The *Humulus lupulus* paradigm

1. Hops – history, cultivation, and morphology.
2. Bitter chemistry – a century of elucidation.
3. Beyond Bitterness – tools for the brewers box.

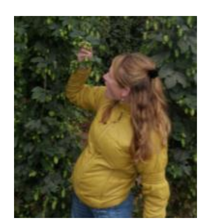
700's -2012

Humulus lupulus

history, cultivation and morphology

Humulus lupulus

Humulus lupulus is the common hop: a dioecious, perenniel, vegetative climbing vine, indigenous to the northern hemisphere.



Origin - CHINA

World renowned Hop Chemist Patrick *Luping* Ting and H. *Lupulus*

Also *H. japonicas,* and *H. yunnanensis,* both of little brewing value

Classification

SCIENTIFIC CLASSIFICATION
Kingdom: Plantae
Order: Rosales
Family: Cannabaceae
Species: H. lupulus

Varietal Subspecies:
H. lupulus var. *lupulus*. – Europe, western Asia.
H. lupulus var. *cordifolius*. – Eastern Asia.
H. lupulus var. *lupuloides* (syn. *H. americanus*). – Eastern N.A.
H. lupulus var. *neomexicanus*. – Western N.A.
H. Lupululs var. pubescens – Midwest N.A.

Cultivation

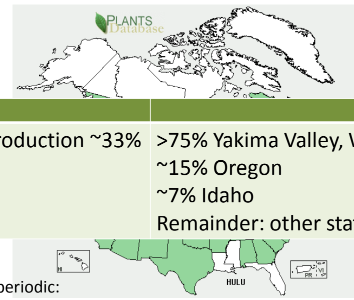
736 Hallertau, Germany
1000 Bohemia, Bavaria, Slovenia
1300 Expanded to Central Europe, Russia and the Ukraine, Flanders and U.K.
1524 England
1629 Massachusetts company imports to N. America
(New Netherlands and Virginia Colony)
1700 - **1800s** extends globally:
S. Africa, New Zealand, Tasmania (1820), Australia(1794) Japan (1860's) , China (1874-1921), India (1880).
1808 First commercial U.S. hop yard in New England
1850 Hops are planted in California, Oregon
1868 Hops are planted in Yakima Valley, WA
1870 Signficant operations in Slovenia, Croatia, Serbia, Macedonia, Bosnia
1878 Hop Picker invented by H.G. Locke, American, patented in Germany
1950 85% of US Hop Harvest picked by machine

Cultivated Varieties -2012

- >80 Varieties Commercially
  - Plus novel varieties through multiple breeding programs
- American, English, German, Noble, Australian, New Zealand, European, Japanese and Ornamentals.

Aroma	% α-Acids	Bitter	% α-Acids	Dual Purpose	% α-Acids	US-European	% α-Acids
Cascade	4.5-7.0	Galena	11.5-13.5	Citra	11.0-13.0	Saaz	3.0-4.5
Willamette	4.0-6.0	Chinook	12.0-14.0	Simcoe	12.0-14.0	Tettnang	4.0-5.0
Mt. Hood	4.0-7.0	Nugget	11.5-14.0	Centennial	9.5-11.5	Hallertau	3.5-5.5
Golding	4.0-6.0	Warrior	14.5-16.5	Amarillo	8.0-11.0	Golding	4.0-6.0
Fuggle	4.5-5.5	Apollo	15.0-19.0	Calypso	12.0-14.0	Perle	7.0-9.5
Sterling	4.5-5.0	Bravo	14.0-17.0	Cluster	5.0-8.5	Magnum	12.0-14.0
Liberty	3.5-6.5	Super Galena	13.0-16.0			Northern Brewer	8.0-10.0
Santiam	5.5-7.0	Summit	16.0-18.0				
Vanguard	4.0-6.0	CTZ	14.5-16.5				
		Millennium	14.5-16.5				

U.S. Agronomy -2012



U.S. Production ~33%	>75% Yakima Valley, Washington ~15% Oregon ~7% Idaho Remainder: other states

- Photoperiodic:
  - Daylight and latitude impact flowering
  - 35^{th} – 70^{th} Parallels

http://plants.usda.gov/java/profile?symbol=HULU&photoID=hulu_001_avd.tif

Hop Picking & Separating

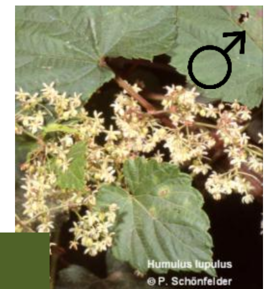
Hop Kilning & Baling

Hop Morphology

- Diploid with 20 chromosomes
- Sometimes triploid, Tournis reported (1
  - Basically male but with occasional female strobiles: XXY
  - Can be bred to be triploid
- Haunold (1972) cloned plants of same genotype at different developmental stages and achieved pollination.
- Dioecious:
  – Recognizable sex chromosomes: X (male) and Y (female)

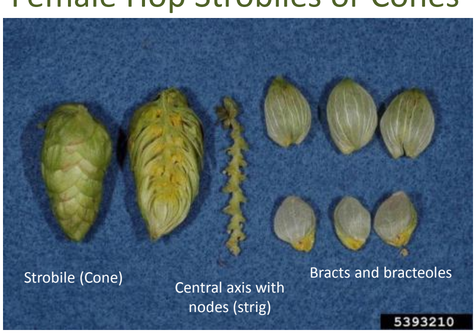
Hop Morphology - Sex

- Male flowers produce pollen.
- Female strobile or cones contain small flowers with ovaries.



