Unintended Over-Attenuation from Dry Hopping Beers

A Presentation of Brewery and Academic Research

Introduction

Why dry hop?

History of Dry Hopping at Allagash

Hop Delivery methods; Temperature variations; Contact time

Bottle Conditioning at Allagash 2016

Basic Relationship Between Extract and CO_2

1.0 g fermentable extract yields ~0.5 g CO_2 and ~0.5 g EtOH

1 °P ≈ 10 g sucrose/L H_2O ≈ 0.5 % w/w CO_2 + 0.5 % w/w EtOH

1 °P ≈ 5 g/L CO_2 + 5 g/L EtOH

1.0 g/L CO_2 ≈ 0.5 CO_2 vol/vol

1 °P (*1.004 S.G.*) ≈ 2.50 CO_2 vol/vol

0.1 °P ≈ 0.25 CO_2 vol/vol

Basic Relationship Between **Apparent** and **Real** Extract

AE: Measurement is relative as not corrected for alcohol presence (lower density/false low)

RE: The actual amount of extract remaining regardless of the presence of alcohol

Anton Paar: DMA 4500 ME and Alcolyzer

Basic Relationship Between **Apparent** and **Real** Extract

AE: Measurement is relative as not corrected for alcohol presence (lower density/false low)

RE: The actual amount of extract remaining regardless of the presence of alcohol

Final Extract (°P)		
	AE	RE
White	1.83	3.87
Saison	-0.25	2.03

Anton Paar: DMA 4500 ME and Alcolyzer

Conditioned from 1.70 to 3.40 CO_2 vol.

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RE: 4.07 °P
CO_2: 2.25 vol.

RE: 3.89 °P
CO_2: 2.25 vol.

RE: 3.89 °P
CO_2: 2.58 vol.

RE: 3.87 °P
CO_2: 2.62 vol.

RE: 4.70 °P
CO_2: 1.28 vol.

RE: 4.31 °P
CO_2: 1.81 vol.

RE: 4.01 °P
CO_2: 1.28 vol.

RE: 4.27 °P
CO_2: 2.07vol.

Target Δ RE = 0.09
Actual Δ RE = 0.27
Over-attenuated 0.45 CO_2 vol.

RE: 2.21 °P
CO_2: 1.90 vol.

Hoppy Table Beer: Batch 1, May 2016

Grains: 2-Row Blend, Maris Otter, Maine-Grown Oats

Yeast: House

Original Extract: 10 °P

Hops: Chinook, Cascade, Comet, Azacca

ABV: 4.8 %

Gravities

date	time	temp	pH	°P	Notes, cell count, etc. . .	by
5/13/2016	8:30				Lab D1	LR
5/13/2016	13:55	69	4.6	7.7	45.2*10^6/72°/Trub	IS
5/14/2016	10:00	73		3	60.4x10^6	TB
5/15/2016	11:15	70		2.5		EC
5/16/2016	12:00	70		2.5	still active	TB
5/17/2016	8:00	71		2.4		TB
5/18/2016	5:54				lab TG	HM
5/18/2016	11:00	71		2.3		TB
5/19/2016	20:20	72		2.3		PER
5/20/2016	12:30	72		2.3		IS
					Dry Hop: Do Not Turn	
5/21/2016	12:09	73		2.3	Dry Hop: Do Not Turn	IS
5/22/2016	10:55	73		2.3	Dry Hop: Do Not Turn	EC
5/23/2016	13:47	71	4.6	2.3	64°/Spund/0.3BAR per JP	IS
5/26/2016	12:00				Dumped yeast. Dry hopped 11# 2015 Comet (P92-YKUCOM5106), 8# of Azacca(14-409-046)	BAI
5/26/2016					found with little to no pressure, brought up to 2psi	EP
5/29/2016	12:45	64			40°	EC

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5/17/2016	8:00	71		2.4		TB
5/18/2016	5:54				lab TG	HM
5/18/2016	11:00	71		2.3		TB
5/19/2016	20:20	72		2.3		?ER
5/20/2016	12:30	72		2.3		IS
					Dry Hop: Do Not Turn	
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5/29/2016	12:45	64			40°	EC

Tracking HTB CO_2 Production through Conditioning

A	B	C	D	E	H	I	J	K	L	M	N	O	P	Q	R
Batch Number	Brand	Package	Date Packaged	Status	Carbonation at Fill	Carbonation testing status (days since packaging)	Week 1 after Packaging Start	Week 1 after Packaging Middle	Week 1 after Packaging End	Week 2 after Packaging Start	Week 2 after Packaging Middle	Week 2 after Packaging End	Week 3 after Packaging Start	Week 3 after Packaging Middle	Week 3 after Packaging End
4532A	hoppy table	12 oz.	6/1/2016	Destroyed	2.15		3.14	3.37	3.35	3.72	3.96	3.94	4.10	4.24	4.30

Target CO_2 vol.: 2.60!

Target Δ RE = 0.13
Actual Δ RE = 0.93
Over-attenuation >2.00 CO_2 vol.!

