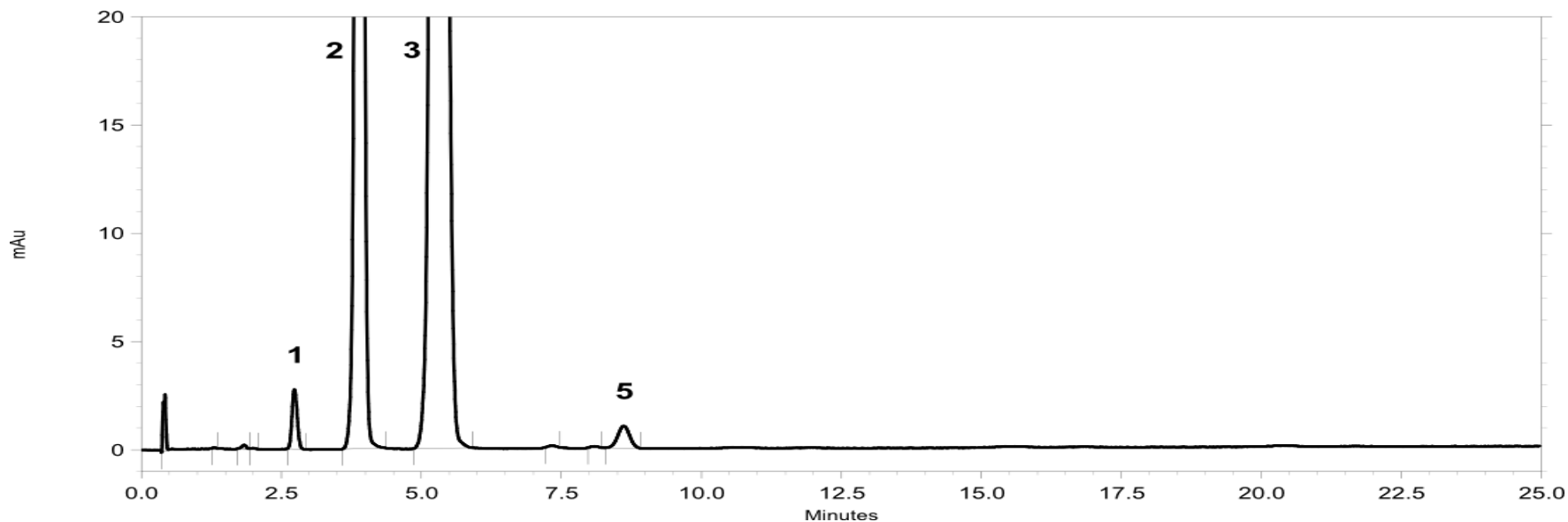
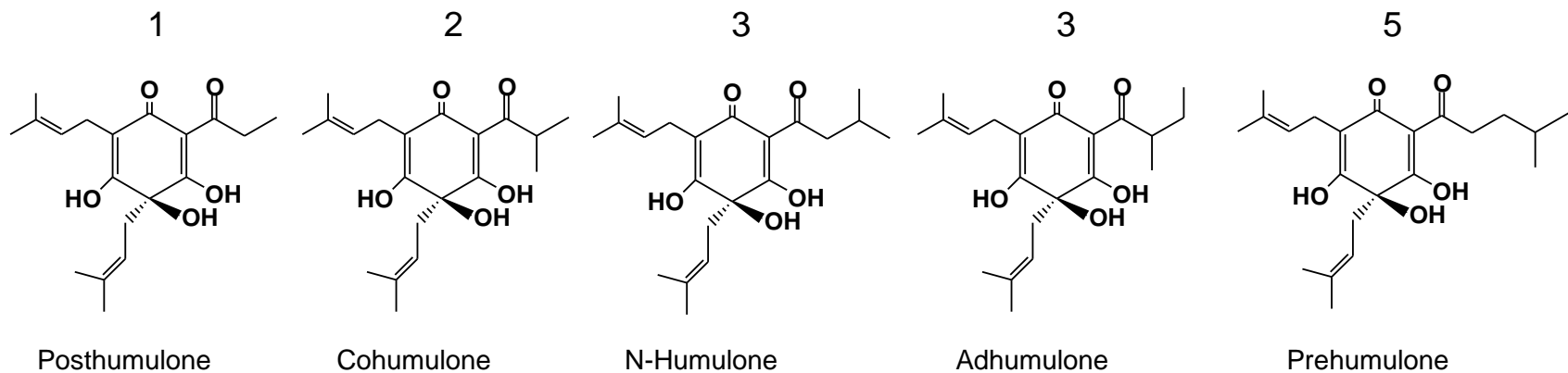
***Note: HSI can be variety dependent, some varieties have higher HSI than others.**

Hop Acids can be measured (more accurately) using High Performance Liquid Chromatography, HPLC

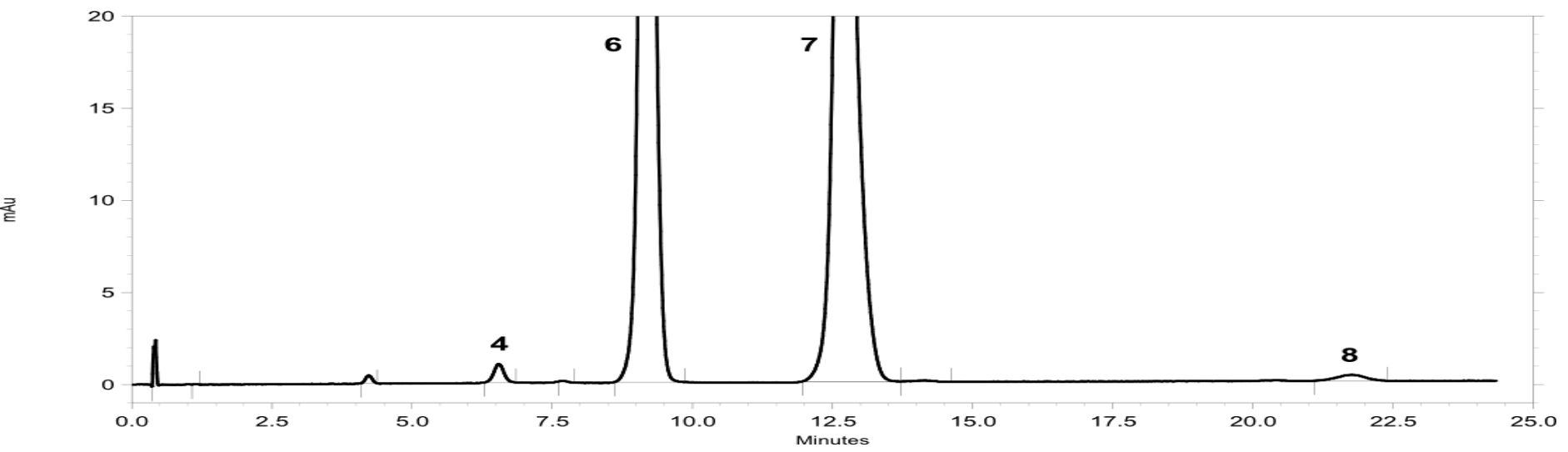
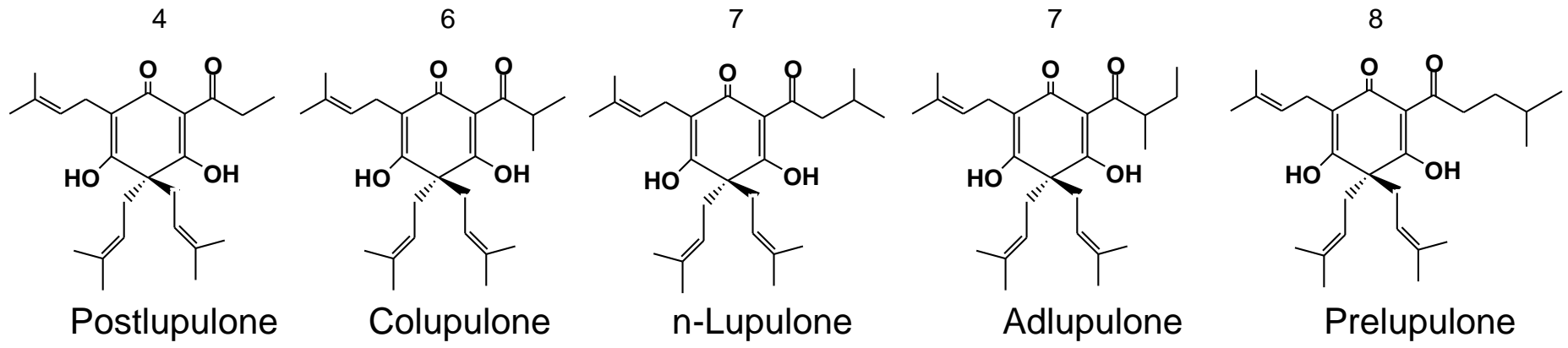




HPLC Chromatogram of The Five Alpha Acids



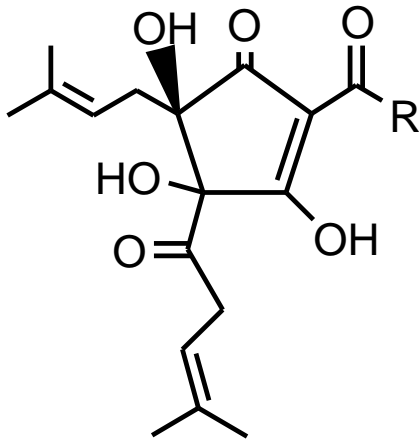
HPLC Chromatogram of The Five Beta Acids



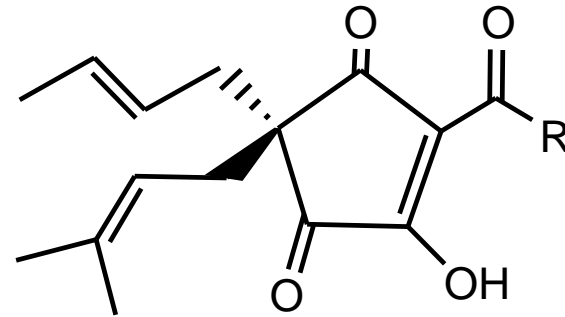
Hop Variety	UV Spectro		HPLC	
	α -acids	β -acids	α -acids	β -acids
Cascade	6.2%	6.0%	5.9%	5.3%
Centennial	9.0%	3.6%	8.6%	3.2%
Chinook	10.5%	2.5%	9.9%	2.5%
Zeus	13.0%	3.8%	12.5%	3.5%

Note: As a general rule the HPLC results will be lower than spectro, however, HPLC only measure alpha acids whereas spectro might be measuring other bitter compounds.

HPLC Can Measure Minor Hop Acids



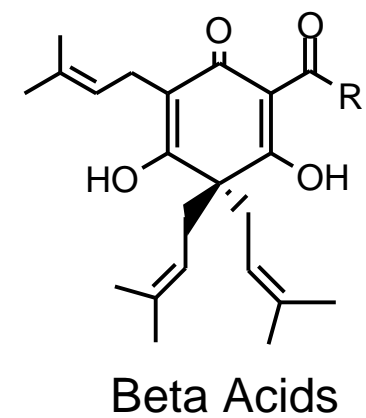
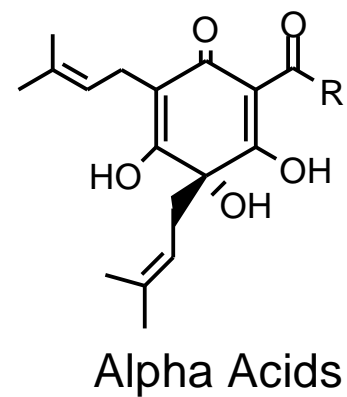
Humulinone



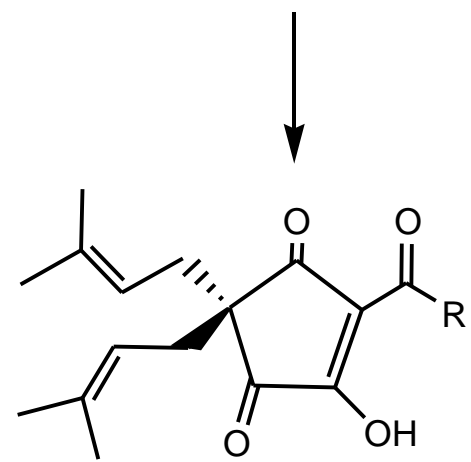
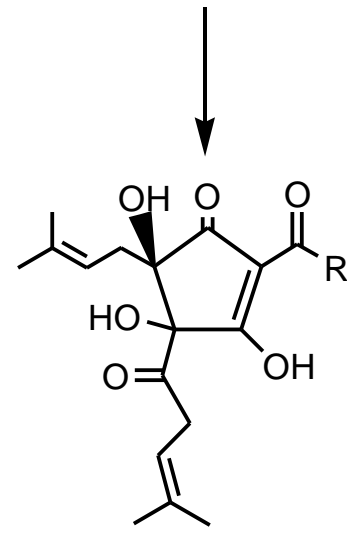
Hulupones