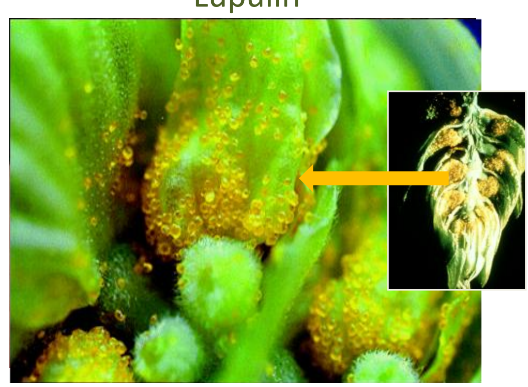
Fertilization:
Genetic diversity
Seeded hops

Female Hop Strobiles or Cones



David Gent, USDA Agricultural Research Service, Bugwood.org

Lupulin



Markets and Breeding

Two Hop Markets:

- Alpha\Bitter
  - Processed hops
  - Yield = kg alpha/acre
  - High alpha / Crossovers
- Aroma
  - Limited Processing
  - Yield = lb hops/acre
  - Low or high alpha varieties

Breeding Objectives:

- -Bitter: High Alpha/High Yield
- -Dual Purpose: High Aroma/High Alpha/High Yield
- -Specialty: Novel aroma with good agronomics
- -Specialty: Low or High Cohumulone/Oil components
- -Organic with good agronomics
- -High Storage Index
- -Disease Resistance

1900's-1970's

Early Characterization of the Bittering Acids

1887

The Alpha Acids

1887 – Hayduck
Hop Constituents precipitate with lead (II) acetate

Precipitate is **green** due to presence of **chlorophyll**

Purified precipitate with silica gel = canary **yellow colored lead salts**

1900's-1930's

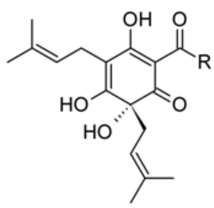
The Alpha Acids

- 1900 – Barth: First Attempt Structural Elucidation
- 1904 – Lintner and Schnell: "humulones" "humulinic acid"
- 1916 – Wollmer: Structure contains enolic group
- 1925 – Wieland: Proposed Alpha Acid Structure

1950's-1970's

The Alpha Acids

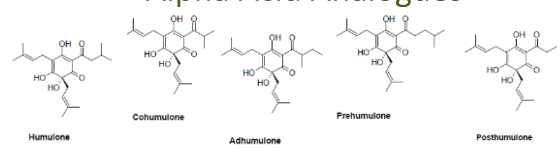
Rigby and Bethune 1952
Proposed several analogues and isomers



Absolute Structure
De Keukeleire 1971
(-) (6R)-2-(3-methylbutanoyl)-4,6 bis (3 methyl-20butenyl)-3,5,6-trihydroxycyclohexa-2,4 –dienone
Empirical Formula: $C_{21}H_{30}O_6$

1950's-1960's

Alpha Acid Analogues



Oxidized Derivatives Alpha Acids
Humulinone: Cook and Harris 1950
Oxyhumulinic Acid: Alderweireldt and Verzele 1957

Posthumulone***	CH_2CH_3	334	1-3

(Rigby, Bethune, *1952, **1953 and ***Verzele 1955)

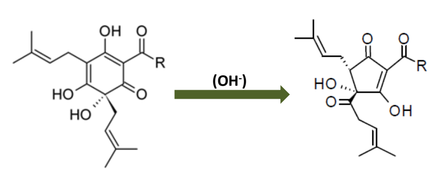
The Alpha Acids

-Not bitter.
-Unstable – oxidize readily in presence of oxygen, heat and light.
-Some oxidized alpha acids form hard resins that do not contribute to beer bitterness.
- Some oxidized alpha acids do contribute to bitterness: humulinones and humulinic acids.
-At 25°C humulone aqueous solubility is low ~6mg/L
-Alpha acids are relatively unsoluble in wort at pH 5, reaching a maximum of about 84 ppm when heated at pH 5.2, and even higher at pH ~6.5.