60 bbls destroyed

Why did we have to dump beer?
Where do we even begin?

⟶ Over 3.00 vol. in 1 week of conditioning

⟶ Over 4.50 vol. in 3 weeks

Experiment A

Goal: Determine if the over-attenuation in Hoppy Table Beer can be explained as a result from dry hopping. Closely observe extract shift pre and post dry hopping.

Method: Observe attenuation in beer at terminal gravity with 3 separate treatments in triplicate

1. Dosing Sugar and conditioning yeast
2. Dry Hop and dosing sugar/yeast
3. Dry Hop only

- 1 mL of our Bottle Conditioning Yeast
- 2.1 g dosing sugar (~0.5 CO_2 vol. increase)
- 10 g/L (2.6 #/bbl) Hop Pellets (5 g Azzaca/ 5 g Comet)
- Beer from 120 bbl FV @ stable extract for 4 days @ 72 °F
- 1 L Erlenmeyer flasks/3-piece airlocks
- Monitor extract pre/post dry hopping over time with Anton Paar DMA 4500/Alcolyzer

Experiment B

Goal: Determine if yeast strain selection, hop variety and base beer affect the over-attenuation observed in Experiment A

Method: Observe attenuation in a standardized base beer, Coors banquet at terminal gravity with 6 separate treatments:

1. Allagash House Yeast
2. Allagash House Yeast and DH
3. Allagash Reserve Yeast
4. Allagash Reserve Yeast and DH
5. Chico Yeast
6. Chico Yeast and DH

- All yeast strains were propped in a DME starter and dosed in @ 1 million cells mL^{-1}
- 10 g/L (2.6 #/bbl) Cascade Hop Pellets
- 1 L Erlenmeyer flasks/3-piece airlocks
- Coors Original Banquet Beer
- Ambient lab temp: 70-72 °F
- Monitor extract pre/post dry hopping over time with Anton Paar DMA 4500/Alcolyzer


Control


Control
House Yeast
Trappist Yeast
Chico Yeast


Control
House Yeast
Trappist Yeast
Chico Yeast
Dashed: Dry-Hopped


Control
House Yeast
Trappist Yeast
Chico Yeast
Dashed: Dry-Hopped

Hoppy Table Beer: Round 2, July 2016

- Same Recipe
- Dry hop schedule adjustment
- 72 °F rather than 64 °F
- 9 days contact rather than 3 days

Gravities

Date & Time	Hours	Temp	pH	°P	Notes, cell count, etc. . .	by
7/15/2016 10:00	22				Lab D1	MB
7/15/2016 10:34	23	70	4.66	8.9	33.4*10^6	IS
7/16/2016 12:20	49	70		3.6	72°/40.6x10^6/trub	EC
7/17/2016 11:00	71	70		2.3		TB
7/18/2016 15:02	99	71		2.3		EP
7/19/2016 12:00	120				1.69 Ea/3.51 Er	zb
7/19/2016 14:37	123	72		2.1		EP
7/20/2016 7:24	140				1.68 Ea/3.51 Er	Hm
7/20/2016 15:18	148	71		2.1		EP
7/20/2016 18:00	150	71			Dry hopped with 13# Comet/10# Azacca	PERC
					8.2%aa p92-YKUCOM5106/14.0%aa 14-409-046	
7/21/2016 7:30	164				Lab TG	MB
7/21/2016 15:44	172	72		2.1		EP
7/22/2016 7:43	188				1.68 Ea/3.54 Er	LR
7/26/2016 14:57	291	72		1.7		EP
7/29/2016 8:58	357				1.30 Ea/3.25 Er	LR
7/29/2016 12:00	360				turned to 40F	JP

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7/29/2016 12:00	360				turned to 40F	JP

Outline of experiments performed at OSU

Exp. 1 – Validating Allagash's lab-based results

- Dry-hopping Coors Original
- Monitoring RE over time using Beer Alcolyzer

Exp. 2 – Source/specificity of enzymatic degradation

- Megazyme enzyme kits to measure activity

Exp. 3 – Conditions affecting enzymatic degradation of "non-fermentables"

- Fermentable production measured via HPLC with sodium azide
- Establishing hops as source of enzyme(s)
- Investigating hop dose response phenomena
- Investigating temperature effects on fermentables production rate

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Exp. 1
Validating Allagash's results

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Monitoring RE over time in dry-hopped beer

Bench scale dry-hopping:

- Coors Banquet (1 L)
- 10 g/L of Cascade pellet hops
- 1 million cells/mL ale yeast
- Incubate at 20° C

Measure RE over time:

1. Beer
2. Beer + Yeast
3. Beer + Hops
4. Beer + Yeast + Hops

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Allagash dry-hopping validation at OSU

Allagash

OSU

Beer + Hops

Beer

Beer + Yeast

Beer
+ Yeast
+ Hops

Real Extract (P)

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Hop compounds stimulate “after-fermentation”