Minor Hop Acids Are Formed Via Oxidation Of Major Hop Acids

Major Hop Acids

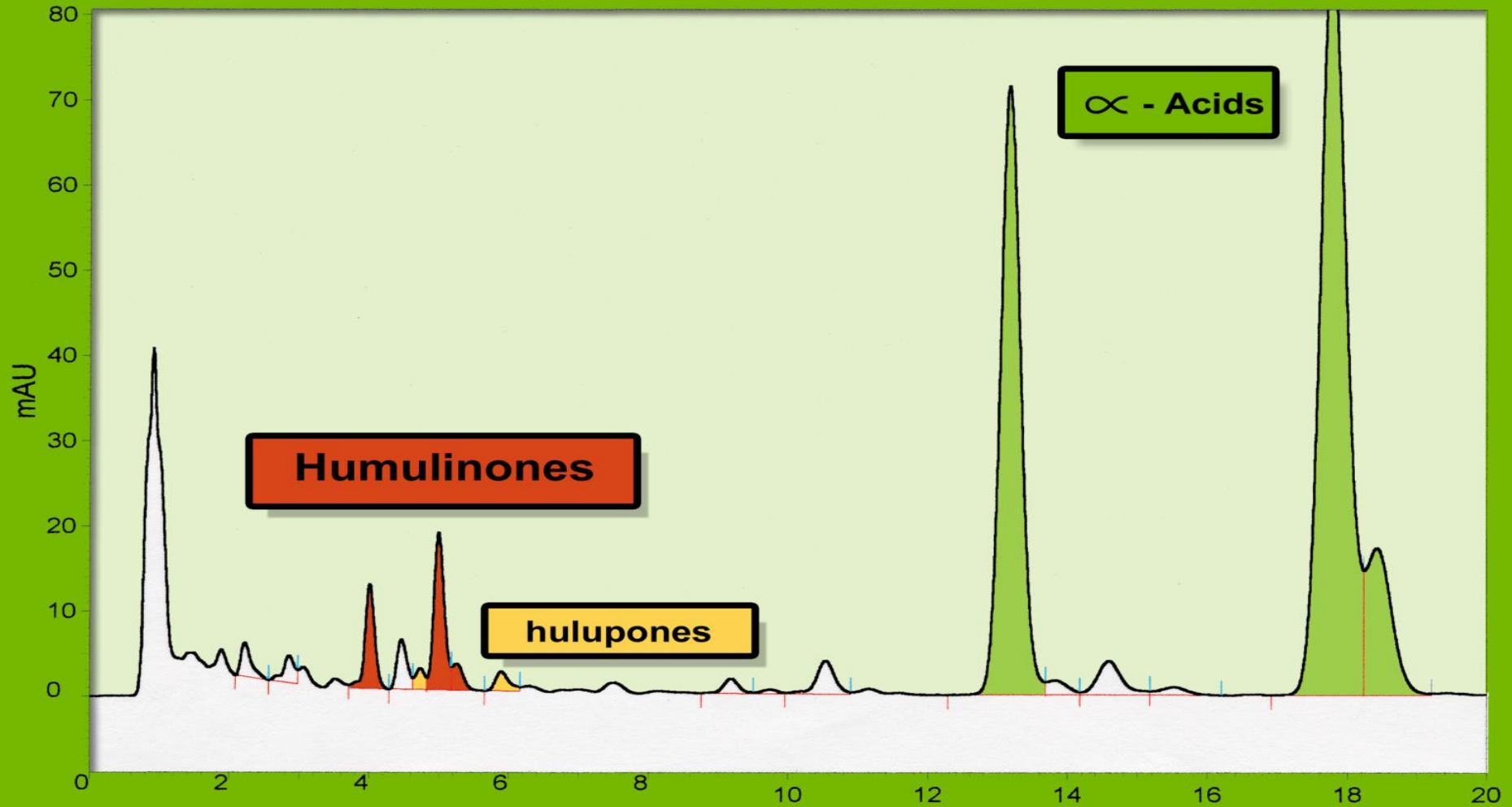


Minor Hop Acids
(Oxidized)

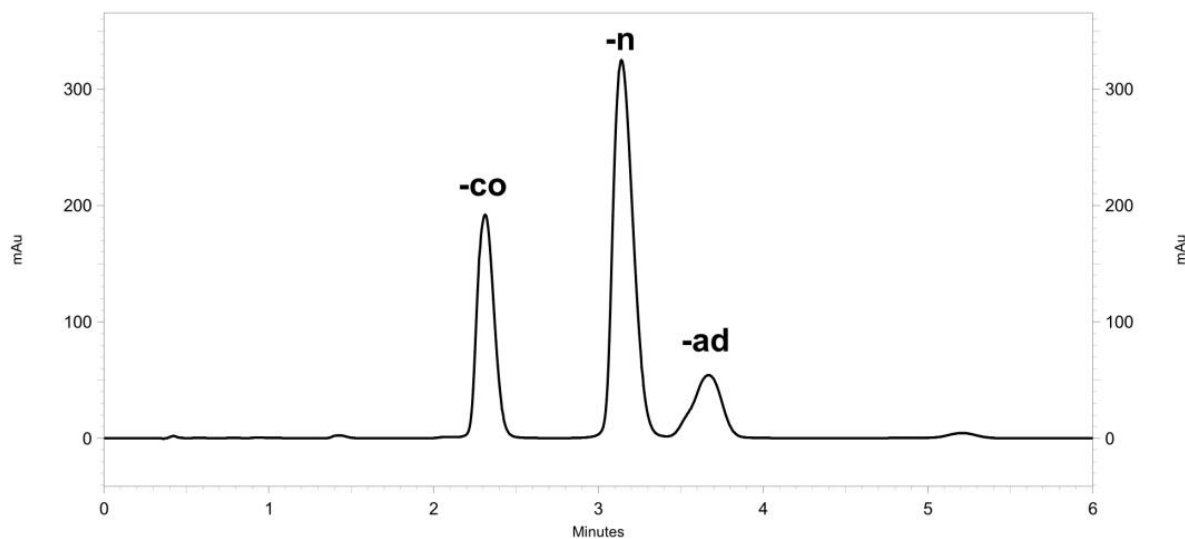
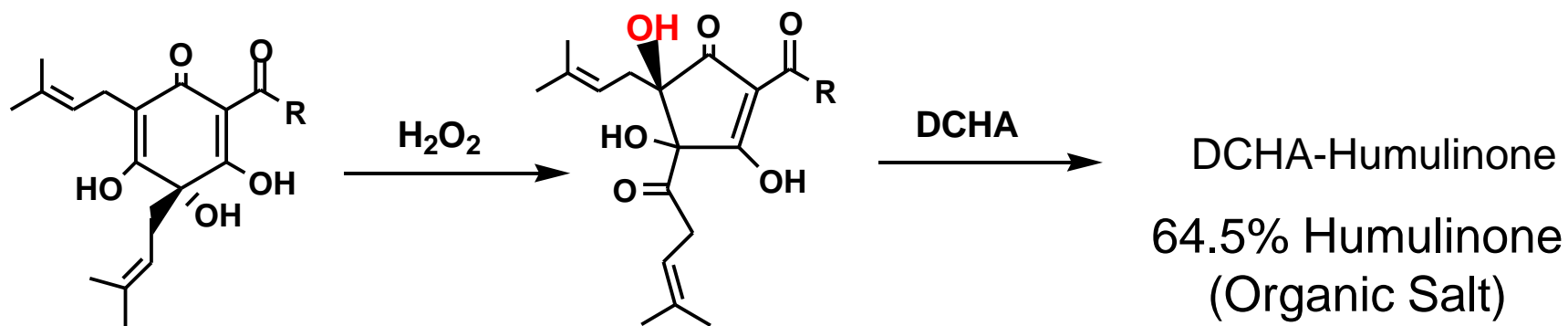


