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A novel beer fining and stabilising agent extracted from hops

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Introduction

- Fining agents are used in the clarification of beers
  - Typically work by cross-linking haze particles to generate larger aggregates of material which settle more rapidly to the bottom of a vessel (N.B. Stokes's law)

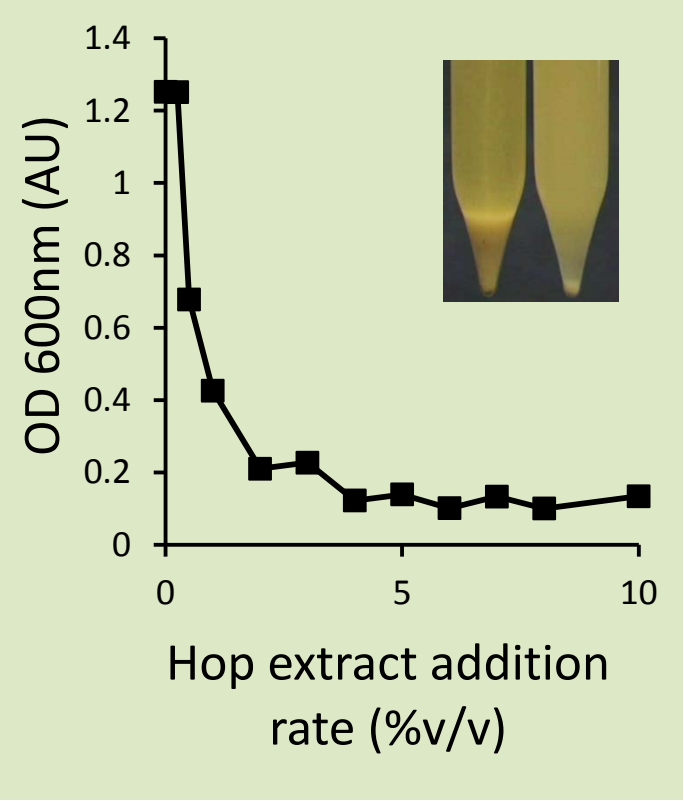
- Reduce the time required to sediment suspended yeast cells and ensure the clarity and colloidal stability of beer
- Traditionally associated with UK cask ales for 'settling' yeast after secondary fermentation
- For lager beers can use as part of rapid maturation to increase filtration run times or reduce centrifuge load

Fining with isinglass: pros and cons

Positives	Negatives
Efficient yeast flocculent/ yeast sedimenting activity	Non-vegan product extracted from fish swim bladders: consumer perceptions?
Active against chill-haze	Solid form isinglass (as opposed to paste) can be difficult to disperse/ viscous
Forms reasonably compact 'tank bottoms' at correct dose rate: helps minimise beer loss	Once prepared in liquid form has a limited shelf-life (few weeks) and must be kept refrigerated
Generally accepted to be beneficial for beer foam	Cost of refined product
Sediment re-settles following transit/ disturbance (important for cask ales)	
Tried and trusted	

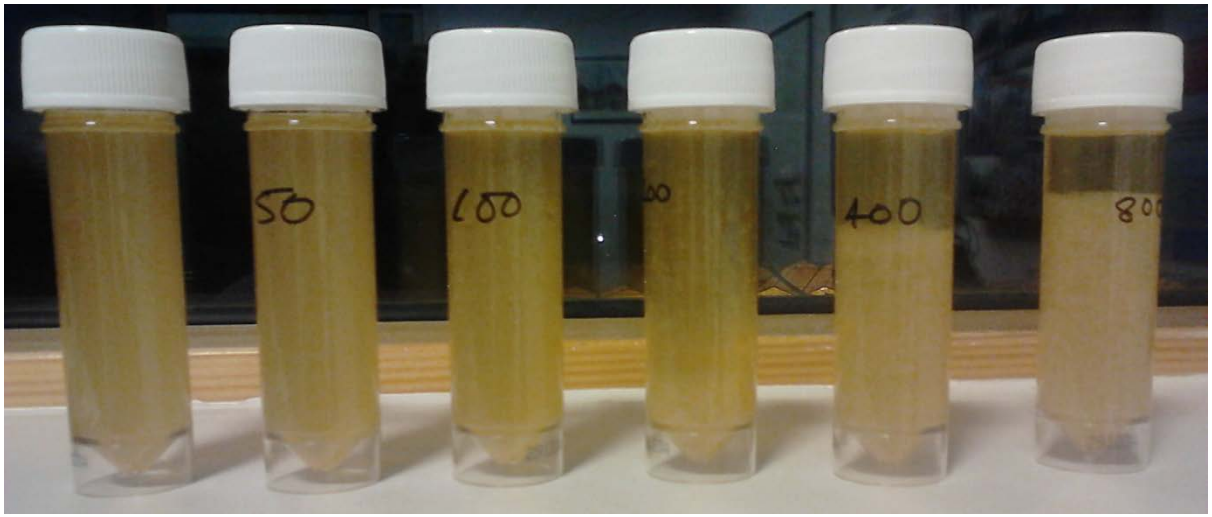
A fining activity associated with hops

- An adventitious observation during dry hopping of home brew with Saaz hops
  - Yeast sedimented unusually rapidly
- Laboratory trials demonstrated that aqueous extracts of Saaz hops could elicit this activity
  - Extracts added at varying dose rates to green beer post-fermentation
  - Yeast cells in suspension monitored using A_{600} measurements
  - Rapid fining activity. At higher dose rates impacts on yeast flocculation are visible within minutes

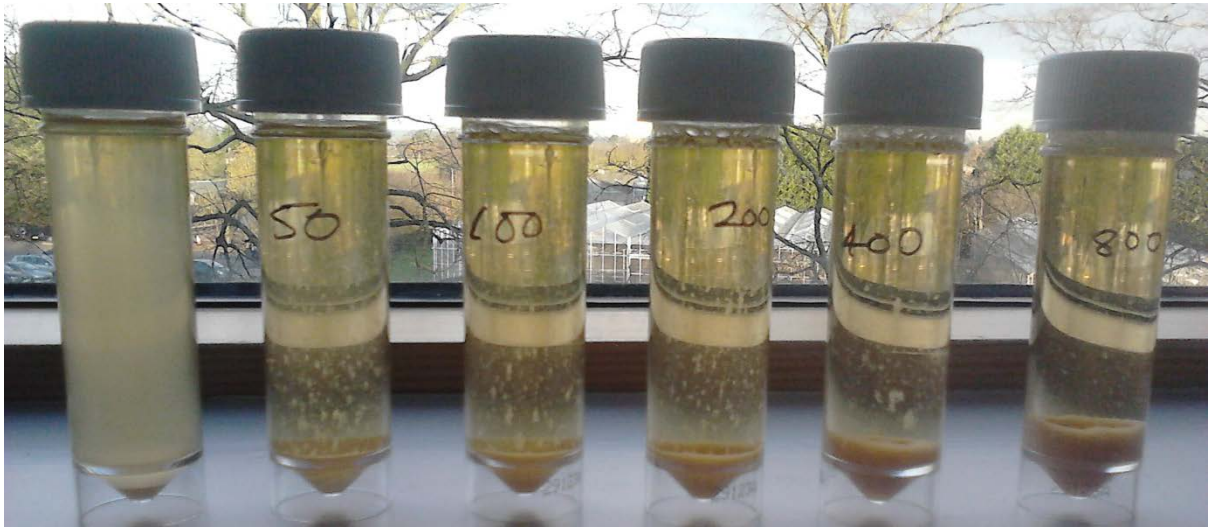


OD 600nm of green beer 2h after treatment with varying amounts of hop extract at 4°C.