Beer + Hops

Beer

Beer + Yeast

Beer + Yeast + Hops

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Exp. 2
Enzyme specificity

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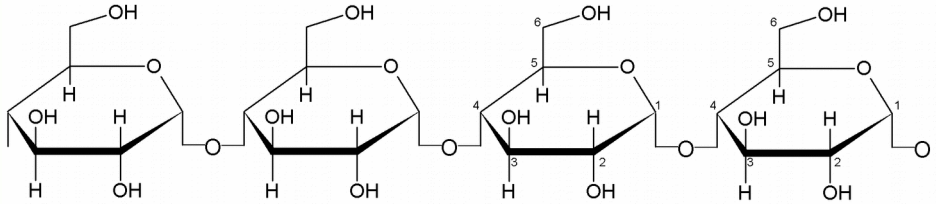
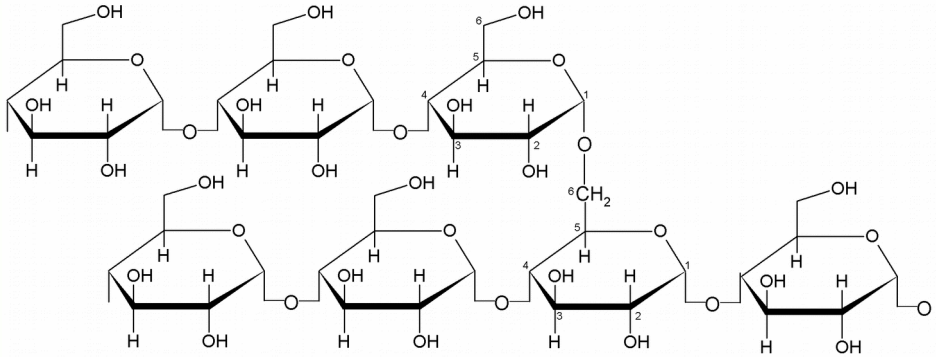
Cascade hops have broad (low) enzyme activities

Enzyme	Hops	Malt (130 dp)
Amyloglucosidase	0.02	NA
α-amylase	0.35	198
β-amylase	0.41	13
Limit dextrinase	<0.01	NA

Starch

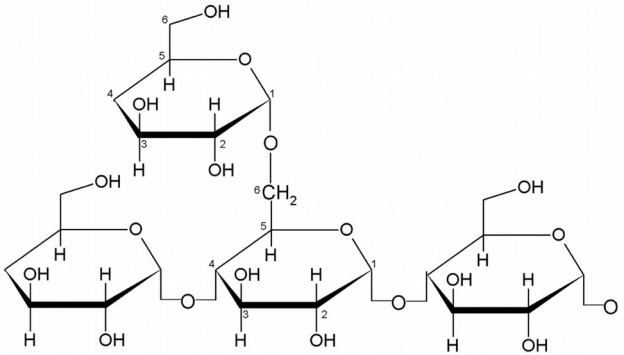


Amylose – straight chain, α 1-4 linkages

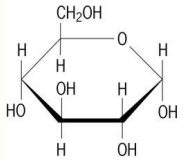


Amylopectin – contains branched α 1-6 linkages

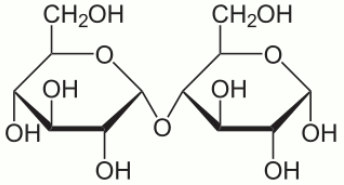
Dextrins and sugars



Limit dextrin (branched), DP 4



Glucose



Maltose

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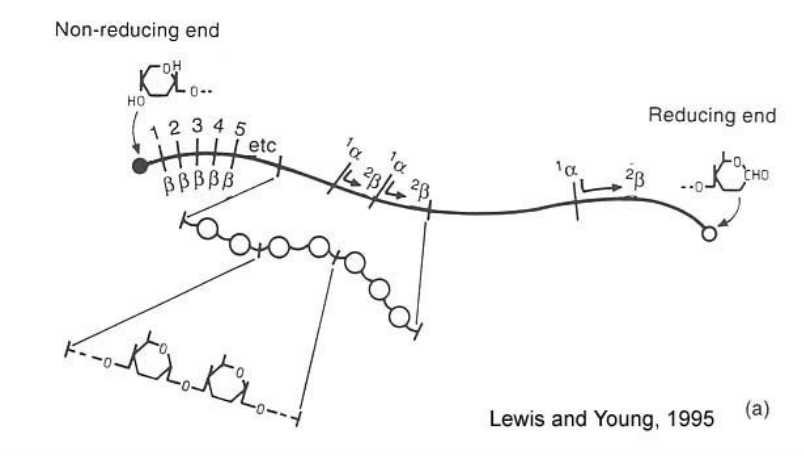
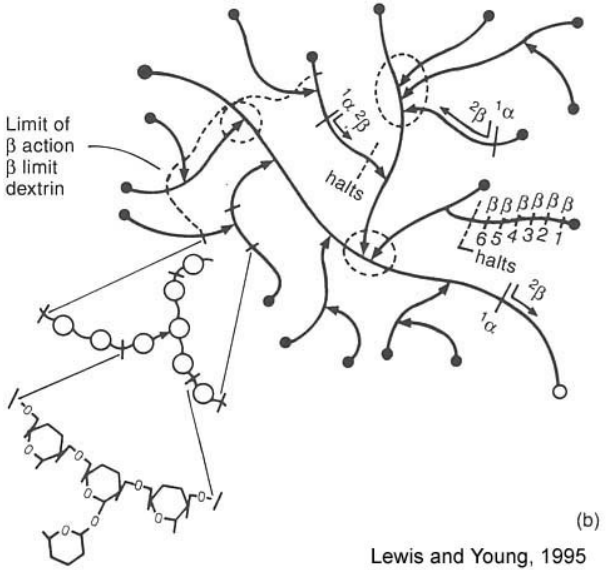
Malted barley enzymes act on starch during mashing