1920's-1930's

Isomerized alpha acids

Wieland Proposed Compound (1925)
Windisch, Kolbach, Schleicher “Soft Resin A” (1927)
Later named ‘Isohumulones’

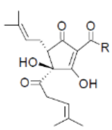


1950's -1970's

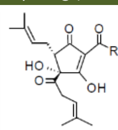
Isomerized alpha acids

1940's – Research recommence with Verzele and Govaert.
1952, 1957 – Rigby and Bethune/Howard et. Al: Bitter substances gave 6 peaks, representing epimeric isomers of each of the three major analogues of alpha acids

Iso humulone A and B, Spetsig (1964)



trans-isohumulone

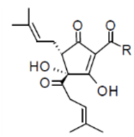


cis-isohumulone

1960's-1970's

Isomerized alpha acids

Structure was confirmed in 1965 by Vancraenenboeck, Vanclef and Lontie



cis-isohumulone

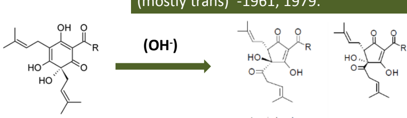
Absolute stereochemistry was achieved in 1971 by De Keukeleire

1950's-1980's

Isomerization

Isomerization from a 6 membered ring to a 5 membered ring:
- Heat >100°C, readily above 180°C (Lance et al. 1975)
- Higher pH yields higher conversion (Anteunius and Verzele 1959)
- Divalent Cations such as Mg^{2+} increase rate (Koller 1968)
- Normal Brewing Conditions yield 32:68 for Trans:Cis

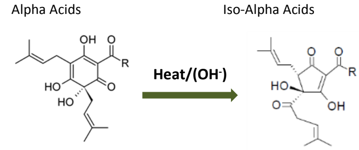
Photoisomerization can also occur under prolonged exposure to irradiation at 254 or 350 nm, 10-12 hours (mostly trans) -1961, 1979.



Utilization

-Isomerized-alpha acids are relatively soluble in water and wort.
- -Boiling in wort utilization is about 25-35%
- -Maximum conversion is about 60% from alpha to iso-alpha
- -Losses due to insufficient boiling time, dispersion, oxidation, adsorption , foaming…….and more in fermentation and filtration.

Alpha Acids — Iso-Alpha Acids

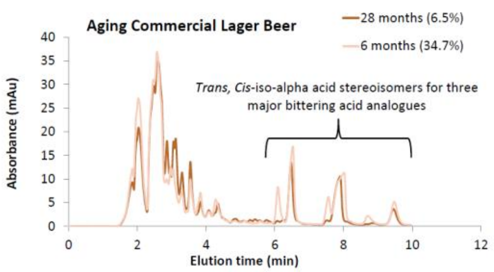


3 mg/L — 120 mg/L

*Peacock 1998

Iso-alpha acid stability

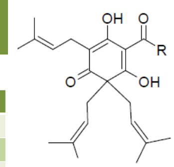
Thermal instability - Cis is thermally more stable, losses of trans occur over time



The Beta Acids -Lupulones

1863 Lermer isolated a colourless crystalline compounds
1916, 1925 Wieland and Wollmer proposed the structure
1957 Complete Separation by Verzele
1973 Modern isolation in hexane by Vandenbossche

Beta Acid	Acyl R	%
Lupulone*	$CH_2CH(CH_3)_2$	30-55
Colupulone*	$CH(CH_3)_2$	20-55
Adlupulone**	$CH(CH_3)CH_2CH_3$	10-15
Prelupulone***	$CH_2CH_2CH(CH_3)_2$	1-3
Postlupulone***	CH_2CH_3	?



Beta Acids

The Beta Acids

-Not bitter unless oxidized.
-Poorly soluble in water and wort.
-Poor solubility as pure compounds (1 g/100mL), but more soluble as a mixture
-Stable to alkaline hydrolysis in absence of oxygen.
-Susceptible to oxidation comparable to alpha acids
- Oxidation results in hulupones – which are said to have undesirable bitterness and can account for loss of alpha in old hops.

1950's-1990's

Improved Utilization
of the Bittering Acids

1950's-1990's

Pelletization

Type 90 - 100 kg of raw hops = 90 kg of pelletized hops
Type 45 - 100 kg of raw hops = 45 kg of pelletized hops

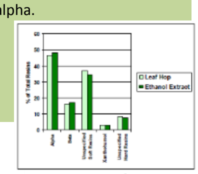
-Retains most of the hop character
-More compressed than whole cones
-Packed under inert gas or vacuum extends life
-Blending can produce pellets of consist alpha acid content

Whole leaf hops: 20-20% utilization
T90 Pellets: 30-40% utilization

1950's-1990's

Solvent Extraction

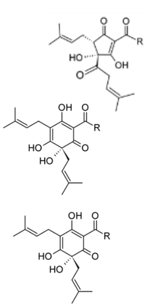
SOLVENTS: Hexane, methanol, methylene chloride or trichloroethylene
-Solvents are evaporated
-Loss of more volatile essential oils may occur.
-Green viscous liquids that contain as high as 50% alpha.
-Canned, very stable during storage
-Standardized with glucose syrup
- Extraction cost is compensated
-elimination of storage losses
-increased utilization (30-40%).



Complete or partial replacement of Pellet Hops

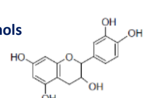
Hopsteiner

Separation of Hop Constituents



Organic Extractable
- Lipids
- Waxes
- Essential Oils
- Soft Resins
- Hard Resins
- Some polyphenols

Aqueous Extractable
- Cellulose
- Lignin
- Proteins
- Polyphenols



1950's-1990's

Isomerized Pellets and Extracts

Chemothermic process converts alpha acids to iso alpha acids.

Pellet Products
-Stabilized by the addition of magnesium salt
-up to 20% iso-alpha ~90% of alpha in original pellet is converted
-Utilization 45-55%

Pre-isomerized hop extracts
-Magnesium or potassium salts

Can be added late in the kettle or even after fermentation, better exploitation of bittering content

*German beer purity laws prohibit usage.