Dose rate trial in pilot brewery lager

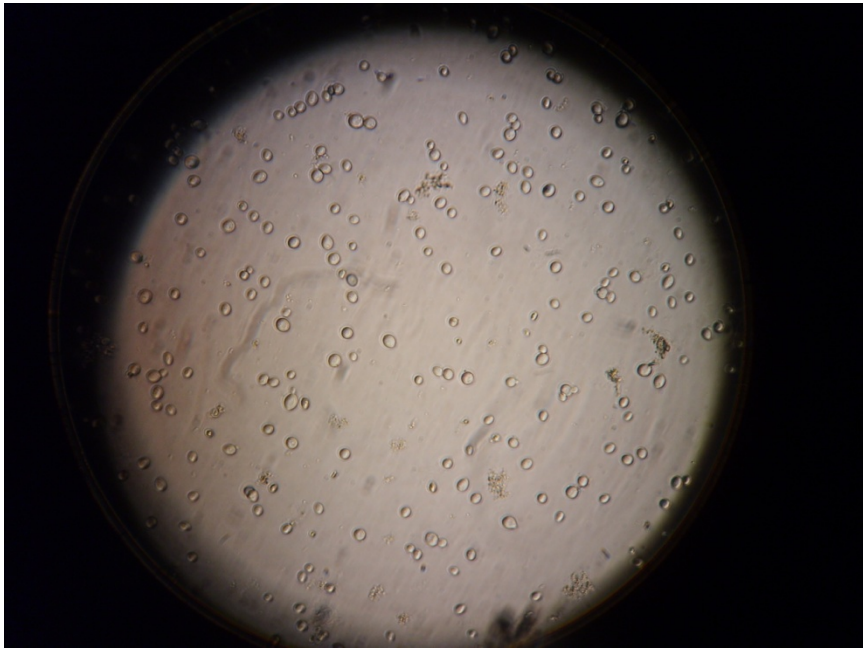


10 minutes after finings addition

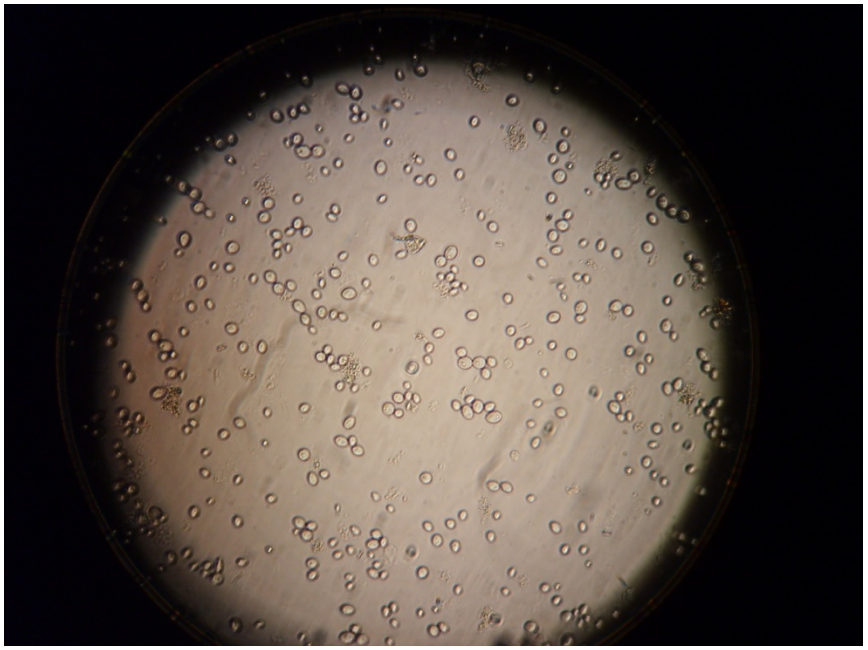


17 h after finings addition

Aggregation of yeast cells by hop extract: light microscopy



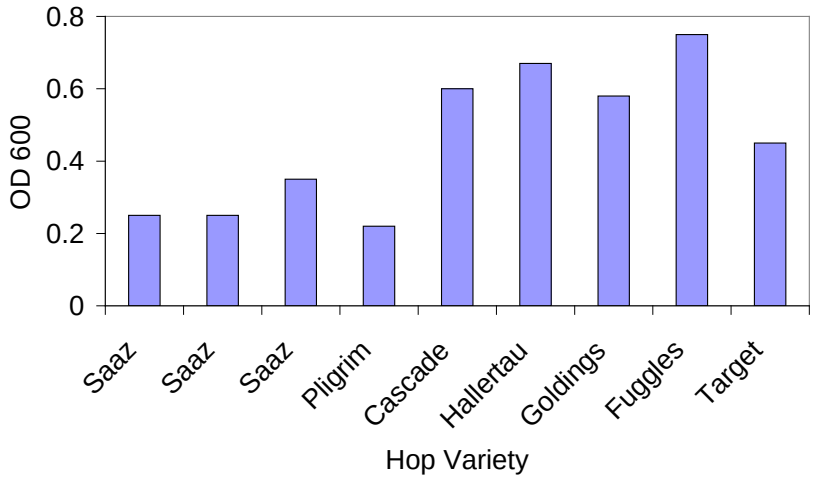
sample of green beer showing dispersed yeast cells



sample of green beer with added hop extract, showing the aggregation of yeast cells

