



To: Macpherson Trustees
Maltsters' Association of Great Britain
31b Castlegate
Newark
NG24 1AZ

From: Maria Josey
Heriot Watt University
Currie, U.K.
EH14 4AS

Date: 1 June 2016

Re: Report on Ph.D. travels to the Young Scientists Symposium in Chico in April, 2016

I would like to thank the Macpherson Trustees for granting the award of £500 for my attendance and presentation at the Young Scientists Symposium in Chico in April, 2016. The generous award covered my registration fees to the conference and the accommodations while at the symposium.

I presented my findings that correlated the increase in certain flavour compound levels post fermentation to how rapid that fermentation had progressed (See Appendix 1). The environment and general aura of the symposium was positive and it served as great practice before I go on to present new research findings at the World Brewing Congress in August, 2016. Presenting at this symposium helped me gain the confidence to present in front of academics and people from the industry. As well, with the supportive crowd at the Young Scientists Symposium, I gained constructive feedback that will help make my research better and improve future presentations. Since the conference, I've been back at Heriot Watt University and giving smaller presentations to colleagues and supervisors. I've already noticed an improvement in my public speaking and presentation skills showing more confidence and clarity than before.

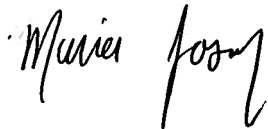
As well as gaining experience presenting, the conference itself was a great experience. I learned about the on-going research in my area at other universities in Germany, Finland, and a few other countries (See Appendix 2). I also got to hear and learn about on-going brewing and distilling research that was outside my area. One presentation that I found very interesting was discussing the use of glycosidic enzymes to optimize hop aroma when dry hopped. There was also wonderful work being done on determining different perceived aromas with chemical compounds and detecting them with GC-MS/MS. Along with the presentations, I thoroughly enjoyed touring the brewery and

laboratories at Sierra Nevada Brewery. It was great to see a brewery at that size and see all their efforts and commitment to becoming as sustainable as possible including growing some of their own hops and installing a composter called the "hot rot" that produced a small amount of biodiesel!

Coming from a new academic perspective, this conference specifically was excellent because it was at a brewery. It provided me the opportunity to see some of the types of jobs in the industry available for a Ph.D. graduate.

Again, many thanks for granting this award allowing me to have this unforgettable experience.

All the best,

A handwritten signature in black ink that reads "Maria Josey". The signature is written in a cursive style with a large, stylized 'M' and 'J'.

Maria Josey
Ph.D Candidate
Heriot Watt University
Currie
EH14 4AS

Appendix 1

PowerPoint Slides from Presentation



RELATIONSHIPS BETWEEN THE SPEED OF FERMENTATION AND LEVELS OF FLAVOUR COMPOUNDS POST-FERMENTATION

Maria Josey, James Bryce and Alex Speers

Young Scientists Symposium 2016
Chicago, California

Yeast Derived Flavour Compounds in Beer

Esters

- Iso amyl acetate
- Ethyl hexanoate
- Ethyl Octanoate

Higher Alcohols

- 3 Methyl butanol
- 2 Methyl butanol
- Iso butanol

Volatile Diketones (VDKs)

- Butanedione
- Pentanedione

CC(=O)OC
Esters

CC(C)CO
Higher Alcohols

CC(=O)CC(=O)C
Volatile Diketones (VDKs)

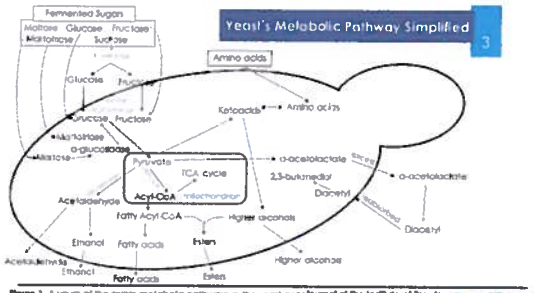
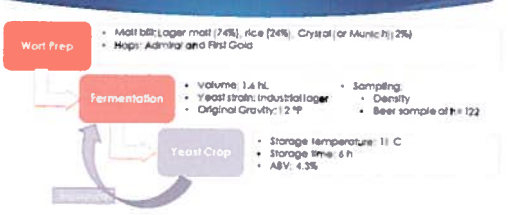