1970's-1990's

Solvent Free Extraction
of the Bittering Acids

Liquid or Supercritical CO_2 Extraction

1975 - Laws et al. of BRF introduced **liquid CO_2 hop extracts** (rich in α-acids, β-acids, and essential oils) under 1000 psi (69 bar) and 50°F(10°C), which was then commercialized by Carlton & United Breweries.

1978, Muller, Vitathum and Huber developed **supercritical CO_2 hop extraction** under 3000 psi (207 bar) and 110°F(43°C).

CO_2 Extracts

-Golden/Amber/Green Semi-fluid Resin or Paste
-Alpha Acid is Variety dependent
 ~ 30% aroma hops
 ~ 50% high alpha variety hops
-Contains hop oil fraction
 -Late addition maintains more volatiles
-Up to 40% utilization in the kettle
 -Late addition = lower utilization

- Ensures more standardized product
- Extremely Stable – up to four years from production date.
- Easy to transport
- Easy to store
- Available in bulk (tote), buckets or cans
- Pre-isomerized CO2 extracts are available

1950's – 2000's

Battling light instability
of the isomerized alpha acids

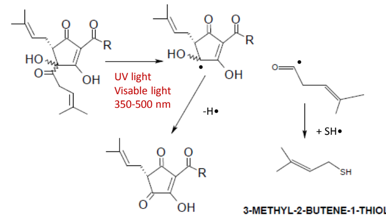
1950's – 2000's

Sunstruck Flavor

1950's, Miller Brewing discovered that under either UV or visible light (in the presence of riboflavin) iso-a-acids and sulfur caused light instability in beer.




Photoxidation of Iso-alpha-acids



The **sun-struck** or **skunky flavor** (3M2B1T) and the mechanism of its evolution was later characterized by Kuroiwa, et al. of Kirin Breweries in the early 1961 and 1963.

1950's and 1960's Miller Research

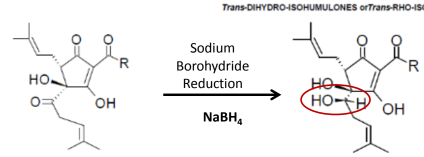
- Light stability……1957…Miller developed a process to produce light stable hops (35% Rho).
- Two products were patented, one for kettle hopping and one for post-fermentation addition (Rho + hop oils).

1961 Light stable beer debuted in flint bottles…. the champagne of beer…MillerHighlife.

Reduced Iso-alpha acids

Miller Brewing patented a commercially viable process to produce **light stable rho-iso-a-acids.**

Trans-DIHYDRO-ISOHUMULONES or *Trans*-RHO-ISOHUMULONES



The sunstruck or lightstruck flavor is not observed when the carbonyl group of the side chain is reduced.

Reduced Iso-alpha acids

- Concentrated resins = reduced storage volume
- Exist as aqueous solutions 10 - 40% Rho, K^+ salt forms
    -Reddish amber in color
- Can be used for light stable applications (clear or green bottles)
- Increased utilzation:55-95% , depending on form.
- Increased consistency
- Can be added in the kettle or post fermentation
- Increased storage stability
    - 1- 2 year shelf life
    - Store at 50-59F
-Available in pails (3 kg), drums and totes (1000kg)

60 -70% as bitter as Iso-alpha-acids

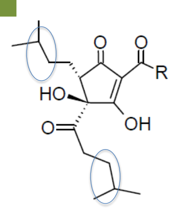
Miller Highlife's clear bottle drove a need for improved light stability…..

- Commercial Rho-iso-alpha acids ultimately replaced traditional Extract as a bittering agent.
- Light stability testing revealed Rho-iso-alpha acids were not perfectly light stable due to purity issues…….

Tetrahydro-Iso-alpha acids

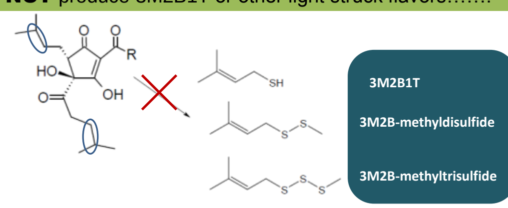
- Another advanced hop product, the Tetrahydro-iso-alpha acids was determined to provide superior light stability……

- 1970 Donnely and Shannon Isolate tetrahydroisohumulones by chromatography.
- 1971 Produced by hydrogenolysis by Byrne and Shaw
- 1975 Kalsec production patent
- 1984 Commercial production of tetrahydro iso-α-acids



Trans-tetrahydro-isocohumulones

Under **UV** or **visible light** the tetrahydroiso-α-acids **DO NOT** produce 3M2B1T or other light struck flavors…….



With Tetrahydro-isohumulones no stabilized 3MBT radical can be formed (Todd et al 1972)

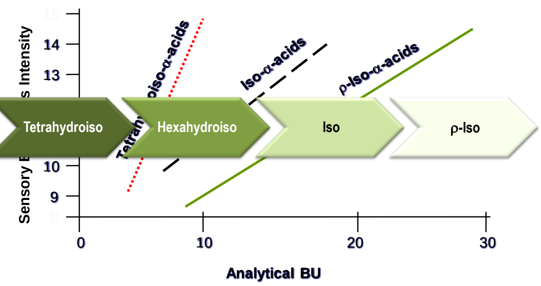
1980's Miller Started using Tetra produced by Kalsec.