Early experimental results: Q&A

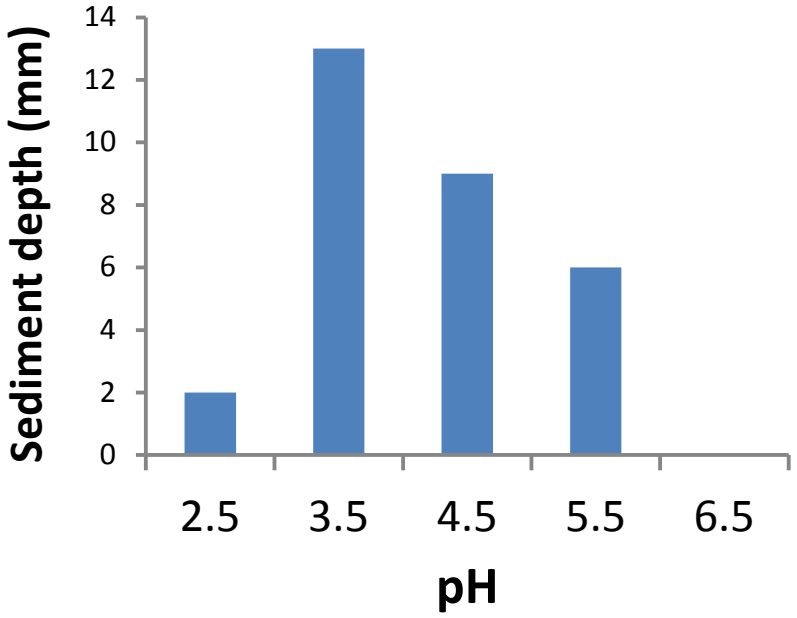
- Can the fining activity be extracted from other, or all hop varieties?
  - Yes, but there were differences in potency when using aqueous extracts of cone hops
- Is the finings material active against multiple yeast strains?
  - It has proven active against all brewing strains investigated, including commercial ale and lager strains



Early experimental results: Q&A

- What is the impact of pH on fining activity?

The effect of pH was tested by combining 2mL of Saaz extract with 12mL of 3 day old green beer, adjusting the pH and then allowing the samples to mix before standing for 15min and measuring the depth of sediment formed.



The active finings can be extracted from spent hop material

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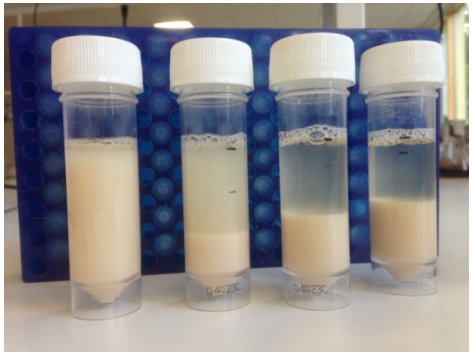
- Eight samples of spent hops sourced from processing facilities: representative of the significant varieties for CO_2 extraction
  - All 8 contained the active with minor differences in yeast flocculation activity
  - Subsequently: spent hops from ethanolic extraction also contain active

Spent hop source	Extract fining activity score
Galena (J.I.Haas, USA)	1 mL
Summit (J.I.Haas, USA)	1 mL
Zeus (J.I.Haas, USA)	2 mL
Target (Botanix)	2 mL
Summit (Botanix)	2 mL
Hallertauer Magnum ($NATECO_2$, Germany)	2 mL
Hallertauer Herkules ($NATECO_2$, Germany)	2 mL
Hallertauer Taurus ($NATECO_2$, Germany)	4 mL



(N.B. a lower activity volume (mL) means greater fining activity as less extract required to observe effect).

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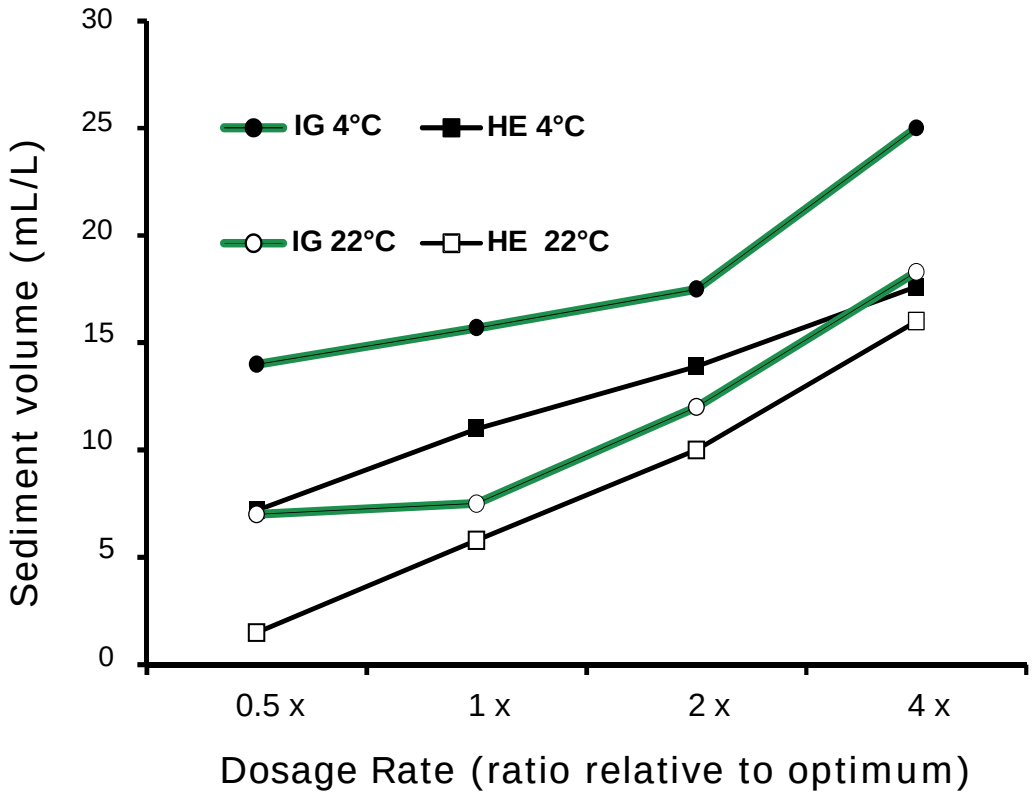
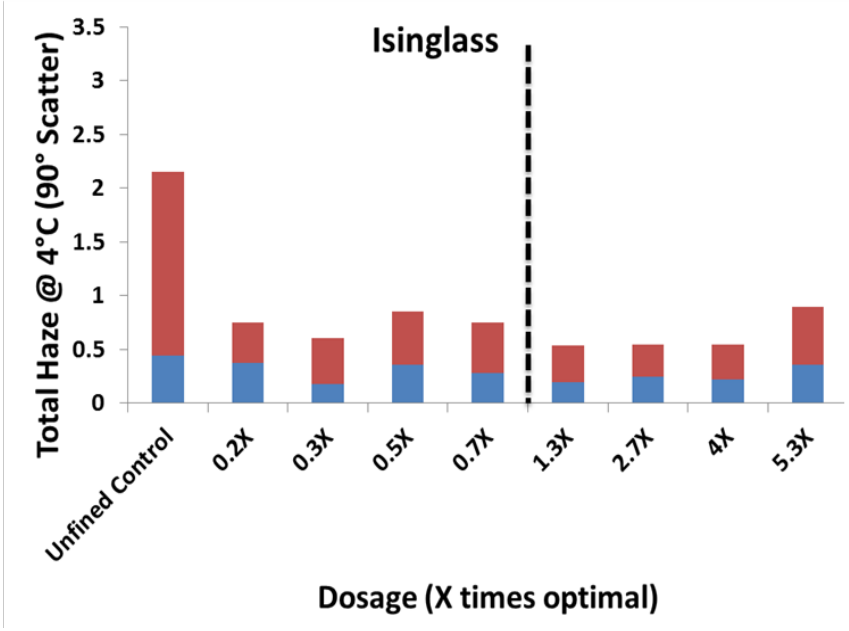
Compact sediment is formed on the fining of beers:

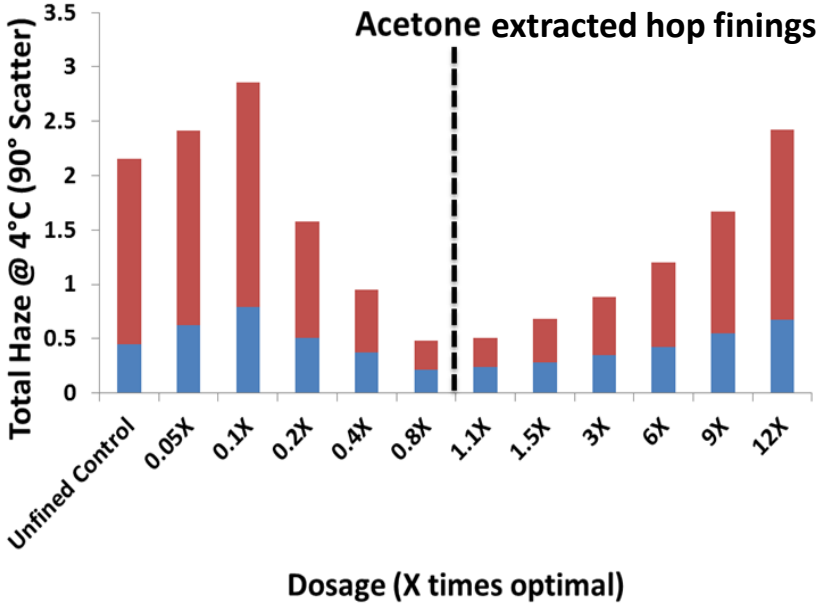


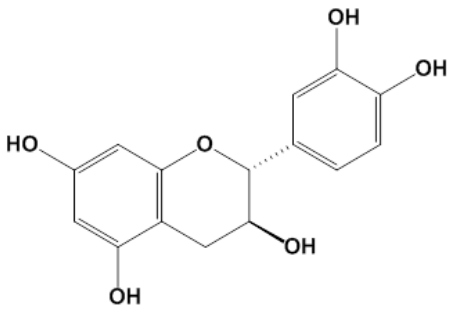
Sediment volumes (ml/L of beer) formed by the addition of varying amounts of isinglass (IG), or hop extract (HE) to green beer at 4°C (solid markers), or 22°C (open markers).

The hop derived finings are active against chill haze:

Chill haze (90°)
Permanent haze (90°)

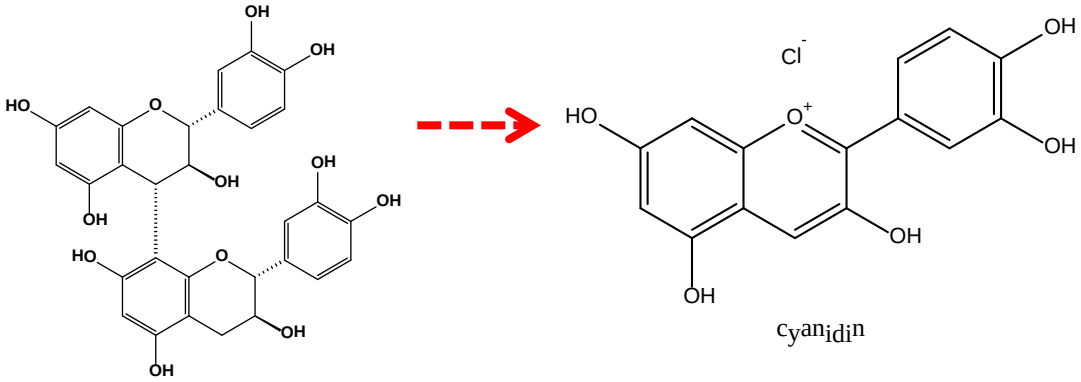






(+)-catechin

The chemical nature of the active finings material



procyanidin

cyanidin

The fining agent is proanthocyanidin in nature

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- Hop extract fractionated using a cyano CN 500/3 mL cartridge and gradient elution with acetonitrile/ water. Active fractions retained for characterisation
- LC-MS analysis of extracts hydrolysed with butanol/HCL (Fig a) reveals a mass spectrometric profile consistent with the presence of cyanidin and suggests that it is catechin and/or epicatechin based.
- The visible spectrum of the hydrolysate (Fig b) is also characteristic of cyanidin. λmax ~ 550nm (typical of anthocyanidins).

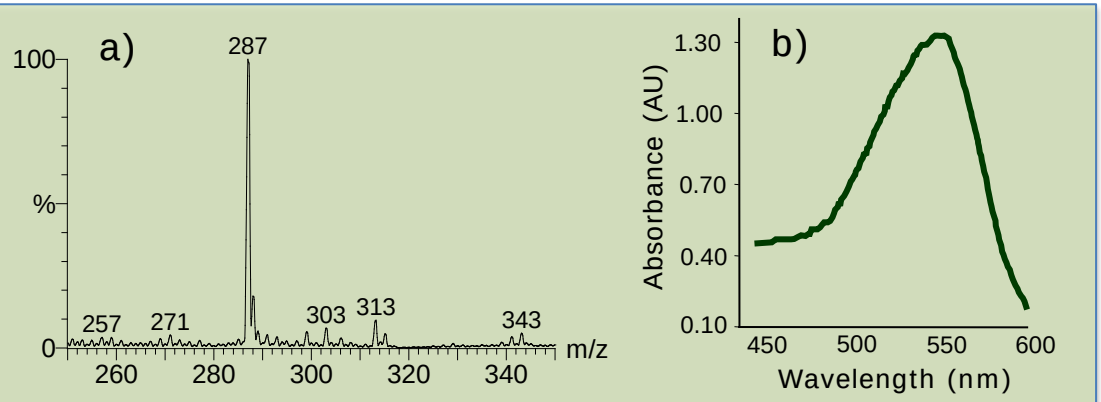

Figure 4 a) Mass spectrum and b) Visible absorbance spectrum of the butanol/HCl-hydrolysed hop extract

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What is the molecular weight range of the active material?