Too small to be assayed
via UV Spectro

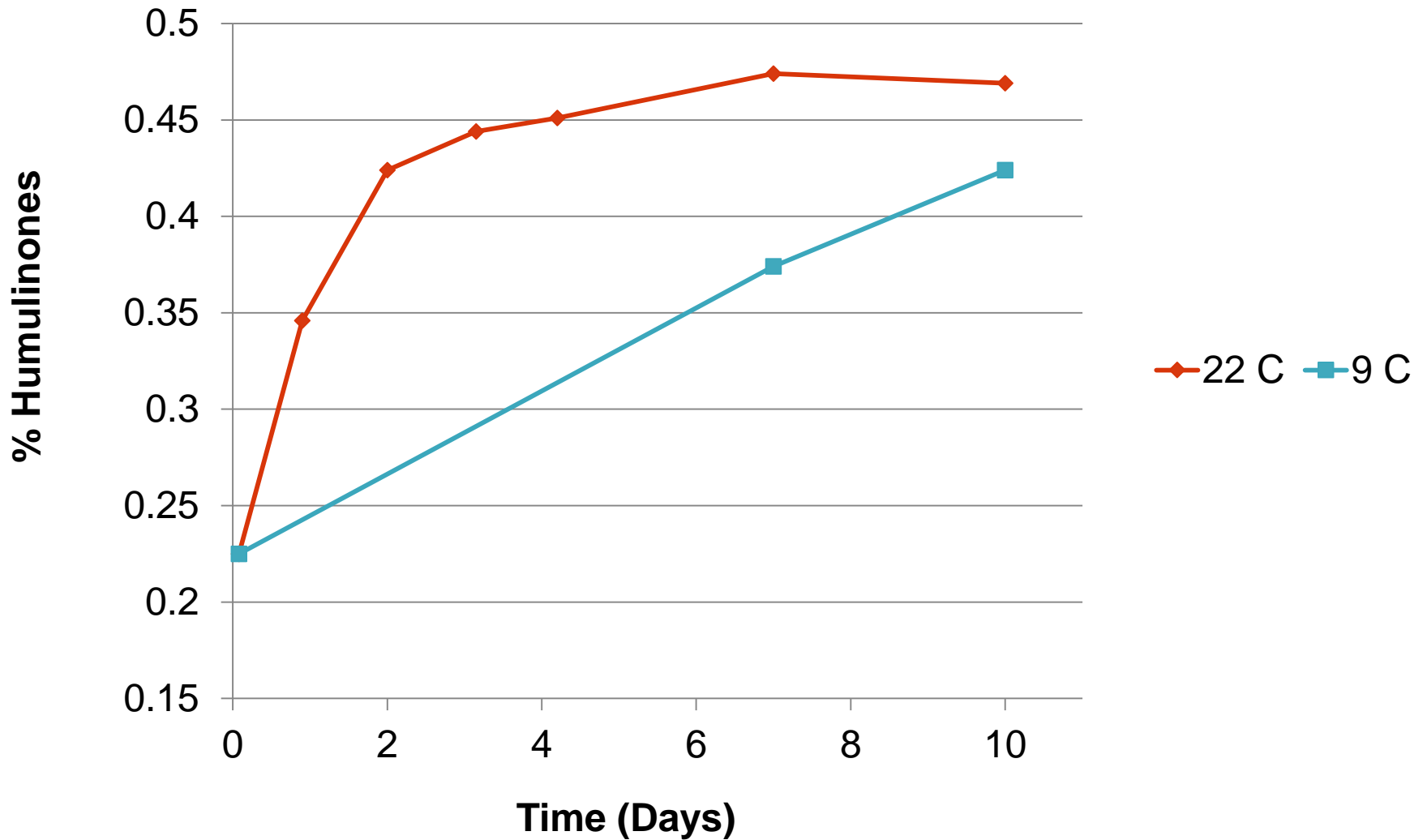
HPLC Trace of Hops



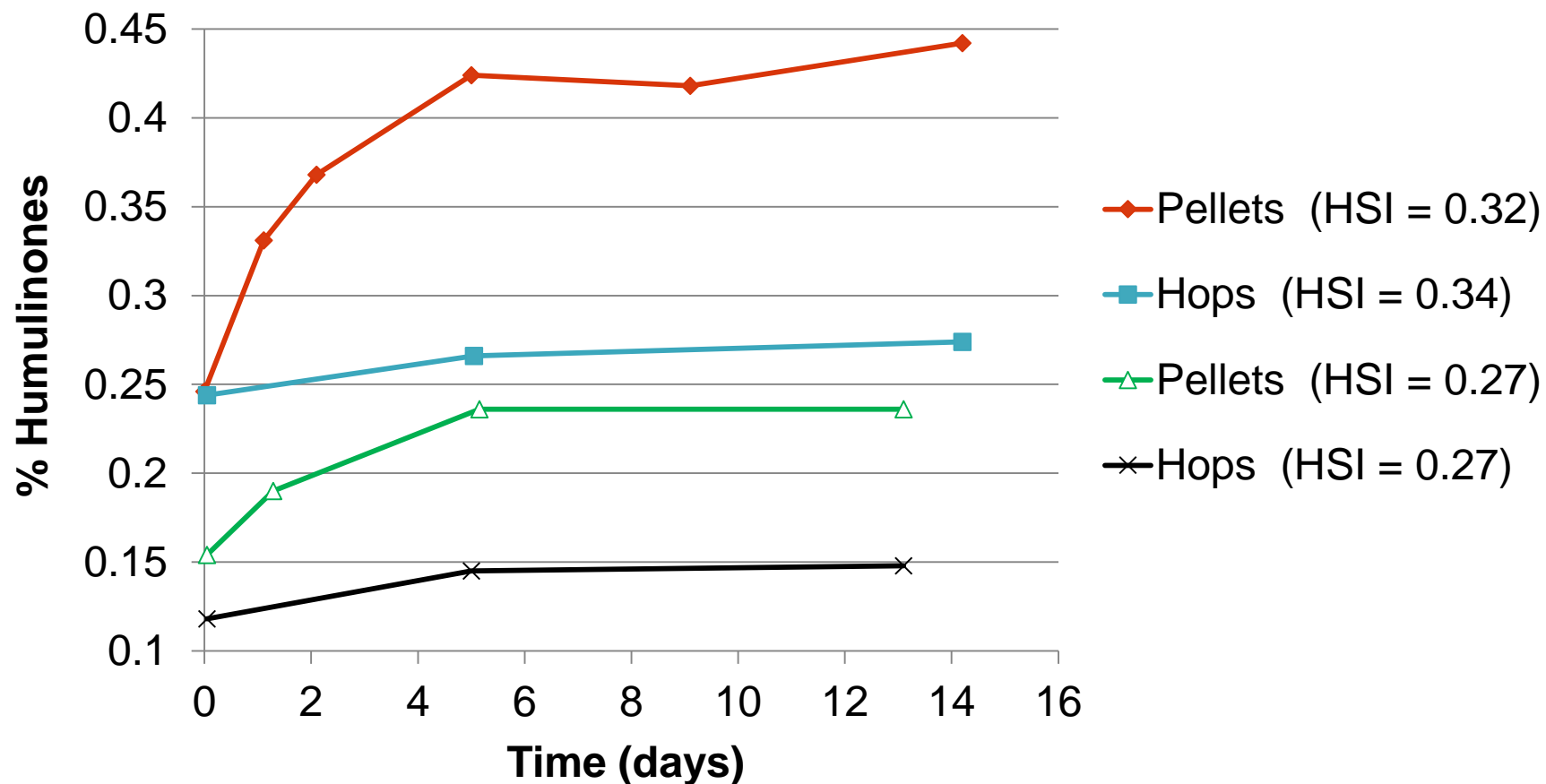
Preparation of Dicyclohexylamine Humulinone A HPLC Calibration Standard For Humulinones



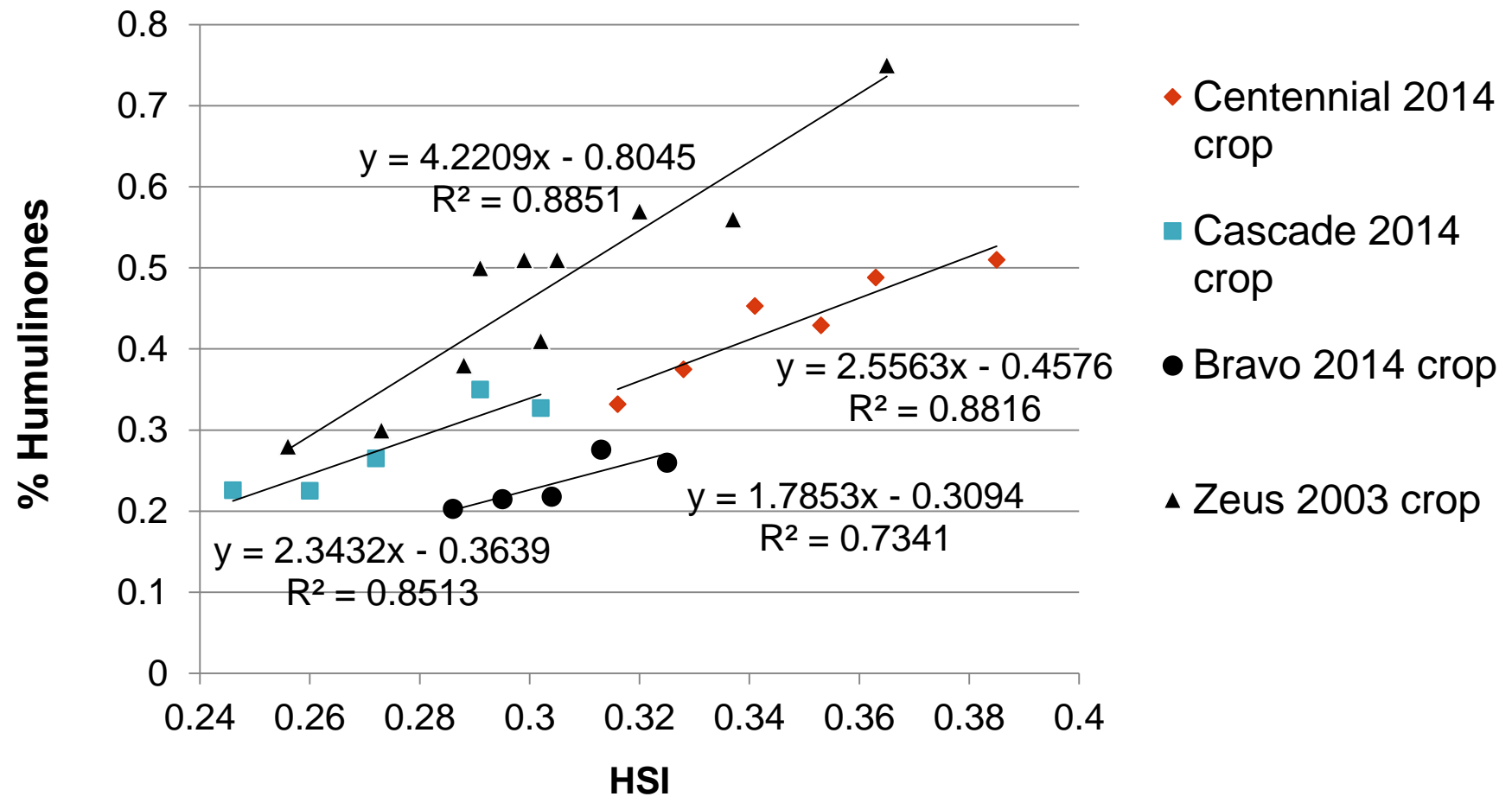
Humulinone Formation in Zeus Hop Pellets vs Temp



Humulinone Formation is Greater in High HSI Hops and Hop Pelle vs Low HSI Hops or Hop Pellets

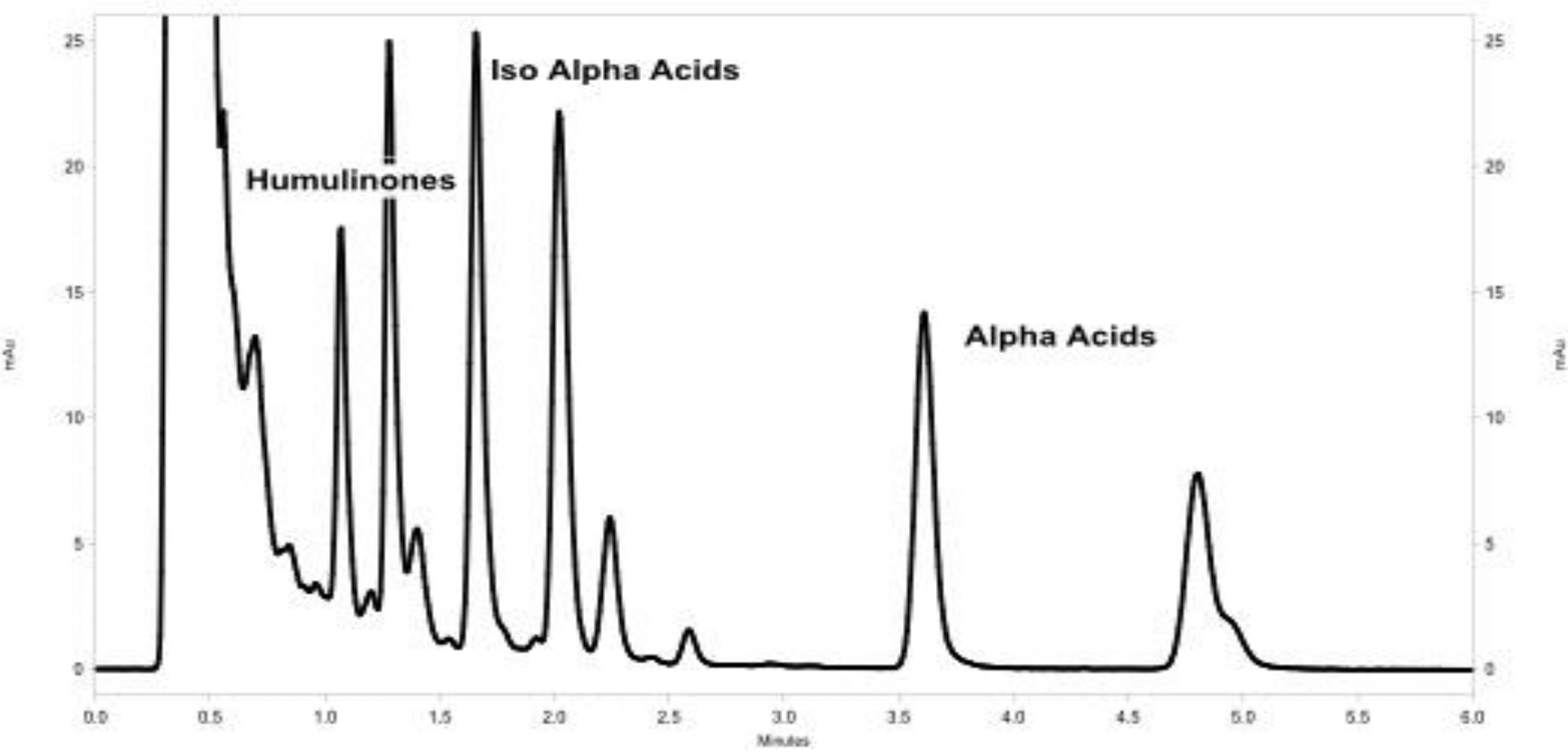


Humulinone Concentration vs HSI

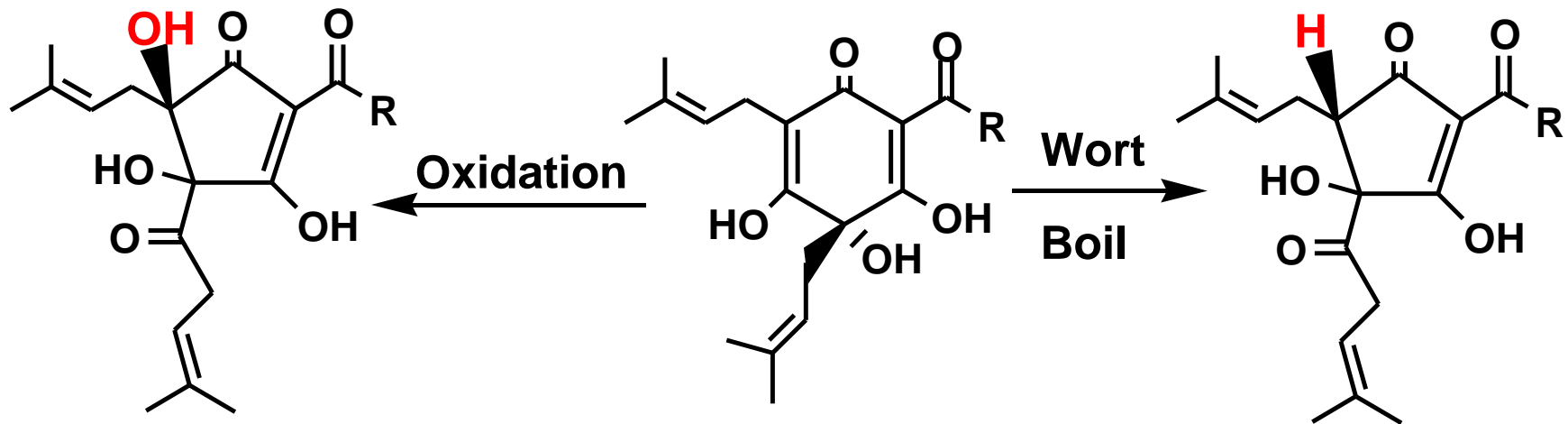


Humulinone Concentration In Commercial Beers Labeled IPA's

We analyzed 30 commercial IPA's by HPLC and found the humulinone concentration to range from 3 ppm to 33 ppm.