Tetra-iso-alpha acids

- Concentrated resins = reduced storage volume
- Exist as aqueous solutions 9-15% Tetra
  - Yellow to amber in color
- Complete protection against lightstruck flavor formation
- Increased utilzation:60-80%%
- Increased consistency
- Can be added in process or post fermentation
- Increased storage stability
  - 1 year shelf life
  - Store at 50-60F
- Available in pails (3 kg), drums and totes (1000kg)

1.0 to 1.7 times more bitter than Iso-alpha-acids

Comparison of Sensory vs. Analytical Bitterness

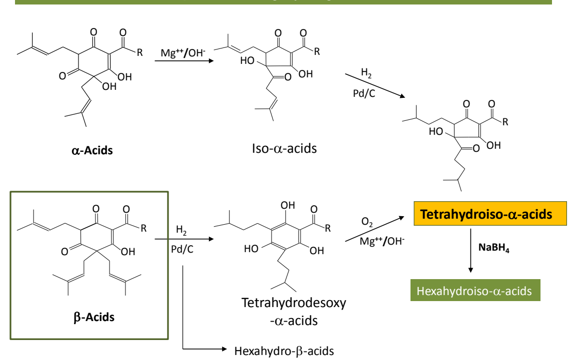


Separation of CO_2 Extract

1982- Miller Brewing Patent:
Process of directly separating α-acids, β-acids, and hop oils from CO_2 extract using pH partitioning.

IMPORTANCE: Solvents were no longer needed, no more hexane or peracetic acid….safer methods of separation and production for advanced hop products using only water, ethanol, and air…..

1985 – Miller Brewing Patents the production of Tetrahydroiso-α-acids from either α-acids or β-acids using hydrogen, air, ethanol, and water.



1960's- 2012

Utilization and Cost Savings.....

from whole hops to advanced hops.

Overall Advancements in Utility through product innovation

Hop Form	Utilization BU from Alpha	Shipping and Storage Volume (100K Hectoliters of beer	%alpha loss during Cold Storage for one year
Baled Hops	15-30%	80m^3	14%
Pellets	25-40%	24m^3	5%
T45 Pellets	25-40%	14m^3	5%
Extract	25-40%	8m^3	5%
Pre-Isomerized Extract	60-85%	3m^3	3%
Reduced Isomerized Extract	50-95%	3m^3	3%
Tetra Isomerized Extract	60-85%	3m^3	<3%

Cost Impact Example – 100% replacement

- Replacing Pellet Hops with Isomerized Extract (30%)

Pellet Kettle Hopping			Post Fermentation ISO-30		
Target	10 mg/L	ISO-Alpha Acids	Target	10 mg/L	ISO-Alpha Acids
Making 40 Liter	400 mg	ISO-Alpha Acids	Making 40 Liter	400 mg	ISO-Alpha Acids
Hop Utilization	25 %		ISO-30 Utilization	80 %	
	1600 mg	Alpha Acids		500 mg	ISO
Hop Alpha Content	10 %		ISO-30 Concentration	30 %	
	16000 mg	Hop Pellets		1667 mg	ISO-30
Pellet Utilization	95 %				
	16842 mg	Hop Cones			
Cone cost $3 /lb	$0.11/batch		ISO Cost $11.34 / lb	$0.04 / batch	
	$0.32/bbl			$0.12/bbl	

37% Hop Cost Reduction

**40 liter batch size with a target of 10 BU

Cost Impact Example – 25% replacement

- 25% CO_2 Extract and 75% Pellets:
  - 19% Savings per barrel
- 25% Iso-alpha acids (30%) and 75% Pellets:
  - 16% Savings per barrel
- 25% Tetra-iso-alpha acids (10%) and 75% Pellets:
  - 12% Savings per barrel

Beyond Bitterness

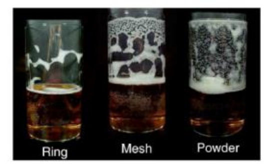
Foam Enhancement

1970's-2009

FOAM STABILITY AND CLING

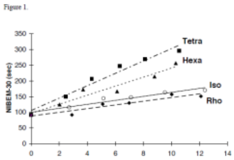
- Beer foam potential requires the interaction of **hop bittering acids** and **beer proteins.**
- Preferentially interact with the most foam stabilizing compound in beer, LTP (barley lipid transfer protein), due to hydrophobic interactions.
- Reduced products retain foam potential because they do not degrade while Iso-α-acids degrade over beer shelf life.
- Protect the foam in non-pasteurized beer even while yeast proteinase A slowly destroys the foam proteins.

Figure 5. Kunimune and Shellhammer 2008



COMPARISON of FOAM STABILITY AND CLING

Figure 1.



Kunimune, 2007

Figure 6.