Figure 1. A view of the major metabolic pathways in the yeast cell (Journal of the Institute of Brewing, volume 117, issue 1, page 157-163, 10 JUN 2014 DOI: 10.1002/jib.449 <http://onlinelibrary.wiley.com/doi/10.1002/jib.449>)

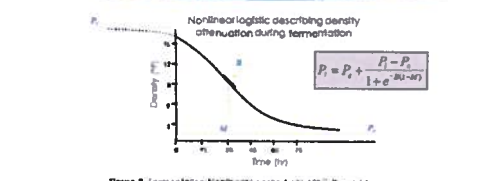
A Few Fermentation Variables Studied That Impact Flavour

- Pitching Rate**
 - Higher pitching rates associated with lower levels of butanedione and pentanedione (Sten et al. 2007)
- Wort Gravity**
 - Higher wort gravity related to increased acetate, ester levels (Verbeek et al., 2009)
 - Sugar ratio impacts flavours (Stewart 2009)
- Wort Aeration**
 - Lower wort aeration related to lower ester levels (Brown, 2003)
- Yeast Strains**
 - Mutated cells vary flavour levels in the final beer product (Emond, 1993)

Experimental Design



Nonlinear Logistic Model

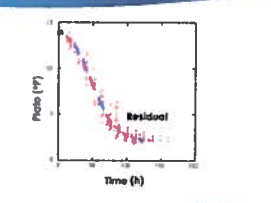


Nonlinear logistic describing density attenuation during fermentation

$$P_t = P_{\infty} \left(1 - \frac{P_{\infty} - P_0}{1 + e^{-k(t-t_0)}} \right)$$

Figure 2. Fermentation Nonlinear Logistic from ABV, Yeast 14

Why use this model?

- To "best-fit" the line through data, computer scores fit by summing error (sum of squares)
 - Repeatedly "gusses" new line lowering error
- $\sum \text{Residual}^2 = \text{RSS} = \text{error}$
- 

Density trends for the nine re-pitched fermentations

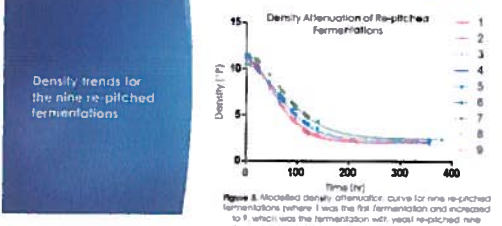


Figure 3. Modelled density attenuation curve for nine re-pitched fermentations (where 1 was the first fermentation and increased to 9 which was the fermentation with yeast re-pitched nine times). The re-pitched numbers noted by individual colours

Density trends for the re-pitched fermentations

With fermentation with Crystal malt

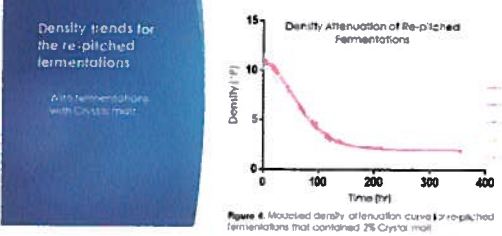


Figure 4. Modelled density attenuation curve for re-pitched fermentations that contained 2% Crystal malt

Density trends for the re-pitched fermentations

With fermentation with Munich malt

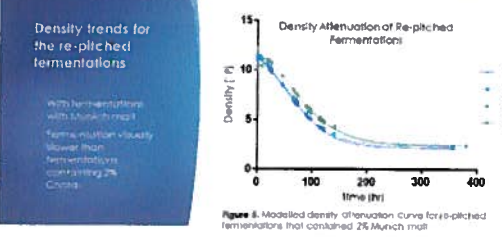


Figure 5. Modelled density attenuation curve for re-pitched fermentations that contained 2% Munich malt

Flavour Compounds and Re-pitched Number

11

- No obvious trend related to the re-pitched number

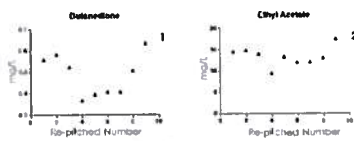


Figure 4. Butanediol (1) and ethyl acetate (2) levels (mg/L) at the end of fermentation for nine re-pitched fermentations

Table 1. The goodness of fit (R^2) for the various levels of slope compared to the functional slope of the fermentation (B) or compared to the ADF at the end of the fermentation. The slope of the plot for ADF has the strongest correlation to the two levels.