Humulinones Are Extremely Similar In Molecular Structure to Iso-Alpha Acids



Humulinone

1 ppm ~ 0.66 IBU

Alpha Acids

Iso-alpha Acids

1 ppm ~ 1 IBU

Humulinones are more polar than isoalpha acids and therefore should be more beer soluble than isoalpha acids. Also humulinones have been reported to be 66% as bitter as isoalpha acids.

**To Better Understand The Incorporation
Of Humulinones Into Beer
A Series of Dry Hopping Experiments
Were Performed**

Experiment #1 : Humulinone Utilization

Conditions:

Centennial hop pellets assayed 0.35 w/w% Humulinone the day of dry hopping.

Dose Rate: 0, 0.5, 1.0, and 2.0 lbs/barrel

Hop pellets were simply dumped on top of the beer.

Beer Type: Low IBU and High IBU

Temperature of dry hopping, 16 °C

Contact Time: 5 Days

Centennial hop pellets assaying 0.35% Humulinone were added to a low and high IBU beer and dry hopped for 5 days

Sample	lbs pellets Barrel of beer	ppm of Humulinone in beer	% Utilization Humulinone	ppm of Iso-a-acid in beer
Low IBU Beer (8.6)	0	0.8	-	8.6
	0.5	8	98	8.1
	1.0	14	91	7.9
	2.0	28	88	7.5
High IBU Beer (48)	0	1	-	48
	0.5	8	98	39
	1.0	14	91	35
	2.0	27	87	30

*Does this loss in isoalpha acids effect the beers bitterness?

Question #2:

How fast does humulinone dissolve into beer?

How fast are isoalpha acids lost?

What else dissolved into beer when one dry hops?

How do these changes effect beer bitterness?

Experiment #2 : What Dissolves Into Beer and How Fast?

Cascade Hop Pellets

Compound	Concentration, %
Alpha acids (ICE-3)	5.6
Beta acids (ICE-3)	5.8
Humulinones (DCHA humulinones, 65.9%)	0.26
Xanthohumol (99.7% pure, in-house std)	0.26
Hulupones (DCHA hulupones, 67.0%)	0.05

54 IBU All Malt Beer

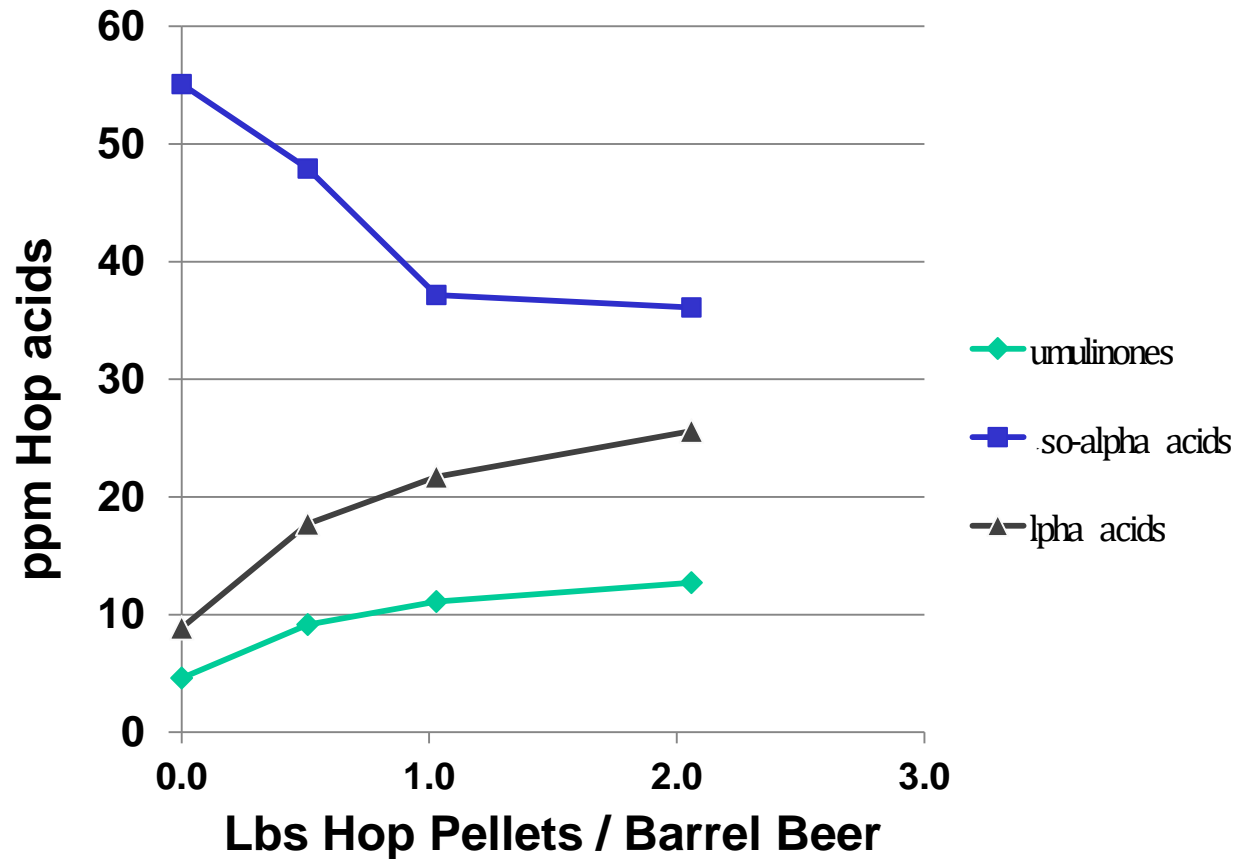
Dry Hop Dose Rate: 0, 0.5, 1.0, 2.0 lbs per barrel

Contact Time: 1, 2, 5 days

Dry Hop Temperature: 16 °C

All Results Reported by HPLC

Hop Acid Concentration After 24 hours of Dry Hopping



1 lb/barrel (Day 1)

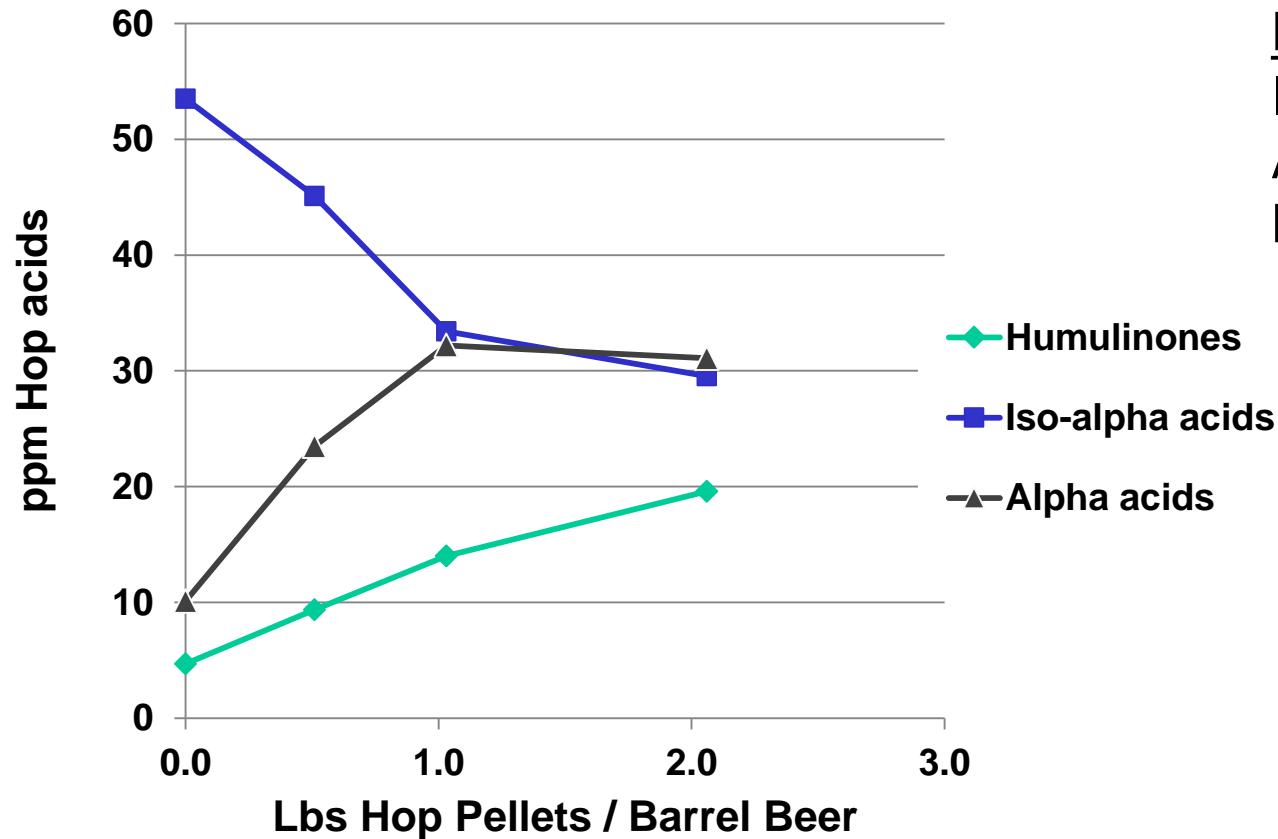
IAA 37 ppm

AA 21 ppm

Hum 11 ppm

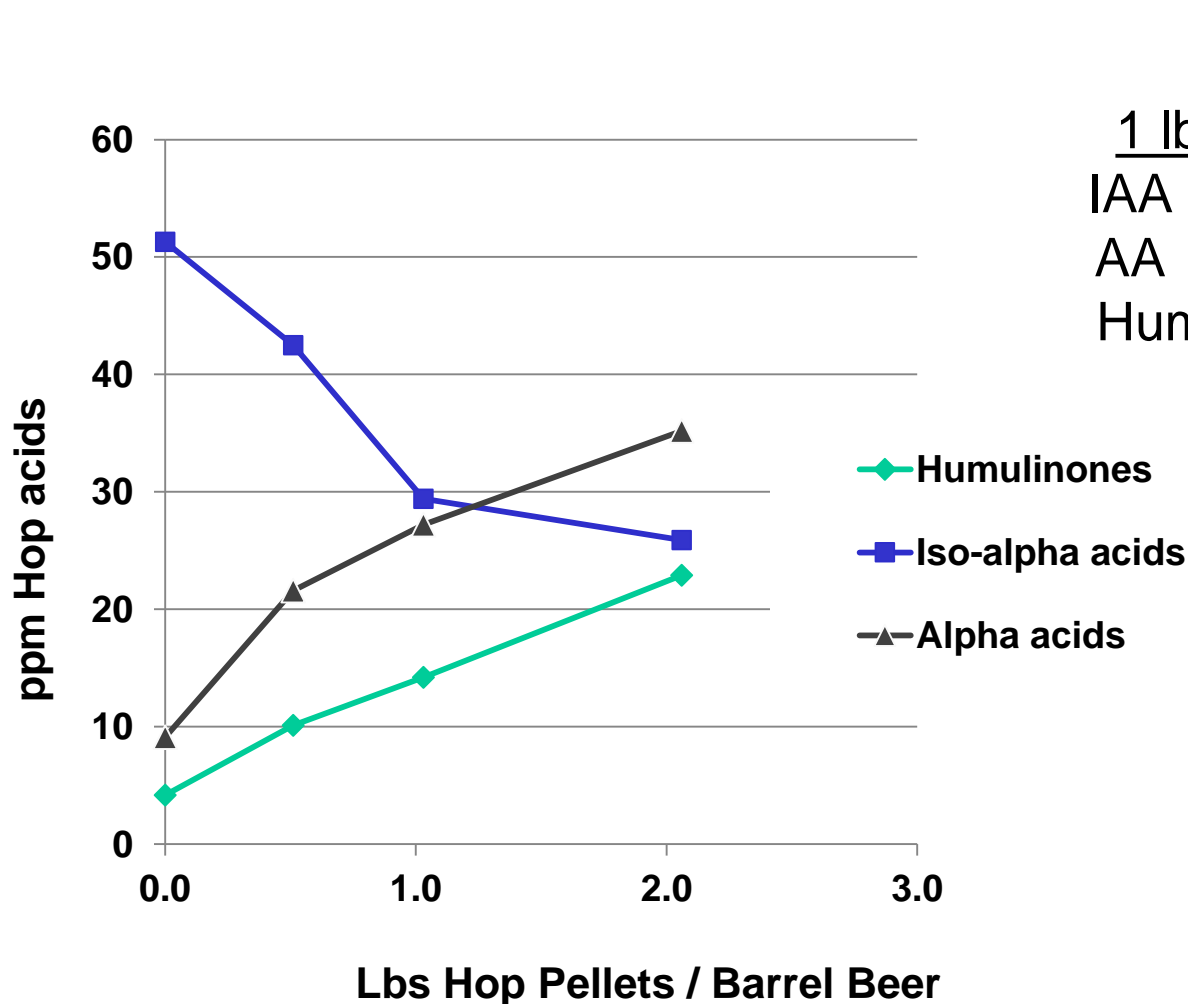
Hop Acid Concentration After 2 Days of Dry Hopping