Beyond Bitterness

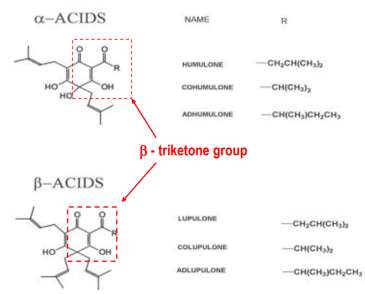
Antimicrobial Power

1970's-2008

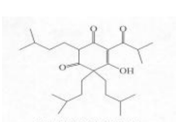
Hop acids inhibit or kill all Gram positive bacteria (Teuber 1970).

Lactic acid bacteria (beer spoilers) are affected differently than a number of other Gram positive bacteria.
Possibly due to differences in maintaining internal cellular pH's

The Key Anti-Microbial Components of Hops



The β-triketone group on the hop acid acts as a binding site for cations. This becomes important for transporting manganese and hydrogen ions across the bacterial cell membrane.



- Hop acids antibacterial against gram positive organisms. (Teuber 1970)
- Act as ionophores transporting ions across bacterial cell membranes, disrupting membrane ion gradients, causing leakage, starvation and cell death. (Teuber and Schmalreck 1973)
- Hydrophobicity of the undissociated form of the hop acids enhances the ionophoric nature = increased antibacterial activity. (Simpson 1991-1993)
- Tetrahydroiso-α-acids and Hexahydro-β-acids are the most antimicrobial of hops acids, but pH dependent. (Miller Brewing 1987 and 1995)
- Tetrahydroiso-α-acids show more effective against microbes than iso-α-acids during the acid washing of yeast. (Miller Brewing 2001)

Relative Anti-Microbial Activity

Hop Compound (optimal effective pH)	Relative Anti-Microbial Activity
Hexahydro-β-acids (pH 7)	⚡⚡⚡⚡⚡
Tetrahydroiso-α-acids (pH 4.2)	⚡⚡⚡⚡
Iso-α-acids (pH 4.2)	⚡⚡
ρ-Iso-α-acids (pH 4.2)	⚡

Applications-Brewery

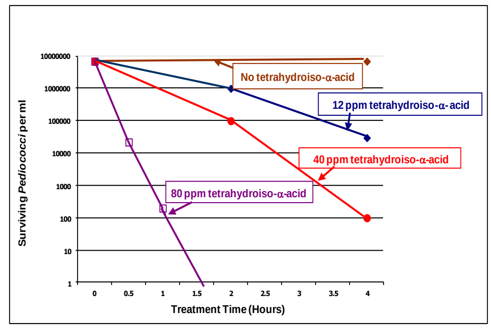
Investigated acid washing with and without hop acids

- Micro control in brewing yeast has traditionally been managed through acid treatment.
  - ➢Sulfuric or phosphoric acid
  - ➢Target pH 2.3 to 2.5

Hop acids were shown to be responsible for killing bacteria during the acid treatment process.

- Hop acid solubility decreases as pH decreases.
- Tetrahydro iso-alpha acids more effectively kill bacteria in acid treated yeast than iso-alpha acids.
- Since tetrahydro iso-alpha acids are so effective at killing lactic acid bacteria at low pH, solubility is not an issue (i.e. fewer ppm are required).

Effects of Tetrahydroiso-a-acids on Disinfecting Brewer's Yeast Seeded with an Acid Resistant Pediococcus (at pH 2.3)



Advanced Hop Products….beyond bitterness.

Product	Bitterness vs. Natural	Light Stability	Foam Enhancement	Antimicrobial
Tetrahydro-iso-alpha acids	1.7 x	Best	Best	Best, pH dependent
Rho-iso-alpha-acids	0.7 x	Good	Minimal	Minimal
Iso-alpha acids	1.0	None	None	Good

Beyond Bitterness

Flavor

1950's-2012

Hop Flavor

Hops also provide spicy, floral, citrus aroma and flavor, and "mouthfeel" characteristics to beer.

Early extensive investigations were conducted to correlate the hop oil compounds to various hoppy flavors in beer.

- Chapman's early studies (1895-1929)
- Howard (1956)
- Howard and Stevens (1959)
- Irwin (Labatt)
- Fukuoka and Kowaka (Kirin)
- Peacock and Deinzer (Oregon State)
- Tressl (Technischen University, Berlin)
- Harley and Peppard (BRF)
- Lam, Foster II, and Deinzer (Oregon State)
- And many others……….

Fractionation of hop oils
Haley, Peppard, Westwood et al. of BRI in 1985

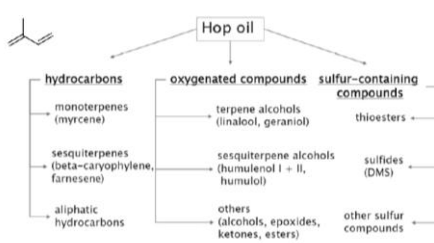
Spicy fraction
Floral fraction
Citrus fraction
Noble Hops
Late Hops
Dry Hops

Commercial post-fermentation products became available to mimic late and dry hopping………..

However, these fractions may not produce desired early kettle hop flavor. …….