Flavour Compound	B vs. Flavour R^2	ADF vs. Flavour R^2
Acetaldehyde	0.498	0.006444
Acetone	0.009491	0.000220
Ethyl acetate	0.2693	0.2344
Isobutyl acetate	0.0001	0.7193
Ethyl butyrate	0.00436	0.3388
Isobutanol	0.3989	0.4388
Iso amyl acetate	0.6111	0.417
2 Methyl butanol	0.117	0.5482
3 Methyl butanol	0.2157	0.4484
Ethyl hexanoate	0.117	0.4896
Ethyl Octanoate	0.117	0.4828
Butanediol	0.117	0.5238
Pentanediol	0.117	0.5398

Key: ■ Significant ($p < 0.05$) ■ Not Significant

16

Flavour compounds correlated to the function of the slope (B) at the midpoint

12

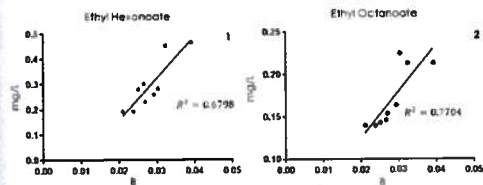


Figure 7. The function of the slope (B) at the midpoint of the fermentation correlated to ethyl hexanoate (1) and ethyl octanoate (2) at the end of fermentation

Summary and Applications

17

- The function of the slope (B) is a better predictor than the ADF to correlate to certain flavour levels
- There is a correlation between the speed of the fermentation and various flavour compounds
 - Butanediol, pentanediol, ethyl hexanoate, ethyl octanoate, iso amyl acetate, iso butyl acetate, ethyl butyrate, 2 methylbutanol.
- Some flavour compounds were not significantly impacted by the slope
 - Acetaldehyde, ethyl acetate, acetone, 3 methylbutanol, isobutanol, and propan-1-ol

Similar trends found with ethyl butyrate, and iso butyl acetate

13

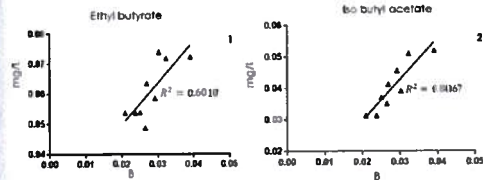


Figure 8. The function of the slope (B) at the midpoint of the fermentation correlated to ethyl butyrate (1) and iso butyl acetate (2) at the end of fermentation

Acknowledgements

18

- IBD
- Mitcherson Memorial Trust
- Jim MacKintley
Analytical Services, Heriot Watt University
- Graham McEwan
Brewer Manager, Heriot Watt University



Effect that the function of the slope (B) has on butanediol and pentanediol levels

14

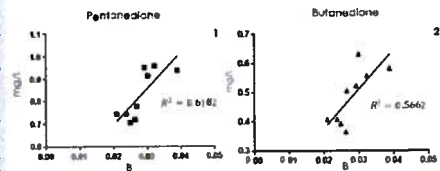


Figure 9. The function of the slope (B) at the midpoint of the fermentation correlated to pentanediol (1), and butanediol (2) levels at the end of fermentation