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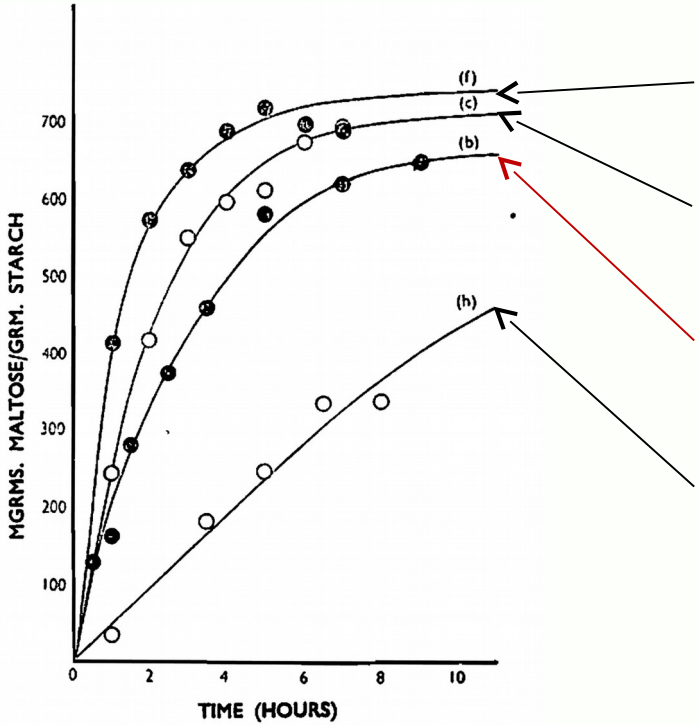
1941: Evidence of “hop diastase” in early cask ales



Worcester hop, Fuggle variety, 6 months old

British Columbian hop, 2.5 years old

Oregon hop, 6 months old

Saaz hop, 2.5 years old

Oregon hop

Time (hours)	mg maltose/g soluble starch
0·0	0
0·5	129
1·0	162
1·5	281
2·5	374
3·5	460
5·0	580
7·0	621
9·0	650
24·0	682

Janicki, J., W. V. Kotasthane, A. Parker, and T. K. Walker. “THE DIASTATIC ACTIVITY OF HOPS, TOGETHER WITH A NOTE ON MALTASE IN HOPS.” *Journal of the Institute of Brewing* 47, no. 1 (1941): 24–36.

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Exp. 3
Conditions affecting enzymatic degradation of “non-fermentables”

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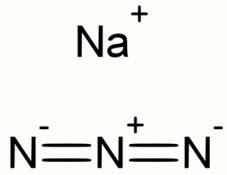
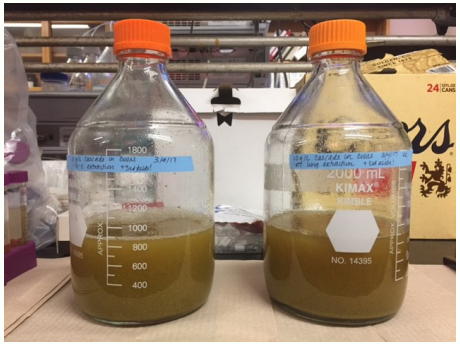
Fermentables measured with HPLC + antimicrobial

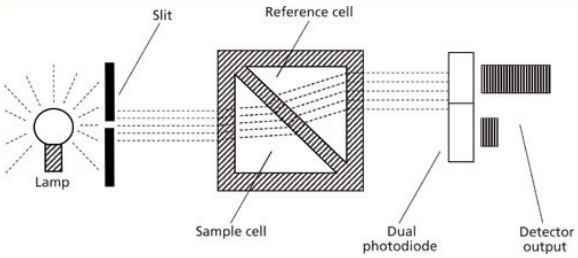
5, 10, 20 g/L



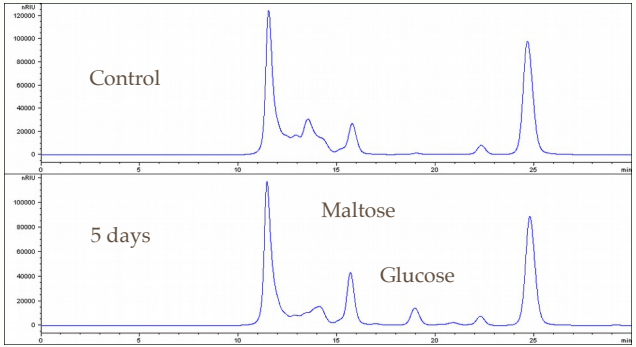
Sodium azide
(antimicrobial)