1 lb/barrel



<u>Day 2</u>	<u>(Day 1)</u>
IAA 32 ppm	(37 ppm)
AA 31 ppm	(21 ppm)
Hum 15 ppm	(11 ppm)

Hop Acid Concentration After 5 Days of Dry Hopping



Day 5

	1 lb/barrel	(Day 2)	(Day 1)
IAA	29 ppm	(32 ppm)	(37 ppm)
AA	28 ppm	(31 ppm)	(21 ppm)
Hum	15ppm	(15 ppm)	(11 ppm)

HPLC Analysis of Beers Dry Hopped for 5 Days with Cascade Hop Pellets

Lbs Hops per BBL	PPM Iso- α -acid	PPM Alpha Acids	PPM Hum	PPM XN	% Utilization			
					Iso- α	α -acid	Hum	XN
0	51.3	9	4.2	0.24	-	-	-	-
0.5	42.5	21.6	10	0.62	- 17%	11%	100%	12%
1.0	29.4	27	14	0.79	- 43%	8%	97%	8%
2.0	25.9	35	23	0.79	- 49%	6%	90%	4%

Hum = Humulinone & XN = Xanthohumol

Note: No Beta Acids or hulupones were seen in any of the dry hopped beers.

How Does This Change In Hop Acid Composition Effect Beer Bitterness?

Lbs Hops per BBL	PPM of Iso-a-acid	PPM of Humulinone	PPM Alpha Acids	Calculated Bitterness*
0	51.3	4.2	9	55.0
0.5	42.5	10	21.6	51.2
1.0	29.4	14	27	41.3
2.0	25.9	23	35	44.6

* **Calculated Bitterness = ppm IAA + (0.66 x ppm Humulinone) + (0.1 x ppm Alpha Acids)**

Experiment #3: Extreme Dry Hopping

What Happens when you dry hop with 3, 4 or 6 lbs of hops per barrel of beer?

Conditions:

Cascade hop pellets assayed 5.7% alpha acids, 5.5% Beta Acids and 0.23w/w% Humulinone the day of dry hopping.

Dose Rate: 0, 1, 2, 3, 4, and 6 lbs/barrel

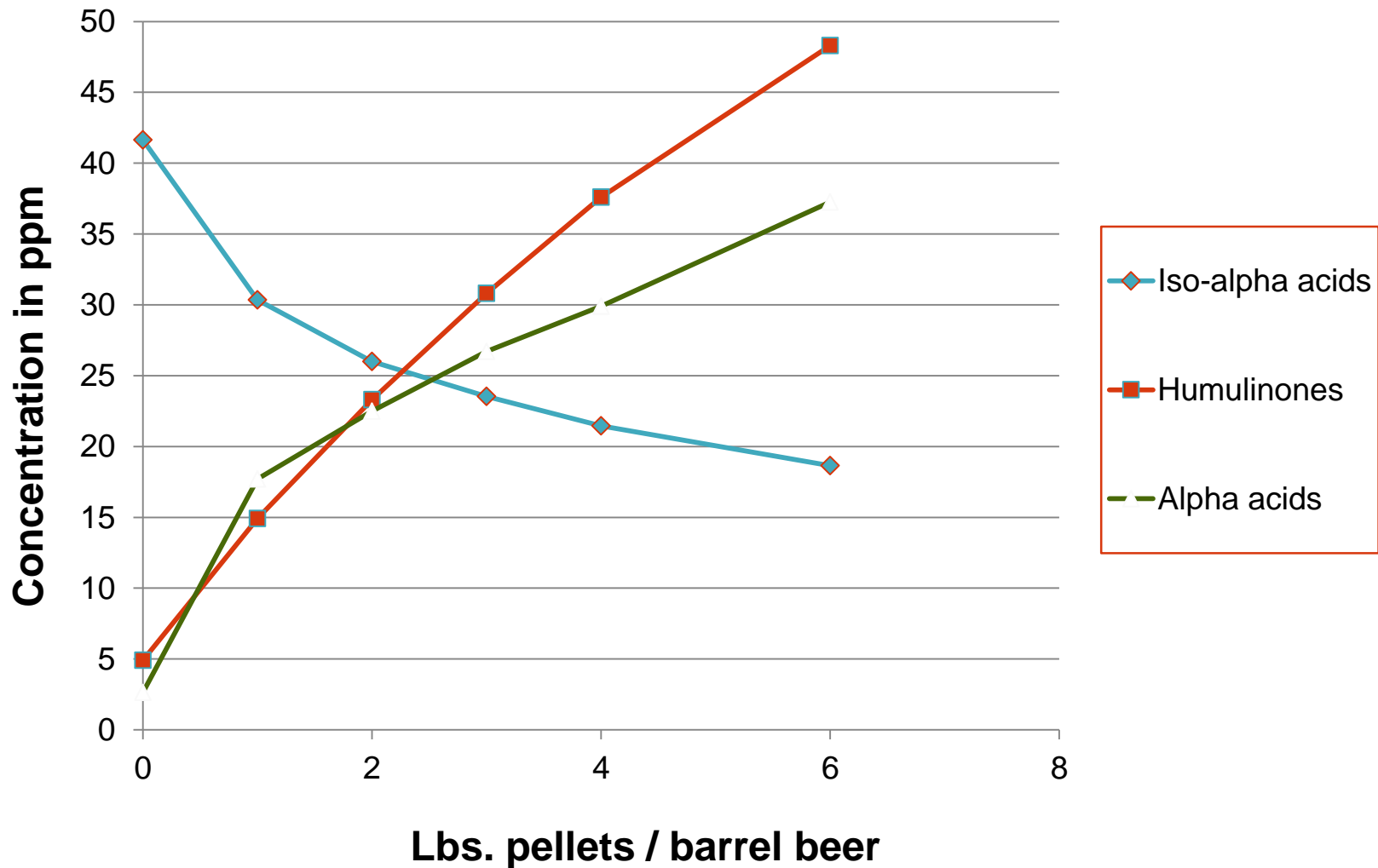
Hop pellets were simply dumped on top of the beer.

Beer Type: 42 ppm Isoalpha Acid Beer by HPLC (High IBU)