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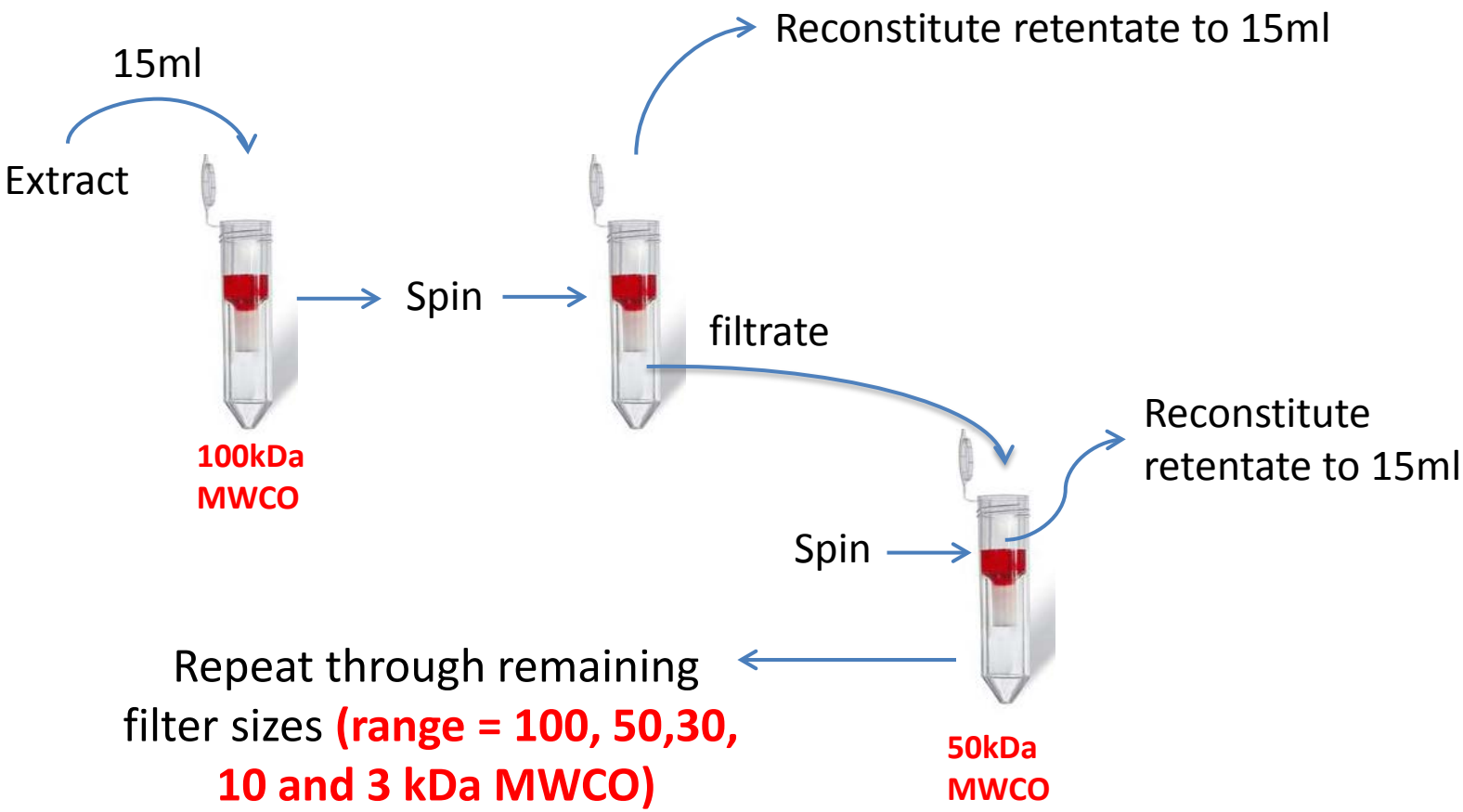
Size fractionation was performed (using centrifuge tubes with a built-in molecular weight cut-off filters)



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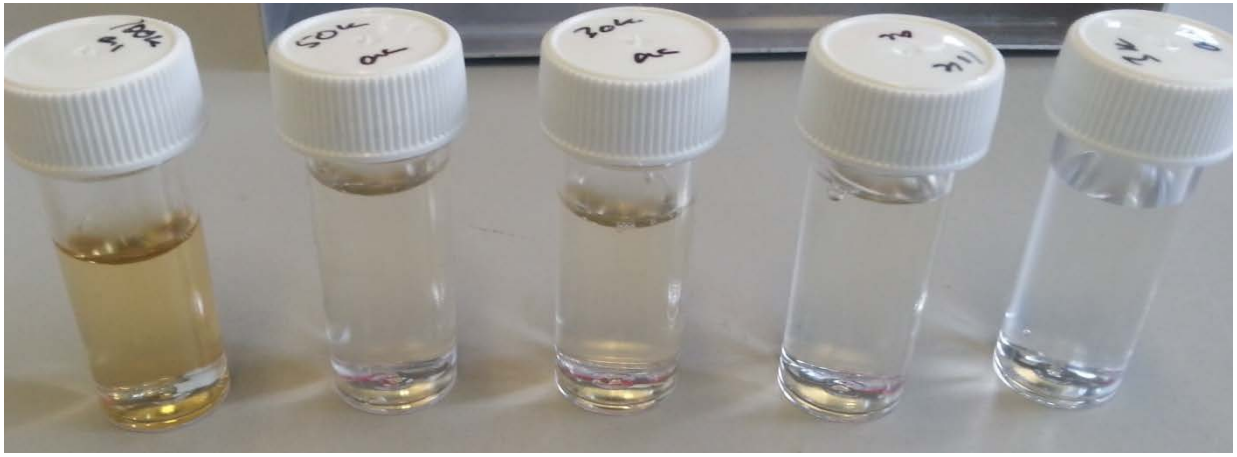
Fining activity of size-fractionated spent hop extract