Hop Oil Fraction

> 300 Compounds
70% Hydrocarbons
30% Oxygenated Compounds

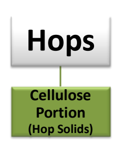


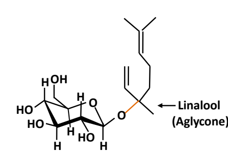
Schönberger, C.; Kostelecky, T. 125th Anniversary Review: The Role of Hops in Brewing. *J. Inst. Brew* **2011**, *117*, 259-267.

Glycosides: A Secret of Hop Flavor Revealed

In 1998, Miller Brewing found that the cellulose portion (hop solids) after CO_2 extraction contains a mixture of water soluble substances composed of 92.4 mole% of glucose with a majority of 55% terminal and other linkages.

- A group of β-glycosides survive the kettle boil because they are **water soluble** and **non-volatile**.

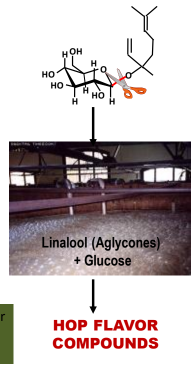




Linalyl β-Glycoside

Glycosides: A Secret of Hop Flavor Revealed

- **Yeast** can hydrolyze β-glycosides and further convert aglycones into hop flavor.
- The **β-glycosides** present in the hop cellulose portion contribute the true kettle hop flavor in beer.
- Further supported by
  - H. Kollmannsberger and S. Nitz, 2002
  - M. Biendl, H. Kollmannsberger and S. Nitz, 2003
  - L. Daenen, D. Saison, L. De Cooman, G. Derdelinckx, H., Verachtert, F. R. Delvaux, 2006



Aglycones are representative of the oxidative and sulfur containing compounds of the Hop Oil Fraction: alcohols, carbonyls and others

Kettle Hop Aroma

No single hop oil component has been shown unequivocally to be present in kettle-hopped beer.

- Sandra and Verzele
- Peacock and Deinzer
- Rigby
- Miller Brewing

…..and the chemistry of hop flavor is still not properly understood.

Beyond Bitterness

Flavor Stability

1990's-2008

FLAVOR STABILITY

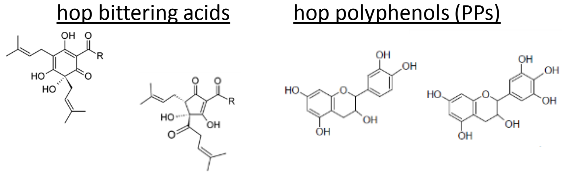
Aged-beer flavor depends heavily on the oxidative degradation of beer compounds by reactive oxygen species (ROS).

ANTIRADICALS and ANTIOXIDANTS

Hop polyphenols (PPs) and hop bittering acids influence oxidative mechanisms

- Metal Chelators
- Free Radical Quenchers
- Reactive Oxygen Species Quenchers
- Enzyme mediators and Inhibitors

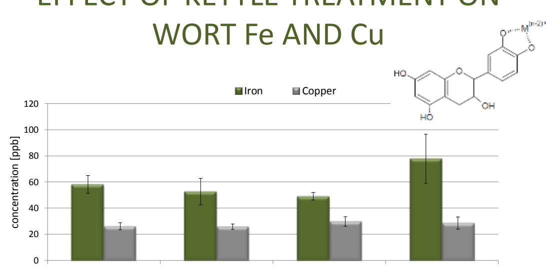
hop bittering acids   hop polyphenols (PPs)



Antioxidants or antiradicals? **That depends..**

- **Humulone and lupulone by a DPPH radical assay** (Motoyuki et al. 1995)
- **Polyphenols are negligible antioxidants in wort and beer by ESR** (Andersen et al. 1999)
- **Polyphenols by the rancimat method; Bittering substances by the fluoro-scan test** (Forster et al. 2001)
- **Polyphenols by AAPH [2,2'-azobis(2-amino propane) dihydrochloride]** (Collin et al. 2001)

EFFECT OF KETTLE TREATMENT ON WORT Fe AND Cu



Kettle treatment with hop pellets resulted in beers lowest in iron content.

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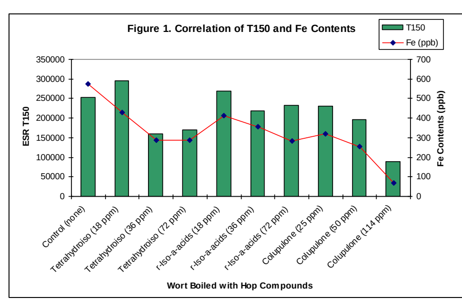
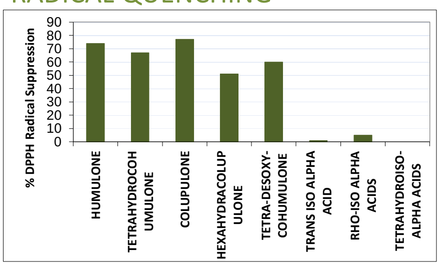
WORT: METAL CHELATION IRON AND T150 CORRELATIONS

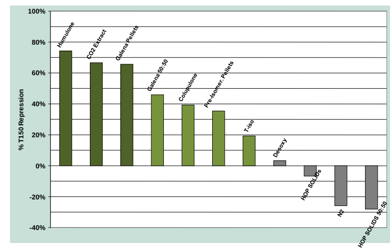


BEER: HOP CONSTITUENTS AND DPPH RADICAL QUENCHING



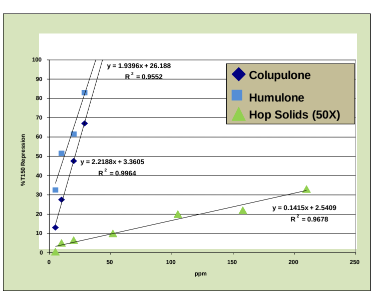
Hop bittering components are effective radical suppressors by DPPH – standalone.