10, 20° C





HPLC with
Ag+ Exchange
Column
& RI Detector
(ASBC Method 18)



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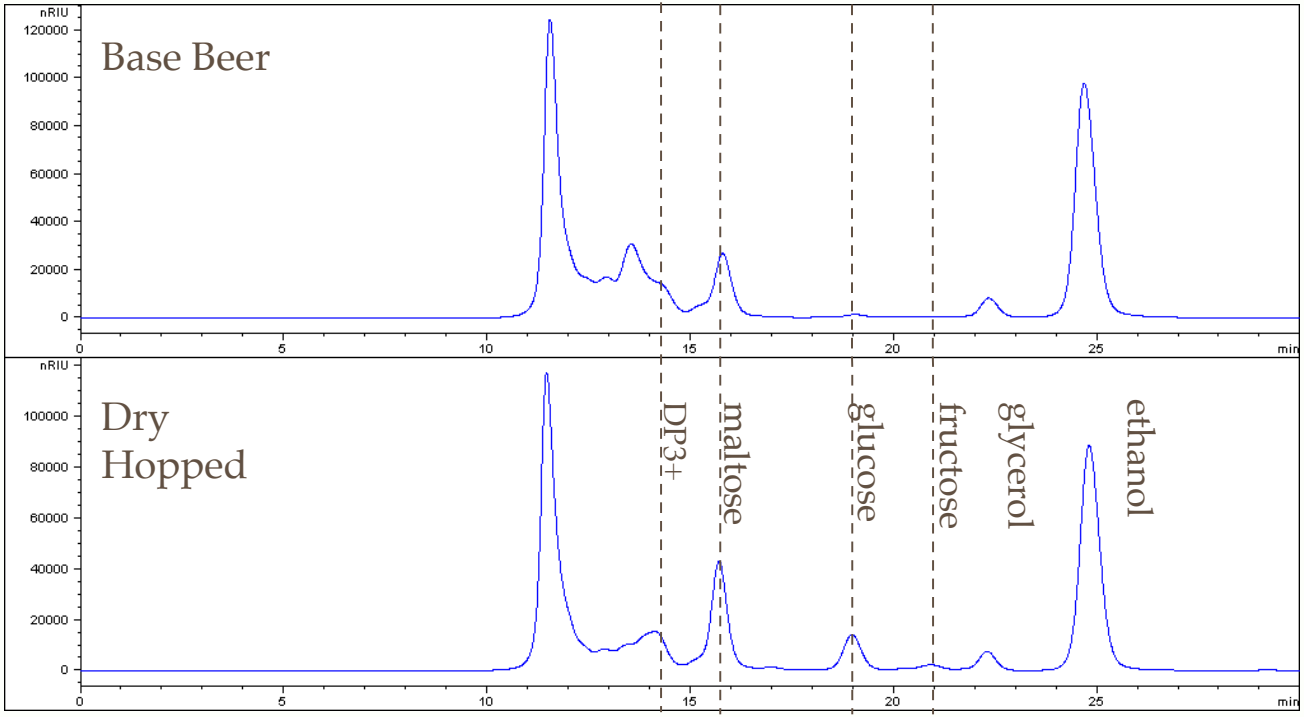
Sugar profile after 5 days on 10 g/L hops at 20° C