References

19

- American Society of Brewing Chemists. Methods of Analysis 20 (2018) ed. Yeast 14. Microbrewery Fermentation Laboratory. The Society. St. Paul, MN.
- Brown, A., & Johnson, J. B. (2014). The effect of yeast strain on the production of ethyl acetate in beer. *Journal of Applied Microbiology*, 116(1), 1-10.
- Brongers, J. K., Williams, J. R., & Johnson, J. B. (2014). The effect of yeast strain on the production of ethyl acetate in beer. *Journal of Applied Microbiology*, 116(1), 1-10.
- Stam, H., Langenhove, H., & De Maessene, H. (2002). The Effect of Pitching Rate on Fermentation and Flavour Compounds in Lager Beers. *Journal of Applied Microbiology*, 93(1), 1-10.
- He, Y., Dong, H., Yu, H., Zhao, T., Chen, B., & Sun, J. (2014). Wort Composition and its Impact on the Fermentation Performance and Beer Quality. *Journal of Applied Microbiology*, 116(1), 1-10.
- Zar, J. H. (2009). *Biostatistical Analysis*. Prentice Hall, Upper Saddle River, NJ.

Apparent Degree of Fermentation (ADF)

15

- $ADF = \frac{P_1 - P_{112}}{P_1} \times 100$
- ADF calculated at P_{112}

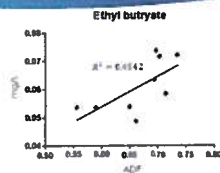


Figure 10. The correlation between ethyl butyrate levels at the end of fermentation and the Apparent Degree of Fermentation (ADF)

Thank you for listening

20

Appendix 2
Scientific Programme

Thursday April 21

	Raw Materials for brewing & distilling <i>Chair: Charlie Bamforth</i>
08:30 - 09:15	Keynote: How Craft Brewing is Transforming the Way We Think About Hops and Hop Flavor <i>Tom Shellhammer</i>
09:15 - 09:45	Towards the release of a 2-row barley variety for California craft malting and brewing <i>Joshua Hegarty</i>
09:45 - 10:15	Impact of barley varieties on malt and beer flavor <i>Lindsay Barr</i>
10:15 - 10:45	Selective pressurized liquid extraction of hop oil from hop cones <i>Katy Orr</i>
10:45 - 11:00	Break
11:00 - 11:30	Pro-oxidative effects on the storage stability of German Perle and Czech Saaz pellet hops <i>Mark Zunkel</i>
11:30 - 12:00	The effect of hopping regime, cultivar and yeast β -glucosidase activity on terpene alcohol levels in beer <i>Daniel Sharp</i>
	Advances in distilled spirits and bioethanol production <i>Chair: Charlie Bamforth</i>
12:00 - 12:30	Creating a gin utilizing novel Scottish Botanicals: A University-Industry collaboration <i>Margaux Huismann</i>
12:30 - 13:15	Lunch
	Advances in distilled spirits and bioethanol production (continued) <i>Chair: Katherine Smart</i>
13:15 - 14:00	Keynote: Impact of brewing practice on yeast performance <i>Katherine Smart</i>
14:00 - 14:45	The Influence of Yeast Handling on Petite Mutant Formation (Cambridge prize lecture) <i>Stephen Lawrence</i>
	Yeast technology and innovations in fermentations <i>Chair: Katherine Smart</i>
14:45 - 15:15	Screening for the brewing ability of non-Saccharomyces yeasts <i>Maximilian Michel</i>
15:15 - 15:45	<i>Lachancea thermotolerans</i> in primary beer fermentations <i>Jen House</i>
15:45 - 16:00	Break

	Tours of the Brewery and its laboratories
	Beer pairing dinner

Friday April 22

	Breakfast
	Yeast technology and innovations in fermentations <i>Chair: Tom Shellhammer</i>
08:30 - 09:15	Gene dosage influences the functional attributes of de novo lager yeast hybrids <i>Kristoffer Krogerus</i>
09:15 - 09:45	Biodiversity of yeast and lab population isolated from Beninese African Sorghum Beer Starter <i>Sedjro Emile Tokpohozin</i>
09:45 - 10:30	Invited Speaker: Yeast culture collections <i>Kyria Boundy-Mills</i>
10.30 - 10:45	Break
10:45 - 11:15	Relationships between the speed of fermentation and levels of flavor compounds post-fermentation <i>Maria Josey</i>
11:15 - 11:45	Uptake and stress release of iron from yeast into beer <i>Will Cayler</i>
11:45 - 12:15	Omics analysis revealed multiple stress responses of lager yeast in the process of autolysis <i>Jinjing Wang</i>
	Packaging <i>Chair: Tom Shellhammer</i>
12:15 - 12:45	Energy state model for bottling plants <i>Isabel Osterroth</i>
12:45	Lunch
	Buses depart for outside visit