WORT: EFFECT OF HOP PRODUCTS ON ESR T150 SUPPRESSION



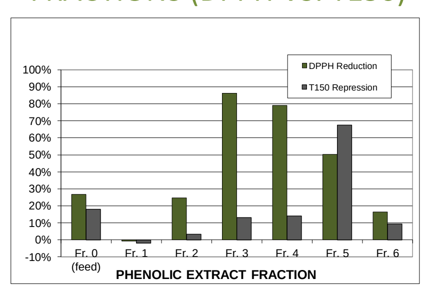
- T150 suppression of boiled wort with the addition of various hop products at 65^{0}C.
- The results are relative to a daily wort control.

BEER: HOP PRODUCTS REPRESS ESR T150



- Hop bittering acids and hop solid extract titrated into lager beer show a linear response by ESR.
- humulone > colupulone >>> phenolic extract

BEER: C18 HOP SOLID PHENOLIC FRACTIONS (DPPH VS. T150)

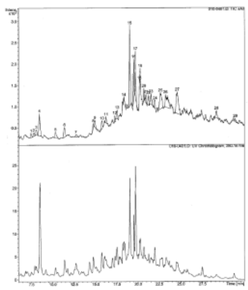


•DPPH vs ESR data conflicting

•Fractions 3 and 4 contain catechin and polyphenol dimers, trimer and tetramers as well as rutin

Compound #[a]	RT	m/z	Proposed Identity	Confirmed Via[b]
*	5.1	584.0	(-)-epigallocatechin-gallate-phloroglucinol	gse
*	6.3	306.0	(+)-gallocatechin	std
1.0	7.4	414.0	ent-(-)-catechin-phloroglucinol	
2.0	7.7	448.0	Unknown	
3.0	8.0	414.0	(+)-catechin-phloroglucinol	gse
4.0	8.4	414.0	(-)-epicatechin-phloroglucinol	gse
*	10.3	306.0	(-)-epigallocatechin	std
5.0	10.3	444.0	(+)-catechin-gallate	std
6.0	11.3	290.0	(+)-catechin	std
7.0	12.7	414.0	Unknown	
*	13.6	136.0	Caffeic acid	std
*	14.7	584.0	(-)-epicatechin-gallate-phloroglucinol	gse
8.0	14.7	368.0	Methylated xanthohumol	
9.0	17.5	206.0	Unknown-phloroglucinol	
10.0	15.7	290.0	(-)-epicatechin	std
11.0	16.2	520.0	Unknown	
12.0	17.2	368.0	Methylated xanthohumol	
13.0	17.5	626.0	(-)-epigallocatechin-3-glucorhamnoside	
14.0	18.3	610.0	Hesperidin	
15.0	19.0	610.0	Rutin	std
*	19.2	444.0	(-)-epicatechin-gallate	
16.0	19.4	464.0	Quercetin-3-glucoside	
17.0	19.6	358.0	Unknown	
18.0	20.1	594.0	Kaemferol-gluco-rhamnoside	
19.0	20.2	534.0	Kaemferol-3-O-C6"-O-malyonyl-glucoside	
20.0	20.7	448.0	Kaemferol-glycoside	
*	20.7	580.0	Naringin	std
21.0	20.9	468.0	Unknown-phloroglucinol	
22.0	21.2	520.0	Unknown	
23.0	21.5	394.0	Unknown	
24.0	22.2	372.0	Unknown	
25.0	22.6	364.0	Unknown	
26.0	23.2	364.0	Unknown	
*	24.5	302.0	Quercetin	std
27.0	24.6	378.0	Unknown	
28.0	29.1	352.0	Unknown	
29.0	31.4	378.0	Unknown	

HOP SOLIDS EXTRACT – POLYPHENOL CHARACTERIZATION



Aron, 2011

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- Craig Poupore

MillerCoors

WATERTOWN HOPS COMPANY

HSI

Hop Storage Index

The method of determining of Hop Storage Index employs the American Society of Brewing Chemists spectrophotometric technique of hop analysis.

The HSI is defined as the ratio of absorbance at 275 nm to the absorbance at 325 nm of an alkaline methanolic solution of a non-polar extract of hops. It is often written as A275/A325.

The absorption values of α- and β-acids extracts are maximal at 325 nm and minimal at 275 nm. Oxidised α- and β-acids extracts have maximum absorption at about 250-280 nm.

Oxidation of the hops is accompanied by a decrease in A325 and an increase in A275, therefore the HSI ratio increases.

This ratio may be used to adjust the hopping rates for old hops that have lost up to 35% of their initial of α- and β-acids.