Method adapted from: ASBC Sugars & Syrups 18

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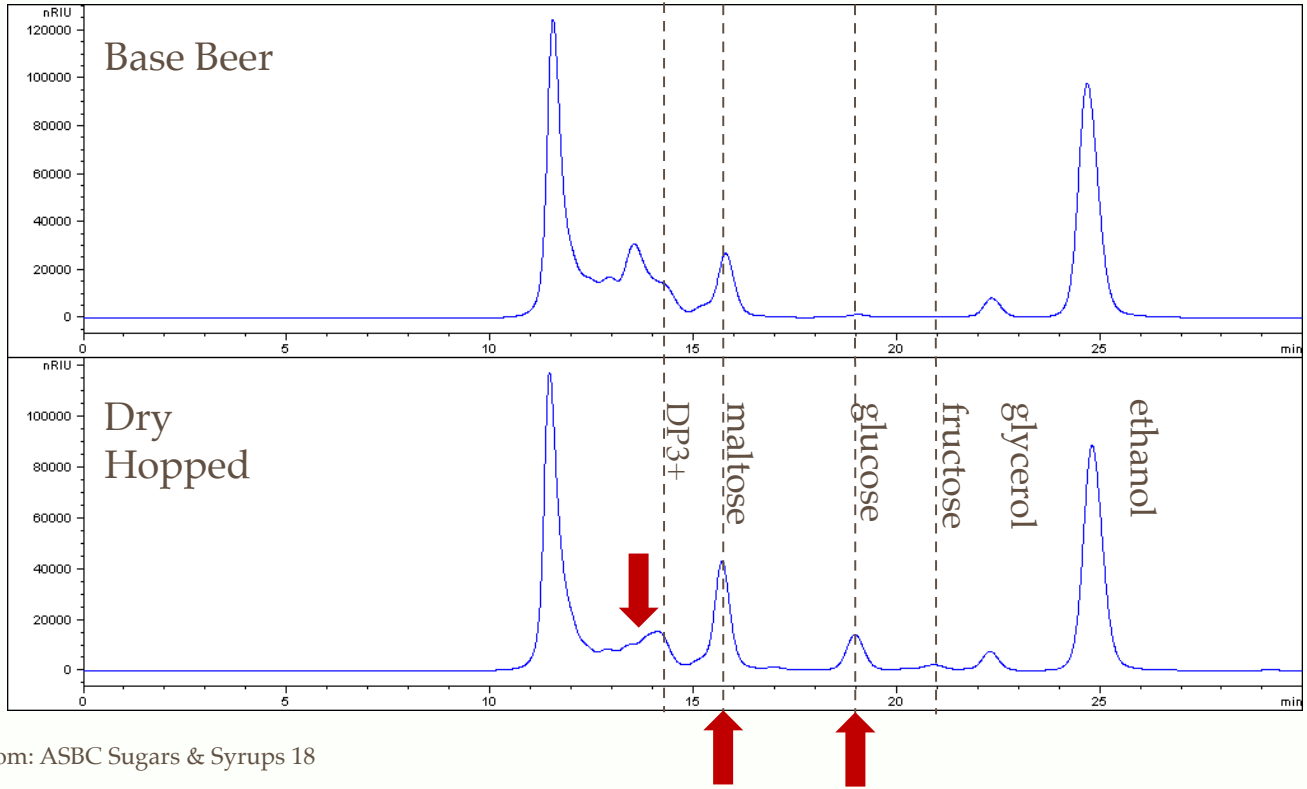

Method adapted from: ASBC Sugars & Syrups 18

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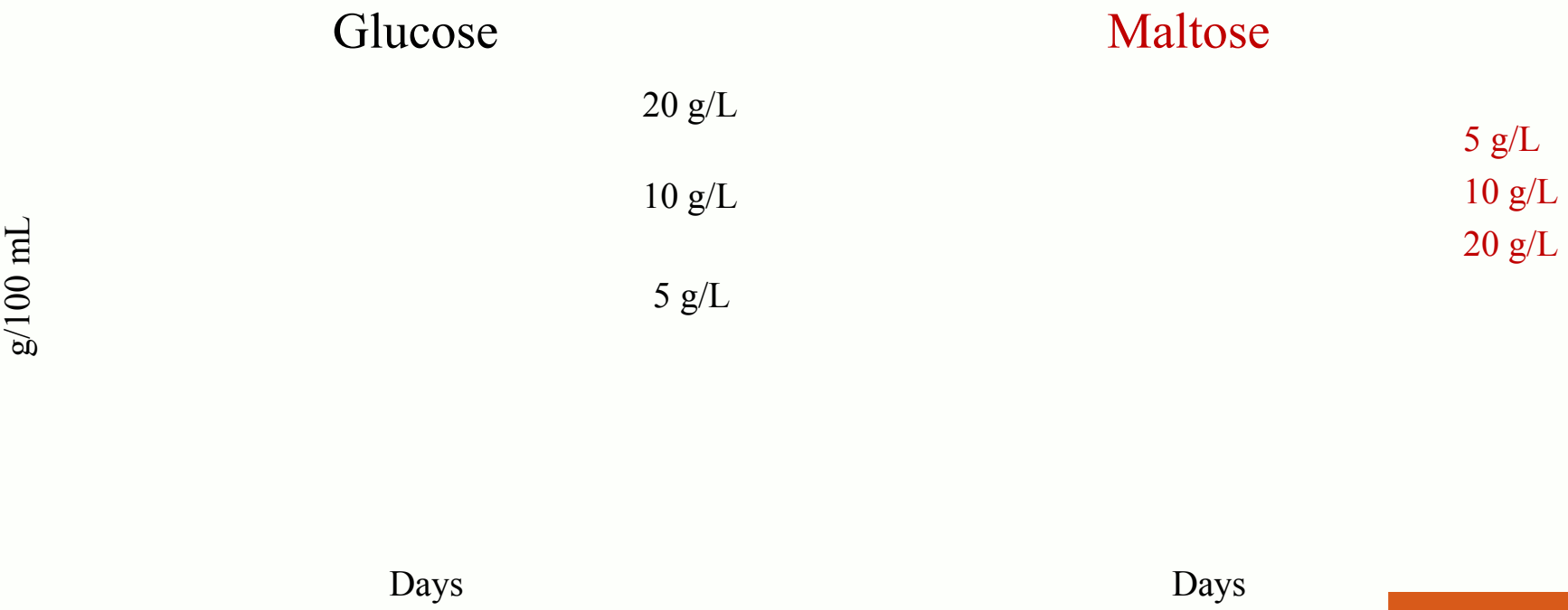
Dry-hopping rate effects final beer concentration of fermentable sugars