Saturday April 23

	Breakfast
	Process Innovations <i>Chair: Gil Sanchez</i>
08:30 - 09:00	Impact of ascorbic acid additions in mashes <i>Joe Williams</i>
09:00 - 09:30	Optimizing hop aroma in beer dry hopped with cascade Utilizing glycosidic enzymes <i>Kaylyn Kirkpatrick</i>
09:30 - 10:00	Investigating sources of variation during dry-hopping <i>Daniel Vollmer</i>
	Other

	<i>Chair: Gil Sanchez</i>
10:00 - 10:30	Linking brain lipidomics to improvement of cognitive function achieved by xanthohumol in a mouse model of metabolic syndrome <i>Parnian Lak</i>
10:30 - 10:45	Break
	Flavor and sensory properties of beverages <i>Chair: Gil Sanchez</i>
10:45 - 11:15	Identifying unique drivers of hop aroma in beers dry-hopped with Cascade, Centennial and Chinook <i>Scott Lafontaine</i>
11:15 - 11:45	Isolation of Foam-positive compounds from roasted barley and black malt <i>Emily Kultgen</i>
11:45 - 12:15	The Digestive Fate of Putative Prebiotics in Beer <i>Abby Kanyer</i>
	Novel analytical methods for raw materials and beverages <i>Chair: Gil Sanchez</i>
12:15 - 12:45	Chasing volatiles - gas chromatography tandem mass spectrometry (GC-MS/MS) in beer flavor analysis <i>Johanna Koserske</i>
12:45	Lunch
	<i>Chair: Alex Speers</i>
13:30 - 14:15	Keynote: Still Life: A Brief History of Artisan Distillation in the US <i>Lance Winters</i>
14:15 - 14:45	A new approach for assessing the intrinsic aldehyde content of beer <i>Jessika De Clippeleer</i>
	Microbiology and hygiene <i>Chair: Alex Speers</i>
14:45 - 15:15	Genetic Strain Differentiation of <i>Lactobacillus Brevis</i> – Know the NUMBER ONE Enemy <i>Robert Riedl</i>
15:15 - 15:30	Break
15:30 - 16:00	Different application methods of <i>Lactobacillus brevis</i> R2Δ and effects on malt and beer <i>Lorenzo Peyer</i>
16:00 - 16:30	Genetic adaptations for lactic acid bacteria BEER-spoilage revealed by rna sequencing <i>Jordyn Bergsveinson</i>
17:00 - 18:00	Poster Chats
	Closing banquet and entertainment

Posters

P01	Differentiating beer aroma, flavor and alcohol content through the use of <i>Torulaspora delbrueckii</i> <i>Panagiotis Tataridis</i>
P02	Determination of method variability in steam distillation of hop essential oils by gas chromatography – flame ionization detection of oxygenated terpenoids <i>Bradley Barnette</i>
P03	From after-the-fact air to instantaneous oxygen: Lessons learned to date from implementing oxygen monitoring technology <i>Lauren Torres</i>
P04	Correlation of fermentable sugar HPLC data to ending gravity and final alcohol content <i>Ami Rose</i>
P05	Evaluation of first-wort hopped beer <i>Christina Hahn</i>
P06	Influence of nitrogen fertilization in hops on nitrate accumulation in cones, pest outbreaks and crop yield and quality <i>Anne Iskra</i>
P07	Mutation Breeding in hop <i>Brooke Getty</i>
P08	A study of the effect of residual extract and ethanol on the partial molal volume of CO ₂ in beer <i>Evening Ferguson</i>
P09	Application of metabolomics to understand barley variety and its contribution to beer flavor <i>Harmonie Akers</i>
P10	Biotransformation of xanthohumol by intestinal bacteria <i>Ines Paraiso</i>
P11	Xanthohumol and derivatives dihydroxanthohumol and tetrahydroxanthohumol improve insulin sensitivity among obese mice <i>Joshua Hay</i>