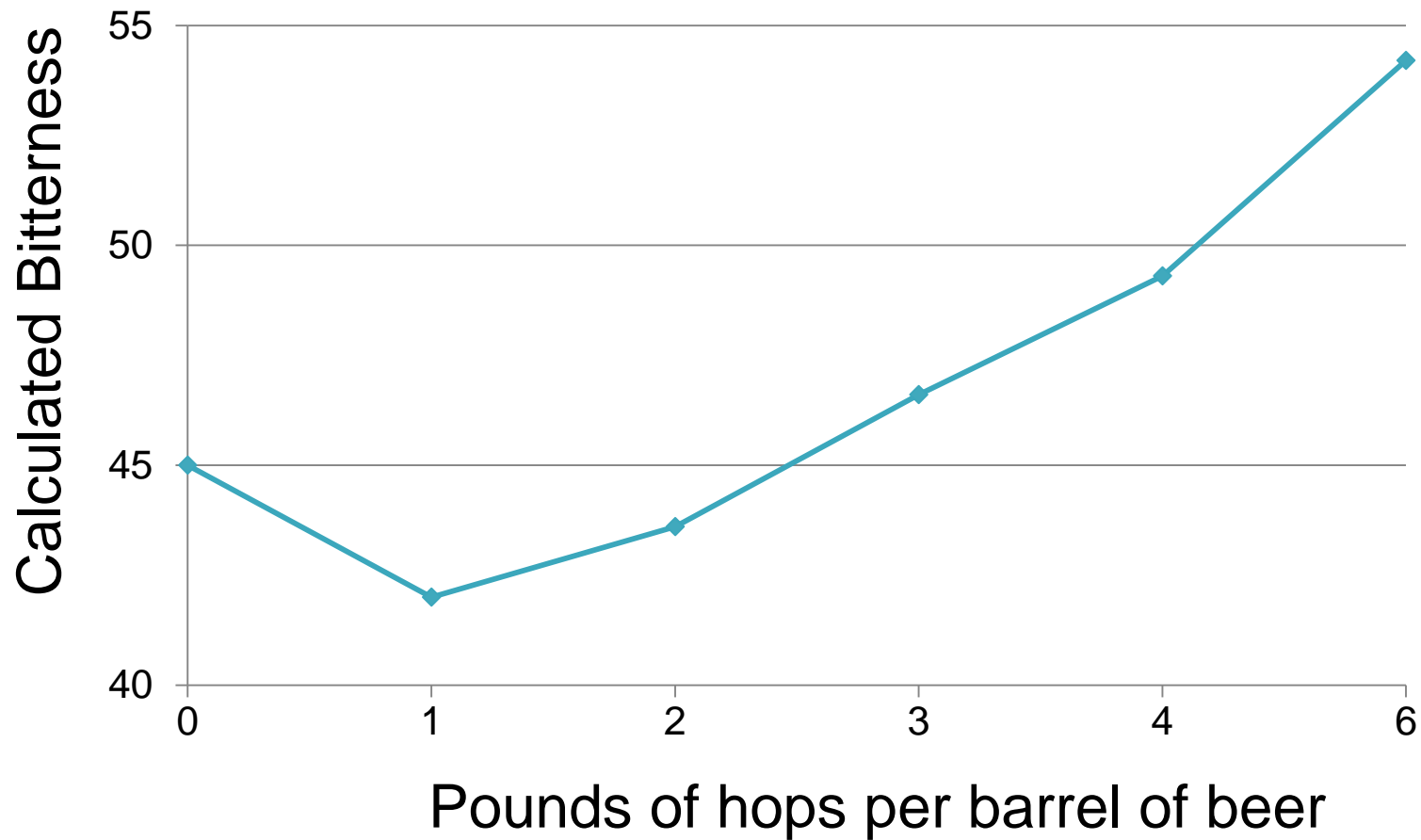
Temperature of dry hopping, 16 °C

Contact Time: 3 Days

Extreme Dry Hopping With Cascade Hop Pellets

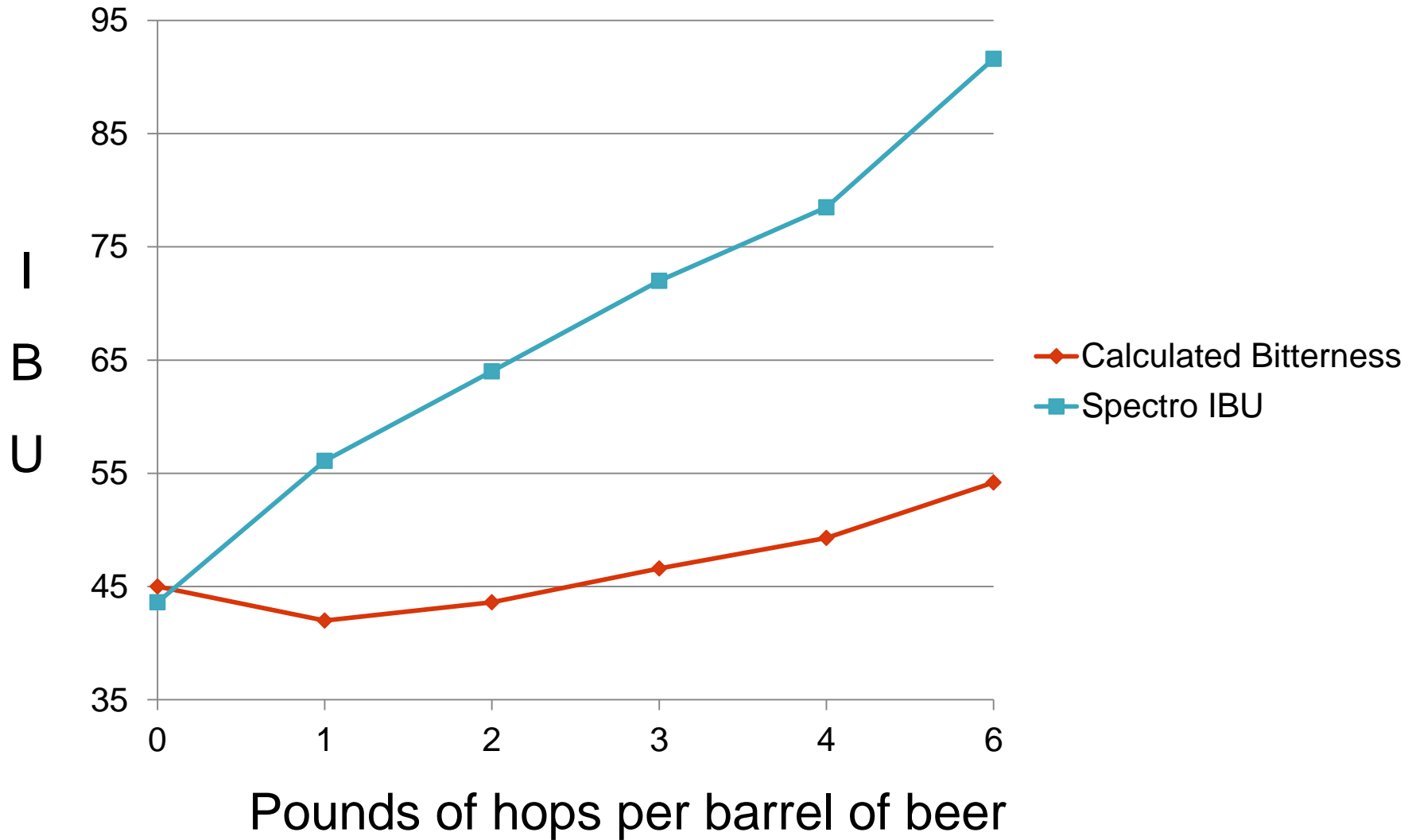


Effects of Extreme Dry Hopping On Calculated Bitterness



$$CB = \text{ppm IAA} + 0.66 \times \text{ppm Hum} + 0.1 \times \text{ppm AA}$$

Spectro IBU vs Calculated Bitterness



Why Dry Hopping Effects the International Bitterness Units Test

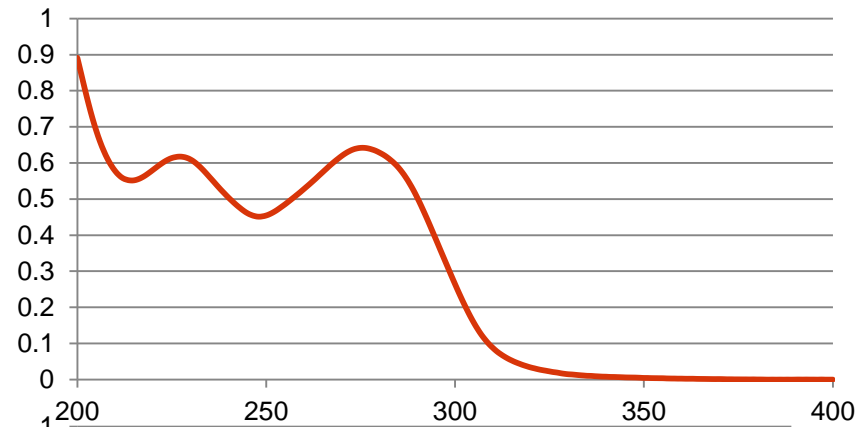
The IBU Test Method Was Developed to Measure Only Isoalpha Acids In Beer & 1 ppm of isoalpha acids ~ 1 IBU.

The Method:

Add 10 mL beer + 20 mL isooctane + 1 drop Octyl alcohol + 1 mL 3N HCl to 50 mL centrifuge tube, shake 15 minutes, centrifuge, transfer upper clear isooctane layer to cuvette, read absorbance at 275 nm.

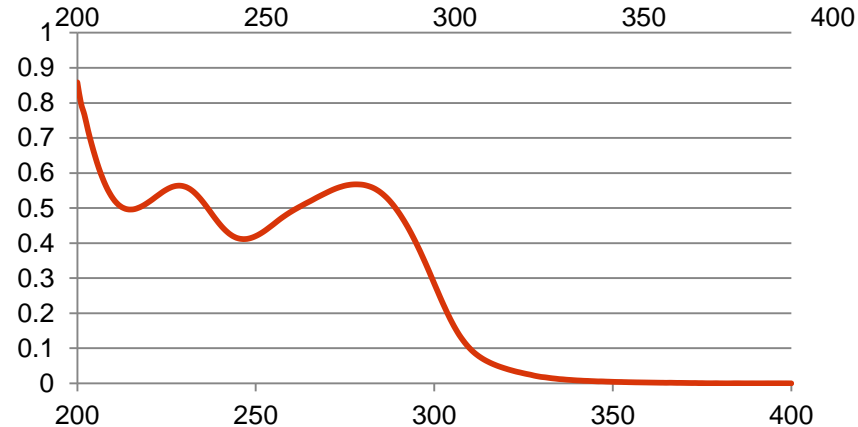
$$\text{BU} = \text{absorbance}_{275} \times 50$$

Note: In addition to isoalpha acids, isooctane will also extract humulinone, alpha acids and other hop compounds that are in beer and these compounds can and do absorb at 275 nm.



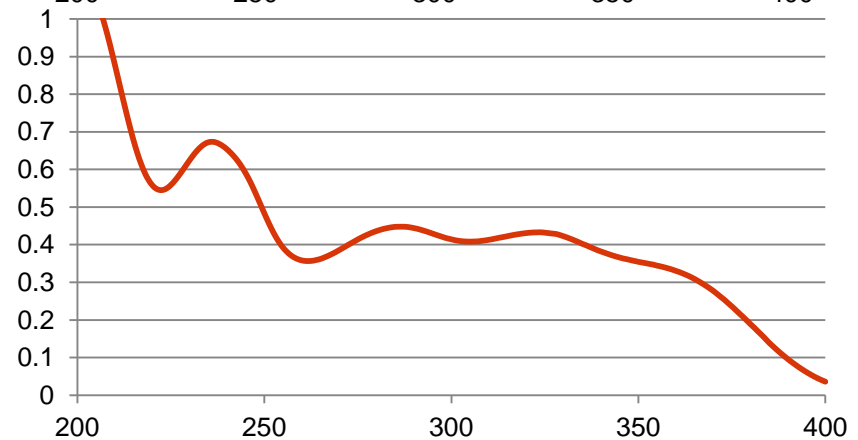
IBU Spectra of Isoalpha Acids

0.70 IBU / ppm IAA



IBU Spectra of Humulinones

0.54 IBU / ppm Hum



IBU Spectra of Alpha Acids

0.62 IBU / ppm AA

The hop acids in dry hopped beers, like Isoalpha acids, Alpha Acids, and Humulinones Absorbs Light at 275 nm Differently And Because Each Hop Acid Has A Different Bitterness Intensity You Can Not Correlate IBU to Beer Bitterness.

	<u>IBU Response Factor</u>	<u>Relative Bitterness</u>
1 ppm Iso-Alpha Acids	0.70 IBU	1
1 ppm of Humulinone	0.54 IBU	0.66
1 ppm of Alpha Acids	0.62 IBU	0.1

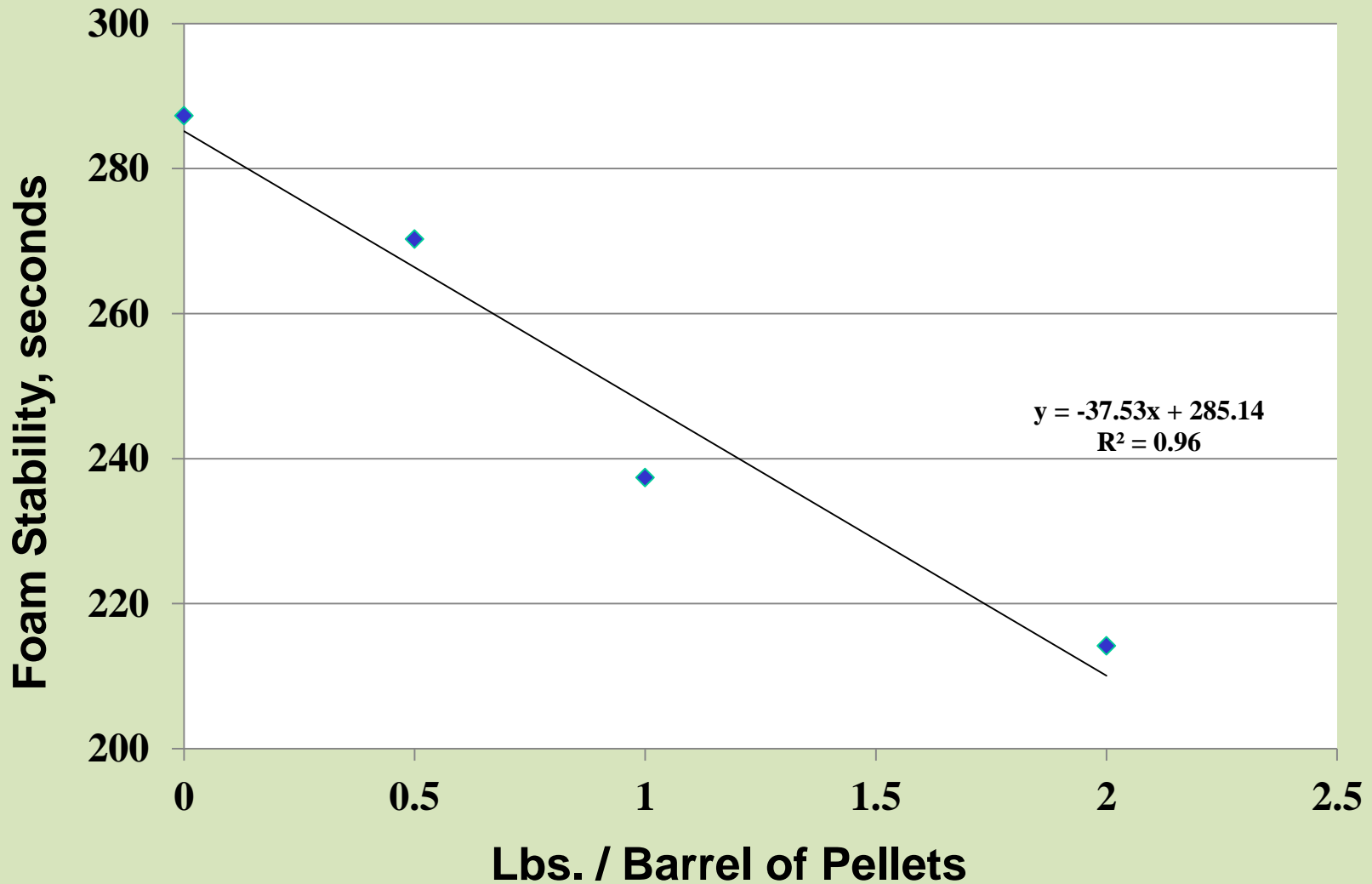
Using HPLC measurements of isoalpha acids, alpha acids and humulinones one can calculate the bitterness.