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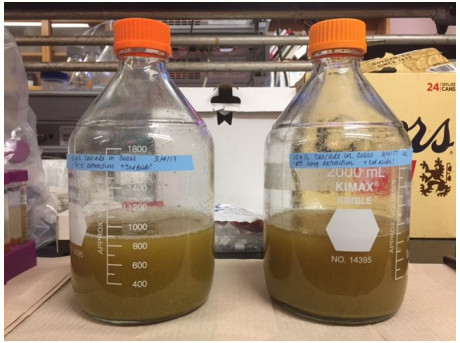
Dry-hopping time/temp: hop removal at 1, 2, and 7 days



1, 2, 7 days of dry-hopping

Centrifuge & remove hops

Measure fermentables **after 7 days** on HPLC

- Coors Banquet
- 10 g/L of Cascade pellet hops
- 0.02% sodium azide (antimicrobial)
- **Incubate at 10 & 20° C**

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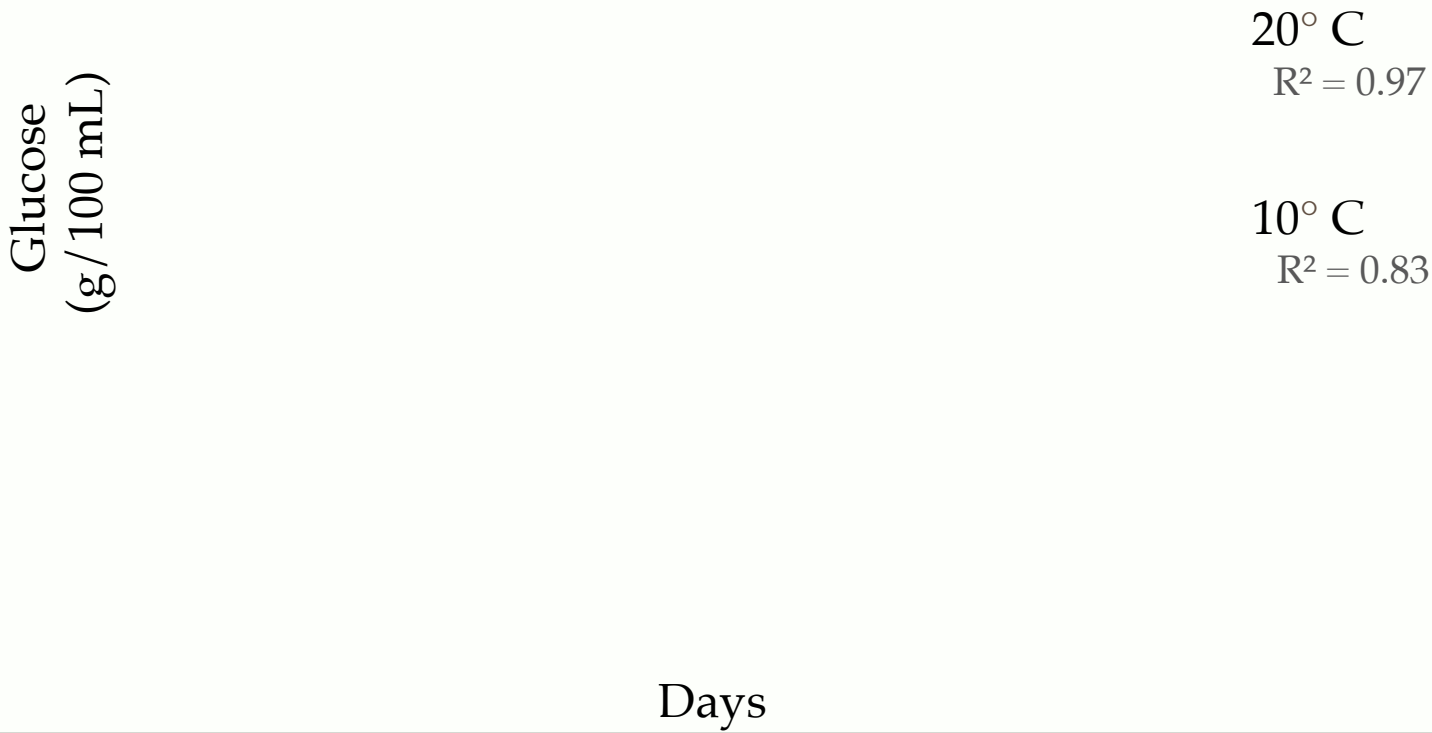
Contact time & temperature effect: Glucose concentration