Size fractionated acetone extracts of Galena spent hop

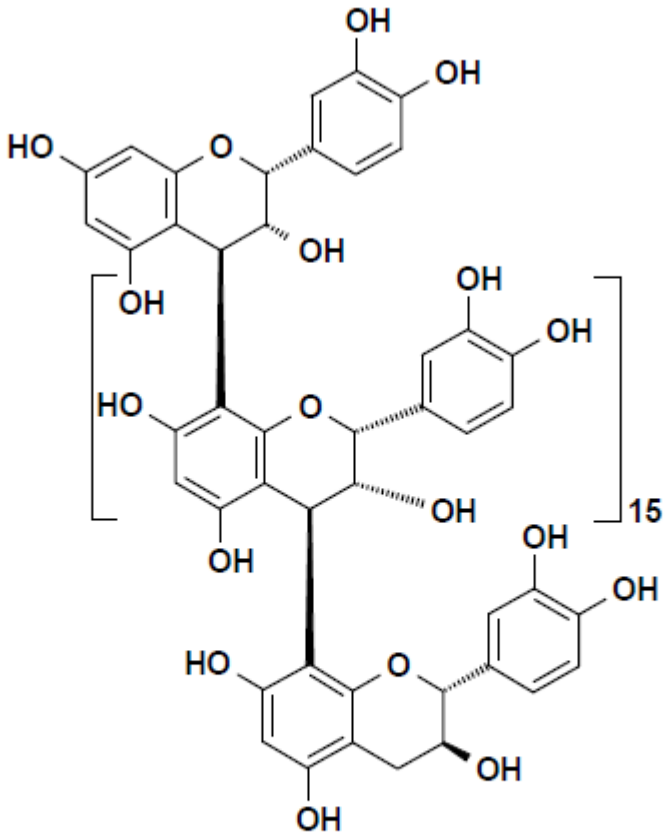


100 kDa 50 kDa 30 kDa 10 kDa 3 kDa

	Volume added to fine (ADY assay)				
	1 mL	2 mL	4 mL	8 mL	15 mL
Intact extract	-	+	+	+	+
100 kDa retentate	-	-	+	+	+
50 kDa retentate	-	-	-	+	+
30 kDa retentate	-	-	-	+	+
10 kDa retentate	-	-	-	-	+
3 kDa retentate	-	-	-	-	-
<3 kDa (filtrate)	-	-	+	+	+

Increased fining activity ←

Proanthocyanidin structures



Sorghum procyanidin
epicatechin-[(4β->8)-epicatechin]$_{15}$-(4β->8)-catechin

70 units would give a polymer mass 20,000

Pilot Brewing/ Storage Trial

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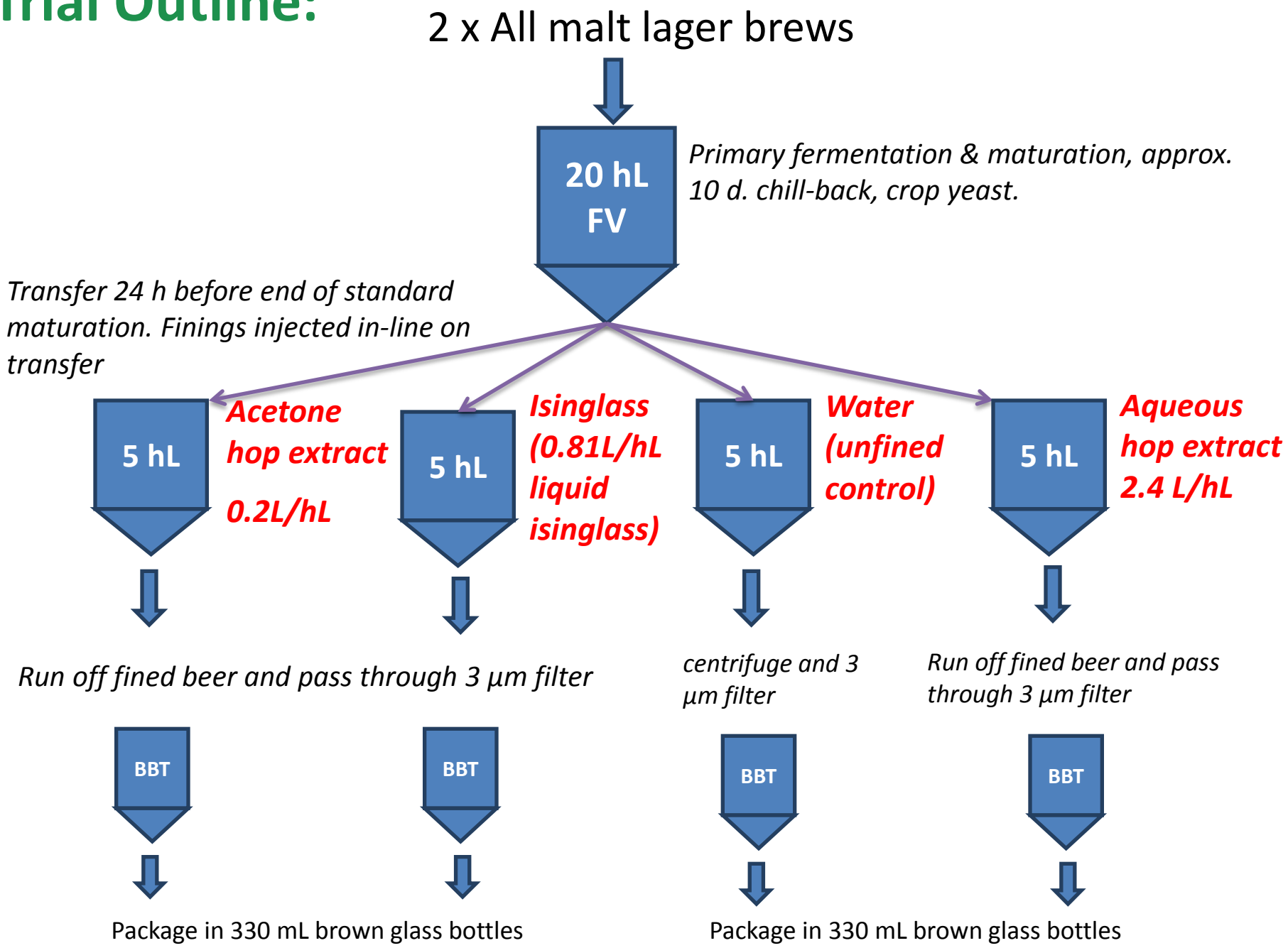
Trial objectives

1. To prepare packaged beers brewed with incorporation of the novel hop finings
   - Conduct sensory evaluation to establish whether there is any impact of finings addition on organoleptics (addition to minimally hopped lager beer)
   - Conduct a storage trial to monitor performance of finings through shelf-life
2. To develop knowledge of the practical application of the finings at pilot scale (10 hL)

Trial Outline:



Trial details

Analytical:

- Chill and permanent haze measured using 90° scatter measurements (Haffmas VOS ROTA 90/25 Turbidity meter) according to EBC 9.29 for all time point samples.
- The fresh beers were analysed in addition for pH, ABV, PG, Bitterness Units (BU) by way of characterising the base beer used in these trials.
- All fresh beers analysed for total polyphenols according to EBC 9.11.