Dry Hopping's & Its Effect on Beer Foam

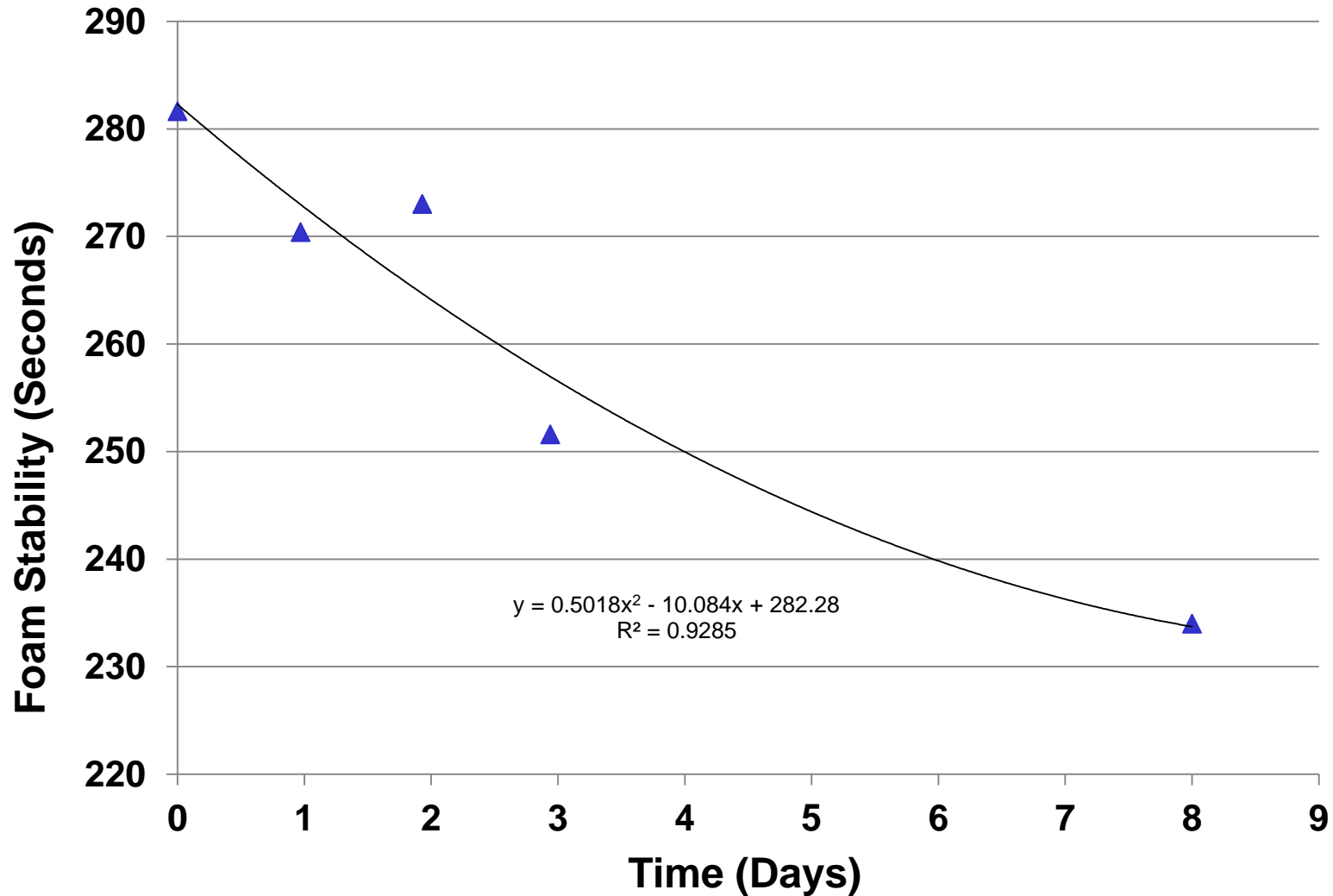
Beer is transferred from a bottle or can to a glass. A flasher will fill the glass with highly reproducible foam. The beer foam collapse is measured over 30 mm and the results are reported in seconds.



Dry Hopped 3 days at 16 °C With Cascade Hops



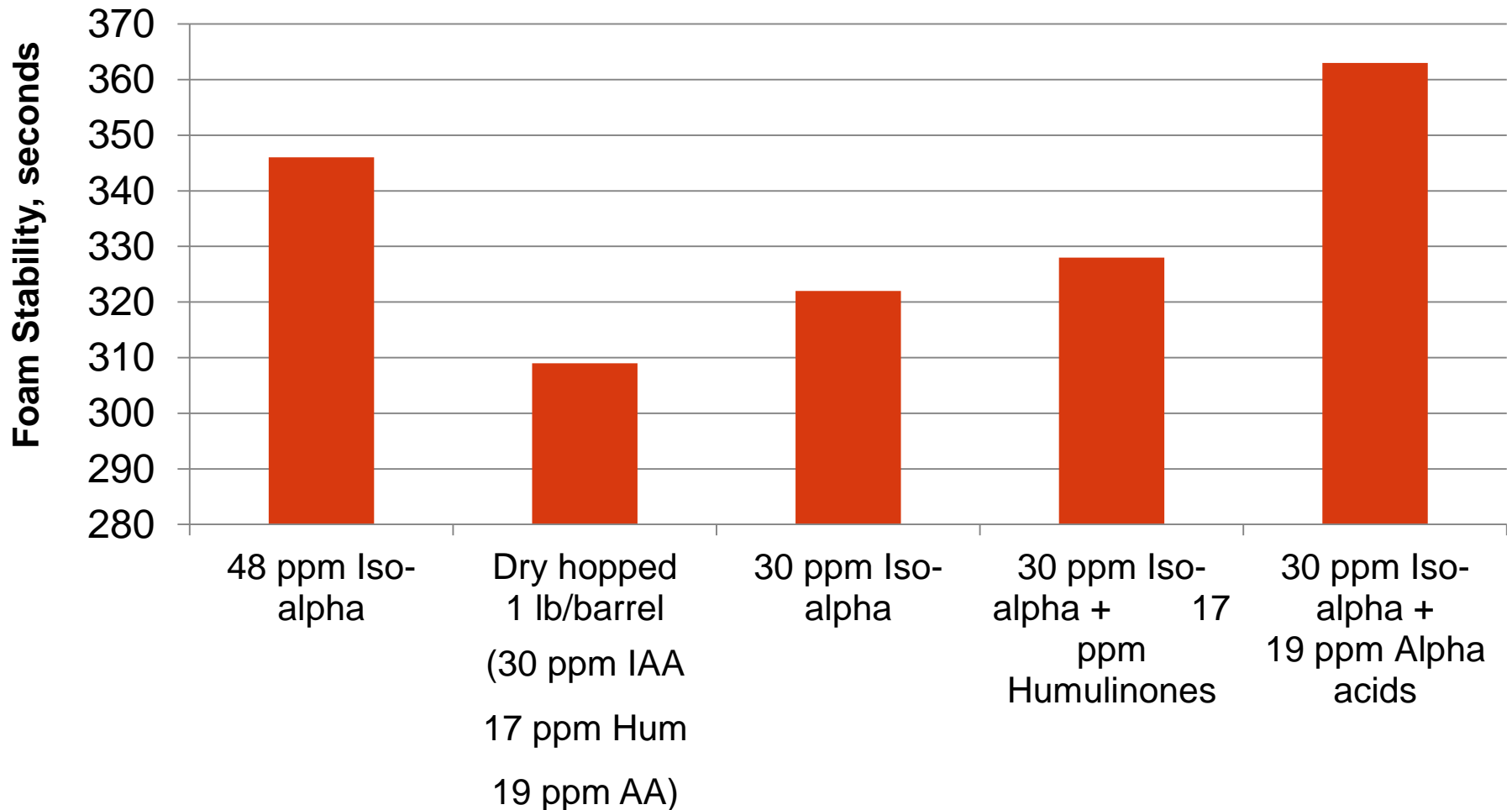
Nibem Foam Results - 1 lb/BBI Cascade Hop Pellets Over 8 Days



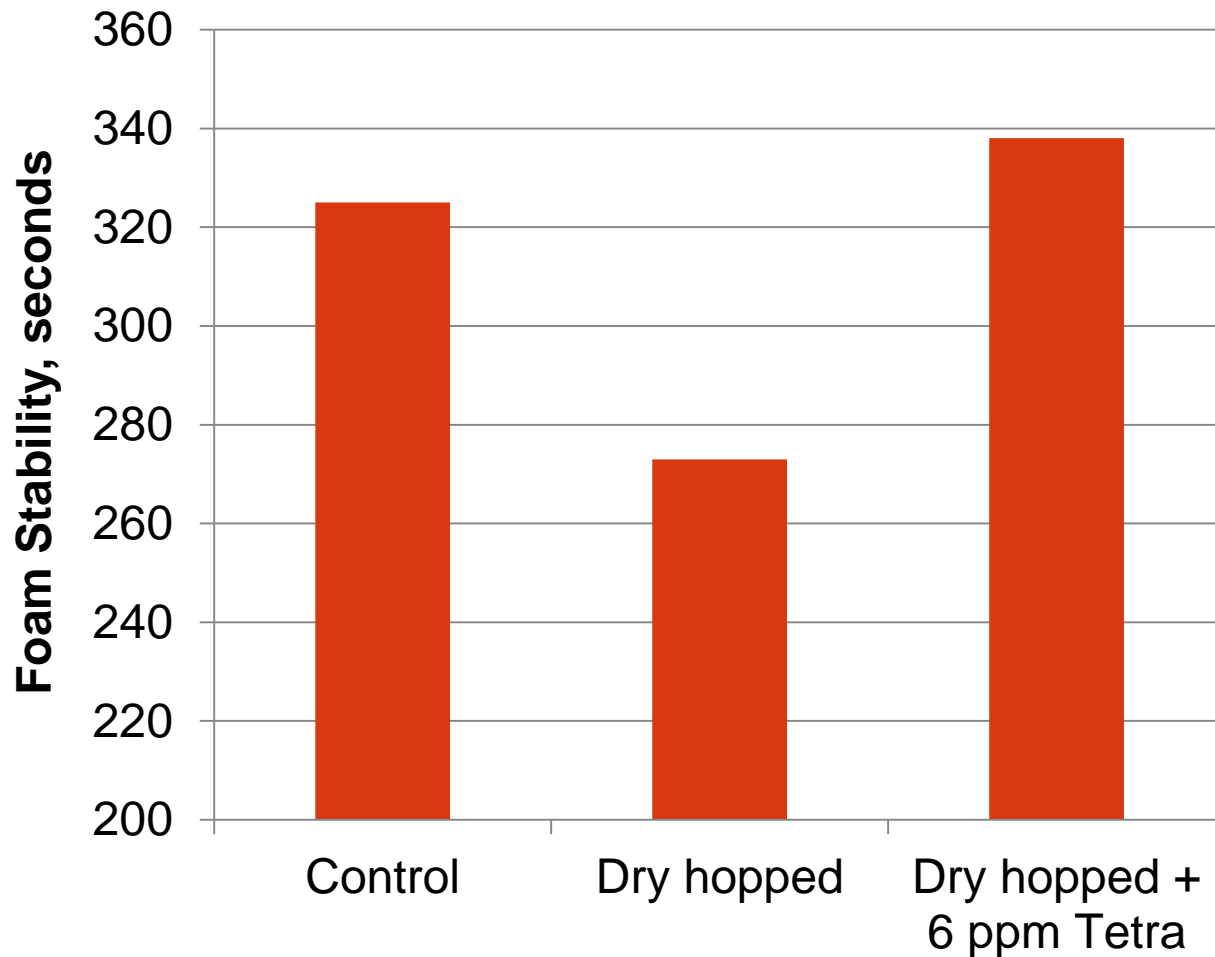
Is The Reduction of Beer Foam Due to The Change In Hop Acid Composition That Occurs With Dry Hopping ?