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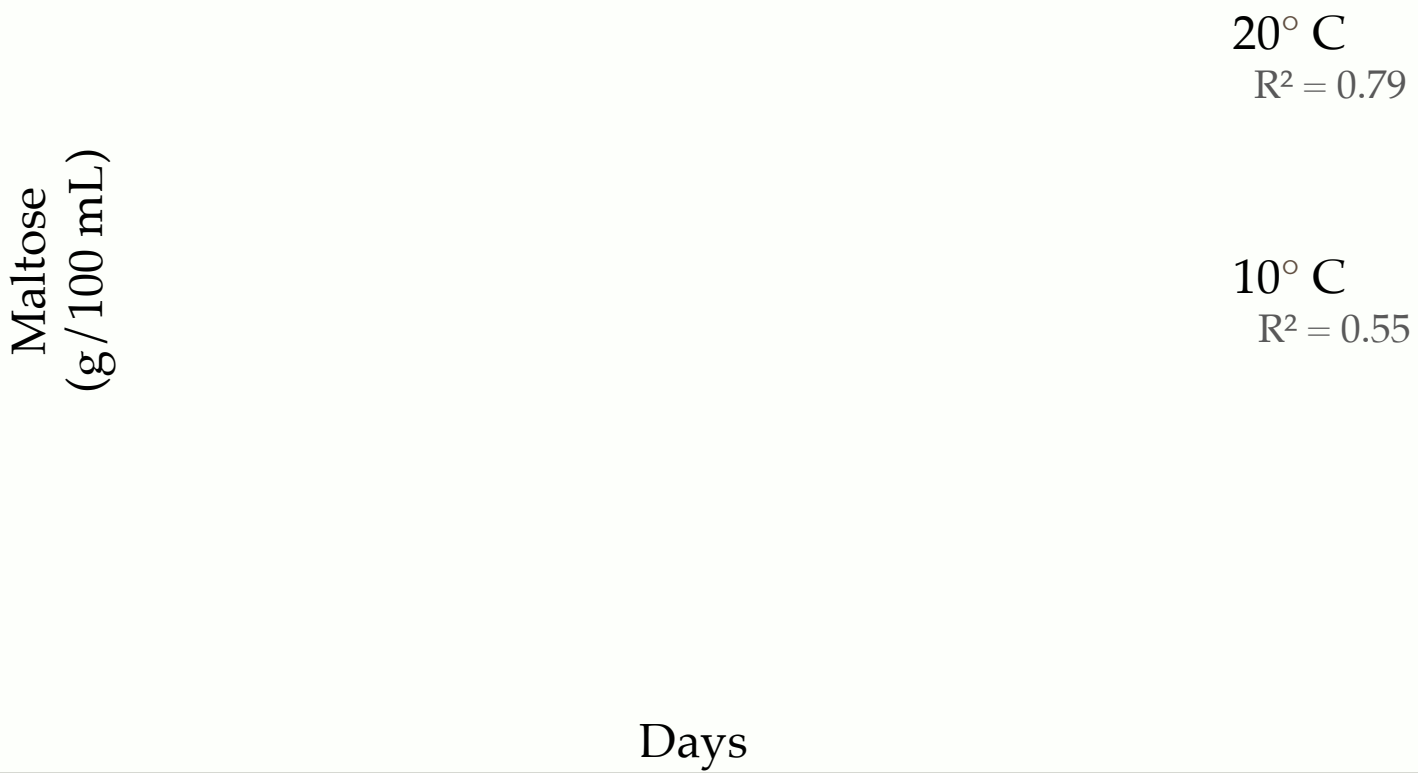
Contact time & temperature effect: Maltose concentration



Complications from changing beer fermentability

Real Extract (P)

	Initial		
RE (°P)	3.5		
ABV (%)	4.9		
CO_2 (v/v)	?		

Complications from changing beer fermentability

Real Extract (P)

	Initial	5 days	
RE (°P)	3.5	2.6	
ABV (%)	4.9	5.5	
CO_2 (v/v)	?	+2.75	

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Complications from changing beer fermentability

Real Extract (P)

	Initial	5 days	40 days
RE (°P)	3.5	2.6	1.8
ABV (%)	4.9	5.6	6.2
CO_2 (v/v)	?	+2.75	+4.75

Conclusions – specific to Cascade pellets

- **Hops are able to stimulate "after fermentation" of finished beer**
  degrading nonfermentable dextrins in beer
- **Hops exhibit low levels of enzymatic activity**
  amyloglucosidase
  α-amylase
  β-amylase
  limit-dextrinase
- **Production of fermentables depends on several factors**
  Temperature
  Exposure time
  Concentration

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Practical Considerations: What does this mean to you?

- Over-attenuation as a result from dry-hopping can lead to dangerous CO_2 levels in the package

- Relevant to all brewers that dry-hop and leave ANY yeast in the package

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Acknowledgements

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Karl Arnberg
Mike Billon
Heather Muzzy
Lee Reeve
Hannah Johnson

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The International Brewers Symposium on Hops Flavor & Aroma in Beer

LaSells Stewart Center | Oregon State University

Corvallis, Oregon | July 25–28, 2017

Keynote presentations – Japan, Germany, Belgium, USA

Roundtable discussions – Dry-hopping, breeding/local, adv. products

Oregon hops farm tour – OSU breeding + Coleman Farms

hopsflavor2017.com

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