Storage Trial design:

Storage Temperature	Beers stored for:	Analyses
4 °C	3, 6, 12 months	Sensory Evaluation – versus control beer (fresh beer + **bold red font samples**) Chill haze & permanent haze (every time/temp point) Nibem foam (FCT 30) fresh beers plus 3, 6 and 12 month samples stored at 20 °C
20 °C	3, **6**, **12** months	
40 °C	**5**, 10, 15, days	

Trial Beer Analytical data (fresh beers)

Sample	CO_2 g/kg	Bitterness Units (BU)	pH	ABV% v/v	Present Gravity (°Sacch)	Polyphenols mg/l
Control, no finings	4.12	2.5	3.96	5.26	3.83	106
Hop Aid (aqueous)	4.07	3.3	4.01	5.13	3.61	120
HopAid (acetone)	4.79	3.6	4.01	5.53	4.05	108
Isinglass	4.27	2.3	3.98	5.55	3.98	113

Sensory Testing of trial beers

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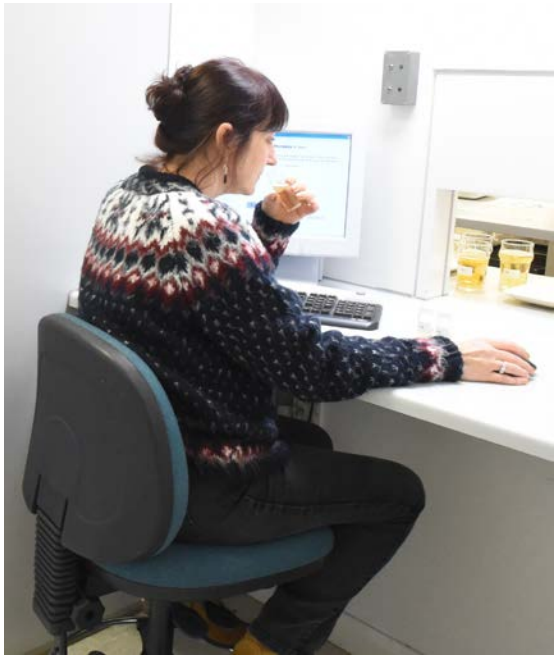
- To determine if the use of the novel finings has a differential effect on the sensory properties of fresh and stored beer
- A series of **same-different tests** were performed by 98 beer consumers to compare the unfined control with each of the other three beers (isinglass, Hop1 and Hop2-fined).
- Samples presented pair-wise:
  - ‘same’ or ‘different’?
  - How sure?: ‘very unsure’, ‘unsure’, ‘sure’ or ‘very sure’.



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Sensory Data Analysis

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- The results from each same-different test, with sureness ratings, were used to calculate the *R* index
- The *R* index is a data analysis technique that allows the calculation of a discrimination index, which is free from response bias.
  - R-Index = 50%: Samples are deemed identical
  - R-Index = 100%: Samples are so different as to be non-confusable
  - According to the critical value tables published by Bi and O'Mahony (2007), an *R*-Index of 58 % or higher indicated a significant difference (α= 0.05)

R index, d' values analysis for Fresh Beers:

Test	Fresh Samples		r-index (%)	d' value	var (S^2)
Test 1	Control	Hop (aq)	46.0	-0.27	0.62
Test 2	Control	Hop (ac)	**58.9**	**1.06**	**0.04**
Test 3	Control	Isinglass	46.3	-0.34	0.40

• Beers containing Hop (ac) extract were perceived as different from control sample, although the difference was minor.
• No consistency in panellist comments on the nature of the difference which confirms the limited relevance of the difference between samples.