Sample	ppm isoalpha acids	ppm alpha acids	ppm humulinone
Control	48	0	0
Control + 1lb/bbl Cascade hop pellets	30	19	17

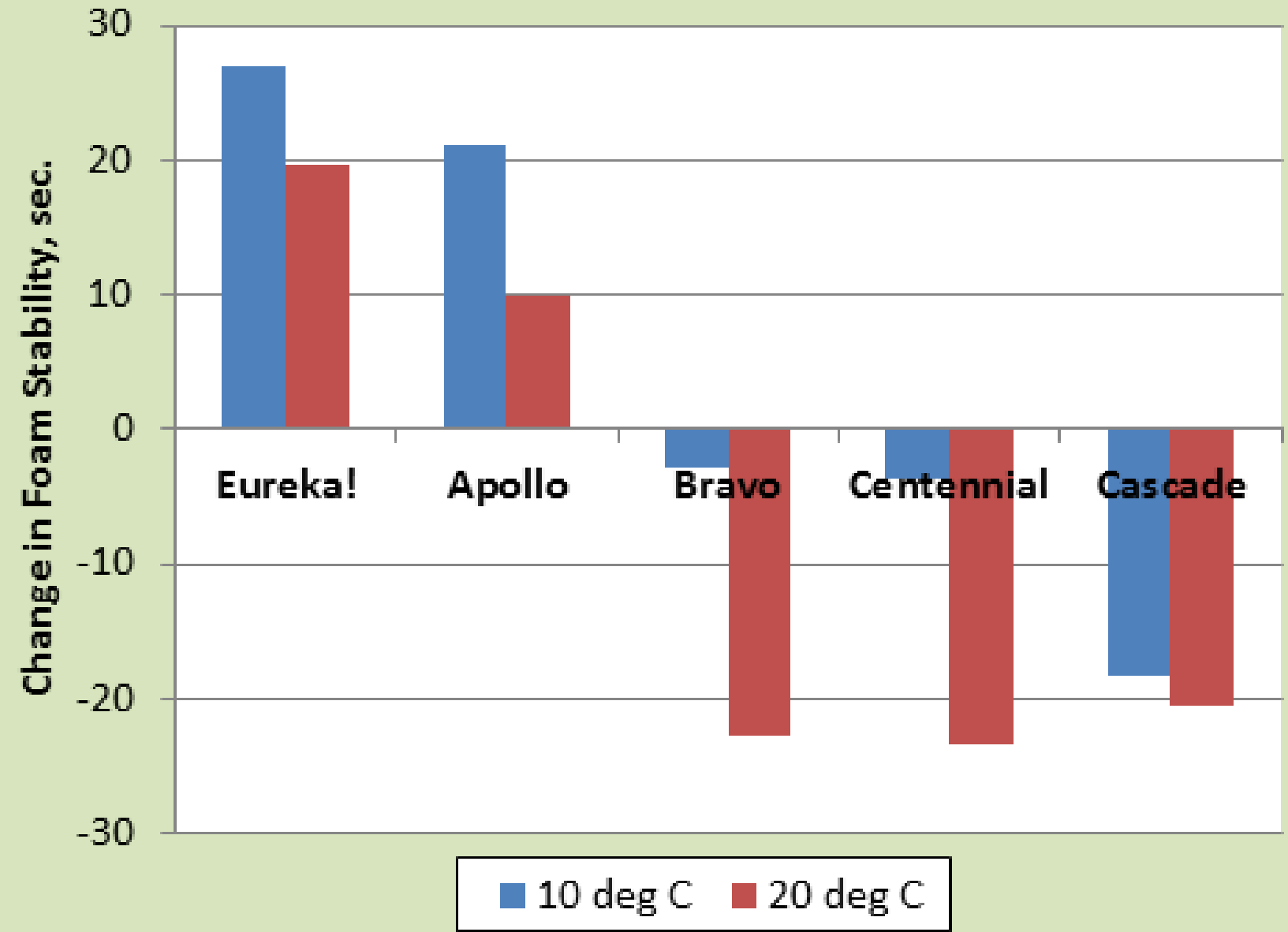
Dry Hopping And Hop Acids Composition vs Beer Foam



Improving the Foam of Dry Hopped Beer With a Foam Enhancing Hop Product (Tetra)

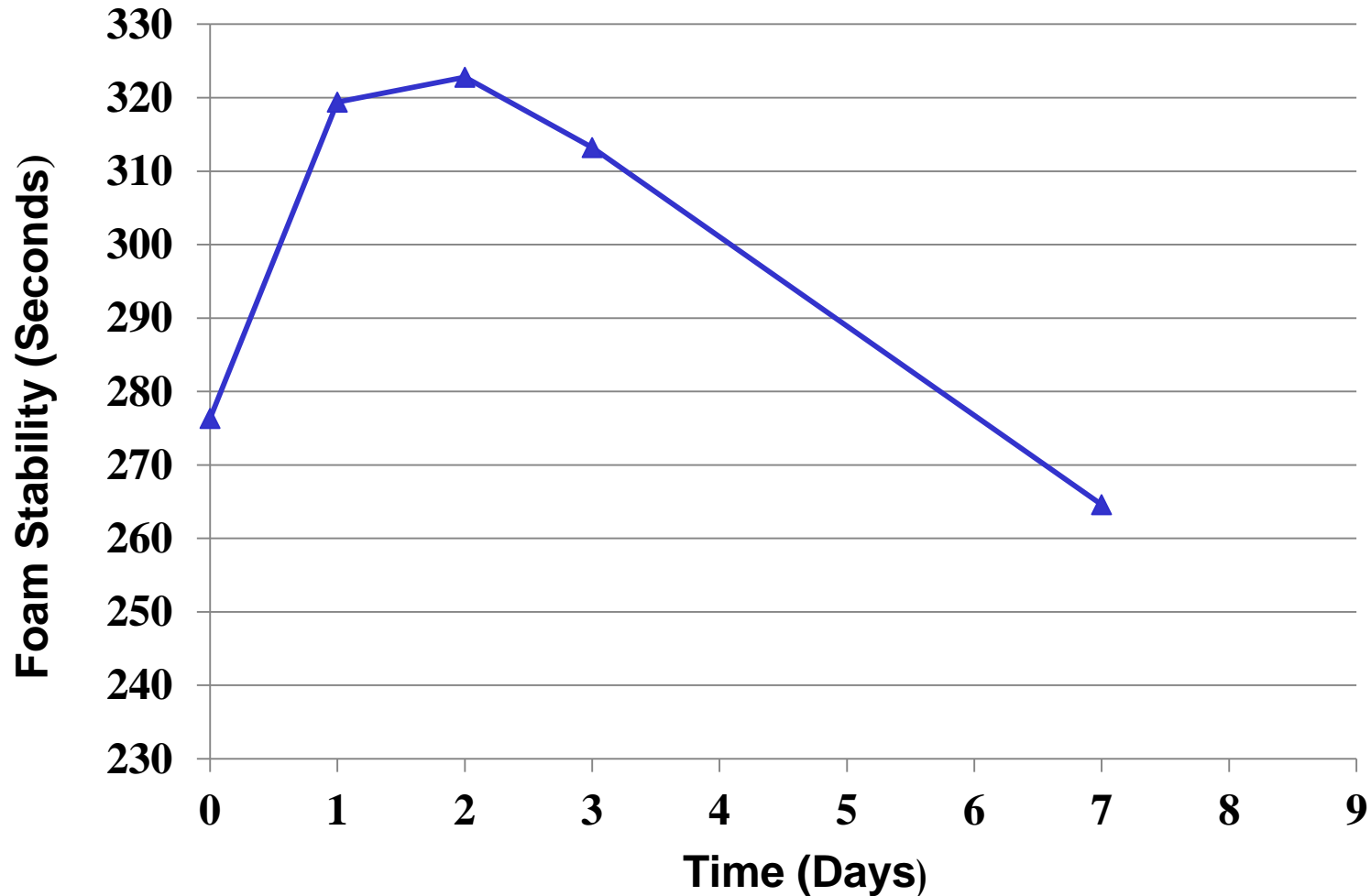


Dry Hopping 1 lb/barrel vs Nibem Foam – Variety Dependent

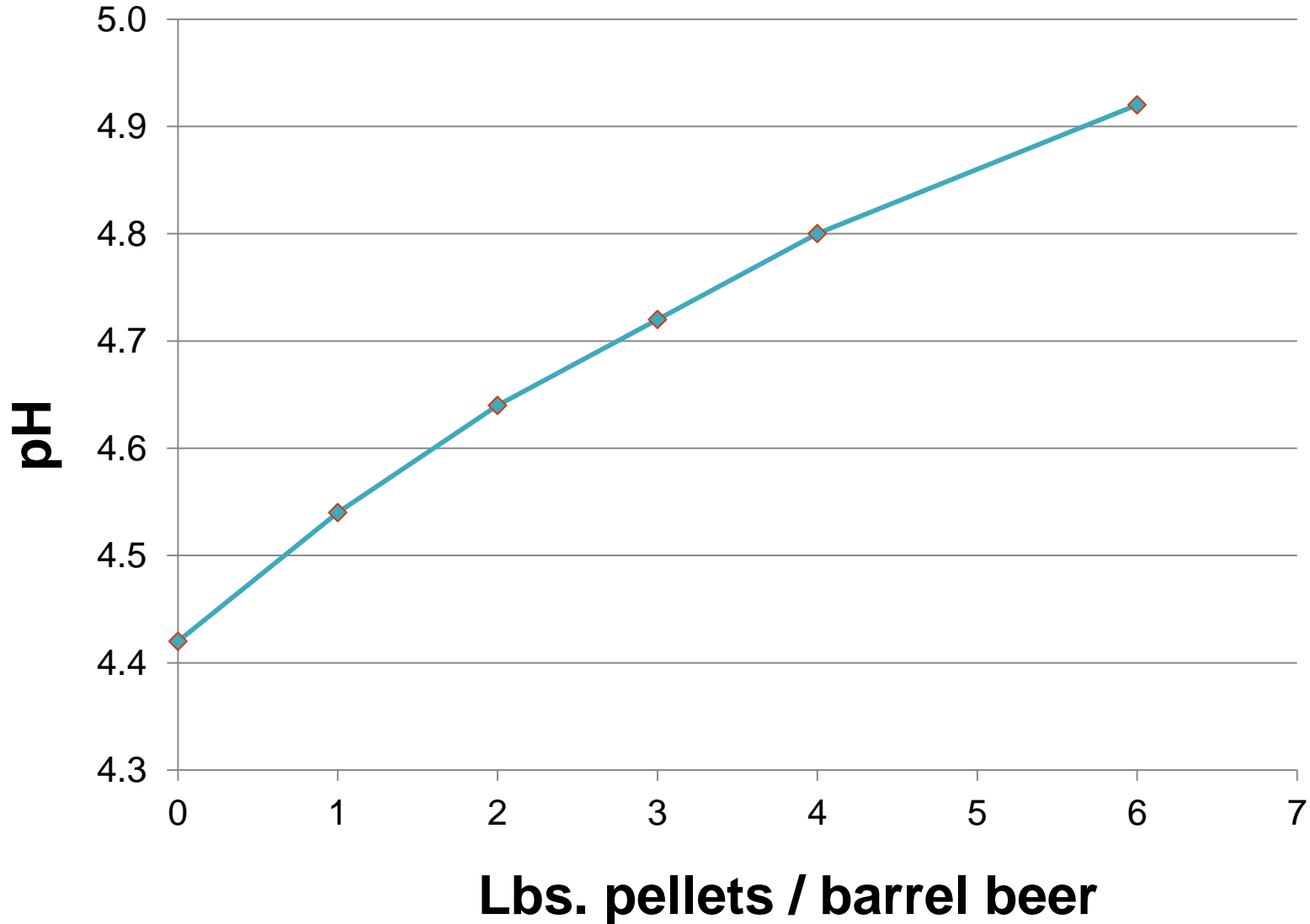


Kinetics of Dry Hopping with Eureka! Hop Pellets

Nibem Foam Stability vs Time



Dry Hopping's Effect On Beer pH



Hops contain small concentrations of humulinones and their concentration increases after pelleting and then stops.

The higher the HSI in hops or hop pellets the higher the humulinone concentration and this relationship is variety dependent.

The humulinones concentration in hops and beer can be measured very accurately using HPLC. Need Calibration Std

Humulinones are more polar than isoalpha acids and are extremely soluble in beer and are reported to be 66% as bitter as isoalpha acids.

Beers that are dry hopped will experience a change in hop acid composition. That is, there will be a reduction in isoalpha acids and an incorporation of humulinones and alpha acids.

Dry hopping a low IBU beer (20 IBU) will remove very little isoalpha acids but add low bitter humulinones and alpha acids and can make a beer more bitter.

Dry hopping a high IBU beer will remove a considerable amount of isoalpha acids and can make a beer less bitter when dosing 0.5 to 2 lbs hop per bbl beer. However due to the high solubility of humulinones, dry hopping any beer with 3 lbs hops/bbl or more can make a beer more bitter.

Because the IBU Test Method was developed to measure only isoalpha acids in beer, dry hopped beers that contain humulinone and alpha acids will give IBU test results that don't correlate with perceived bitterness. That is, the sensorial bitterness will be significantly lower than the IBU test result would suggest.

HPLC can be used to accurately measure the various hop acids in dry hopped beer. By using that data one can calculate a beer's bitterness which should correlate more closely with the perceived bitterness.

Dry hopping can reduce beer foam however this appears to be variety dependent. Also, extended dry hop times can reduce beer foam even further. Foam enhancing hop products like Tetra can be used to enhance the foam of beers that are negatively impacted due to dry hopping.

Because beta acids are extremely non-polar we saw little to no beta acids in any of the dry hopped beers we tested and very small quantities if any of hulupones.

Dry hopping increases a beer's pH by about 0.1 pH units per lbs of hops per barrel dosed and that increase in pH can increase a beer's bitterness by ~ 2 to 3 IBU.

Acknowledgements

Bob Smith & Jeremy Leker

Hopsteiner

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Thank You For Your Kind Attention