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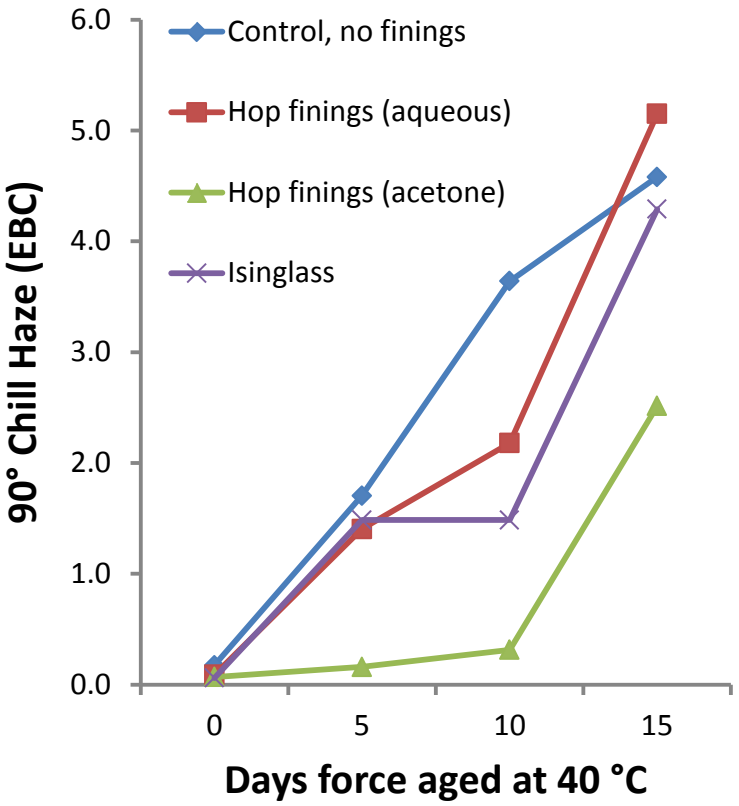
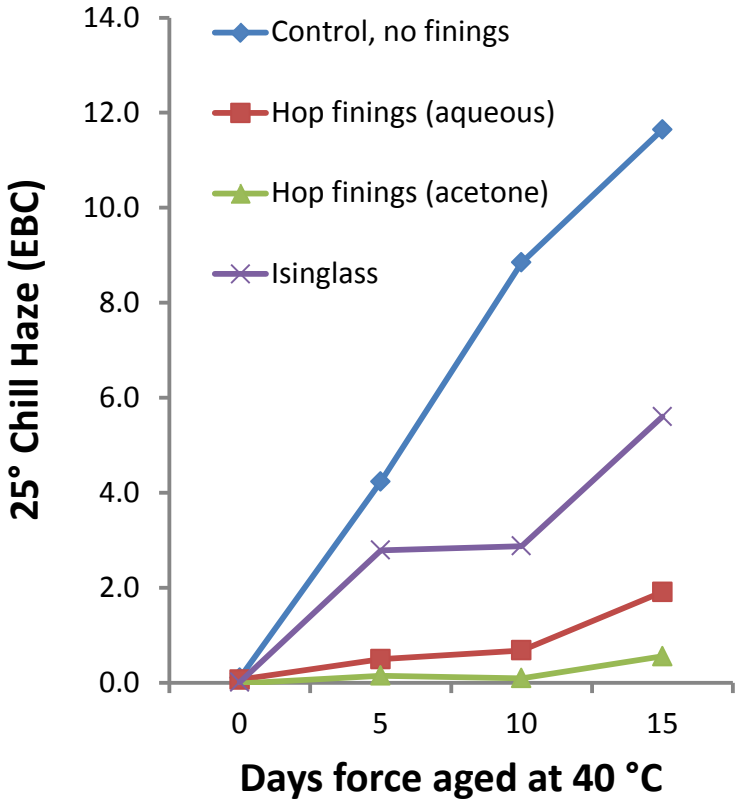
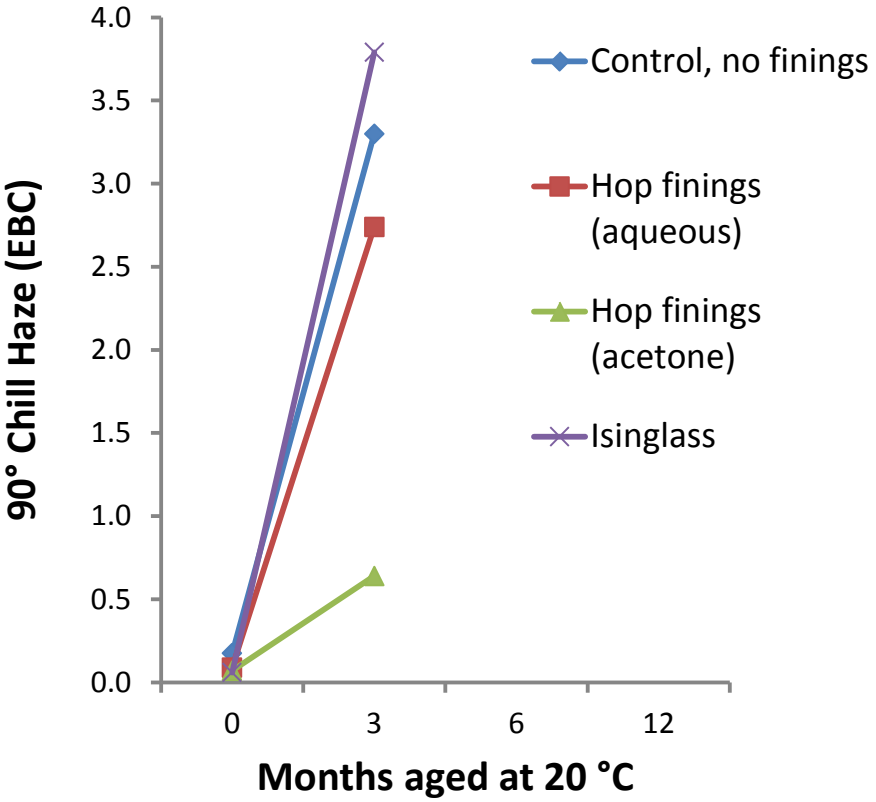
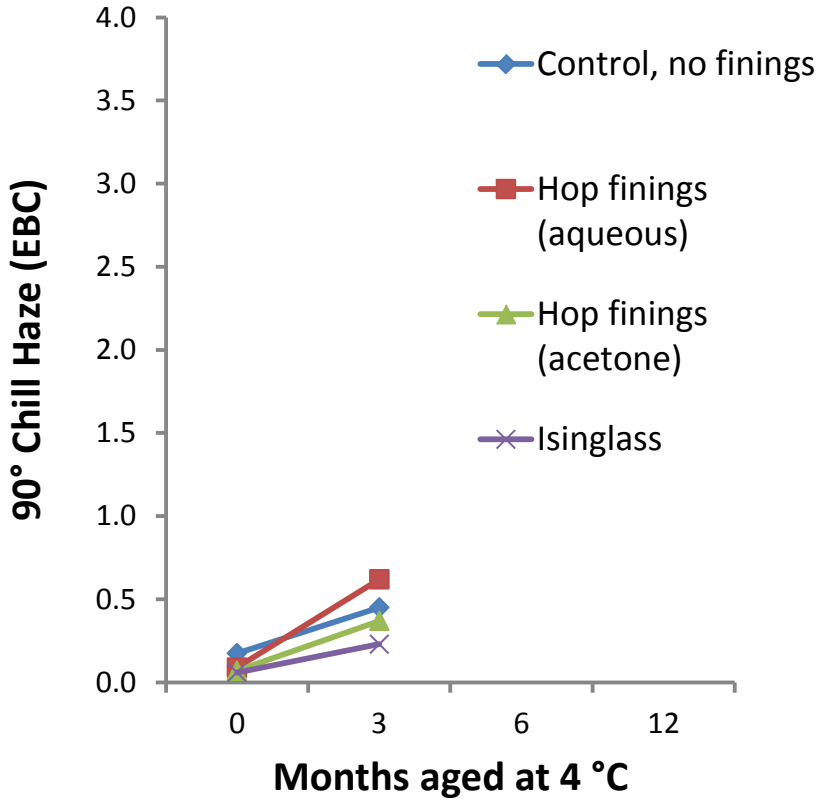
Evolution of chill haze: Accelerated ageing trial





Evolution of chill haze: 20°C and 4°C storage trial





Summary

- The feasibility of preparing a fining and stabilising agent from hop or spent hop extracts has been demonstrated
- Extraction protocols have been optimised (data not shown for commercial reasons)
- The active is tentatively identified as a polymeric proanthocyanidin of varying molecular weight range (e.g. > 100 kDa but also activity at < 3 kDa)
- **Yeast fining activity is rapid and compact tank bottoms are formed**

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Summary (2)

- The hop finings is **active against chill haze** and can match the performance of isinglass in this regard provided dose rate is in the range of optimal fining activity.
- Active can be spray dried or in liquid form. **Powder re-constitutes easily** at room temperature and solutions have low viscosity
- Recommended dosing: in-line during beer transfer (as for isinglass)
- **The finings are prepared from natural ingredients of the brewing process.** Process economics appear favourable.

References

- Cook, D. J., & Linforth, R. S. T. (22nd March, 2013). *Novel Agents and uses and methods for the preparation thereof*. UK Intellectual Property Office, Application number: 1305297.2
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- Linforth, R. S. T., Westwood, K., Somani, A., Doherty, N. & Cook, D. J. (2015). *Hop proanthocyanidins for the fining of beer*. Submitted to: J. Inst. Brew.

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Questions?

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