Malts of different origin influence on the volatile compounds produced by *Brettanomyces bruxellensis*

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Introduction and Objectives

Malted barley is the primary grain source used by the brewing industry because it can provide all the necessary nutrients need yeast bioprocessing and termination. Wort composition is influenced by the raw ingredients and processing techniques of those ingredients. Wort, is primarily an aqueous solution of fermentable sugars, while the remaining components are dextrins, nitrogenous materials, vitamins and minerals, ions and trace elements. Wort, serves multiple purposes for the yeast, 1.) it's a growing medium for new budding yeast and 2.) a fermentation matrix for yeast to be able to produce ethanol, carbon dioxide, and other secondary metabolites that can ultimately influence the final sensory profile of the beer. A standard brewer's wort contains roughly 90% carbohydrates, which is made up of sucrose, fructose, glucose, maltose, and maltotriose, along with dextrins. *Brettanomyces bruxellensis* can ferment all fermentable sugars along with some dextrins. The primary sugar found in wort is maltose accounting for approximately 60 – 65% of the total fermentable sugars. Nitrogen plays a key role in the success of the fermentation process. Yeast requires that the malt used in the wort to have a total nitrogen content between 10 – 15% of the grain dry weight, otherwise not enough free amino nitrogen (FAN) will be released for the yeast to undertake several different metabolic processes. Several different types of nitrogenous compounds can be found in wort. The minimum established FAN levels for a satisfactory fermentation are between 100 – 140 mg/L. Previous studies have looked at the impact FAN concentrations have on the development of medium chain fatty acid (MCFA) esters for *Saccharomyces;* however, few have looked at the impact FAN levels have on *Brettanomyces.*

The overall goal of this project is to see if barley malt of different origins influences the production of metabolites which in turn compounds *Brettanomyces bruxellensis* fermentation productivity. Due to the complex nature of the fermentation process and the chemistry involved, only the major metabolites were studied.

METHOD

- Omega Yeast Company's *Brettanomyces bruxellensis* propagated following White and Zainasheff[3] method.
- 10° Plato (1.040) 20 IBU wort was brewed by single infusion mash (67° C) made the control or test malts.
- Samples (n = 5) fermented at 21 °C for two week
- Fermentation activity was monitored using Plaato digital airlocks.
- Quantification of sugars (sucrose, fructose, glucose, maltose) analysed using Budner and Fries 2017[2]. (n = 5)
- SIU Fermentation Science Service lab analysed Total Nitrogen (TN) on the malt, wort, and beer (n = 3).
- Free amino nitrogen (FAN) analysed in the wort and beer following the NOPA method (Dukes and Butzke)
- VOC and sVOC analysed using Thompson-Witrick et al[1]. 2015 (n = 5).
- One-way ANOVA coupled with Tukey-test or Kruskal-Wallis one-way analysis of variance used to analyse the data.

CONCLUSIONS

- The variation in the different varietals and geographical growing locations did have an impact on yeast growth.
- FAN and TN do have an impact on ester compound produced
- More research is required to further explore geographical growing locations and their impact on beer.

FUTURE WORK

- Look at the impact nitrogenous compounds have on the production of phenolic compounds
- Further research to look at minimum and maximum FAN requirements for *Brettanomyces.*

Acknowledgments

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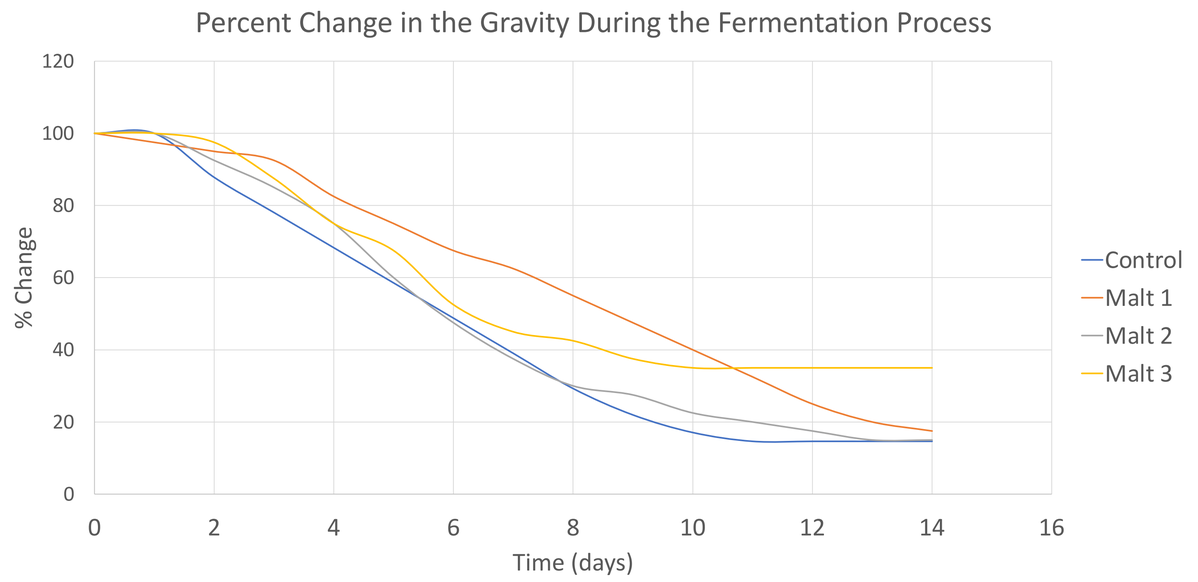
Results and Discussion



Comparison of Beer measurements between the different malts.

	Control	Malt 1	Malt 2	Malt 3
Wort pH[G]	5.5	5.5	5.6	5.6
Beer pH	3.95 (0.02)[A]	4.13 (0.03)[B]	4.05 (0.01)[C]	4.14 (0.001)[B]
OG[G]	1.041	1.040	1.040	1.040
FG	1.0064 (0.001) [A]	1.0074 (0.001) [B]	1.0064 (0.001) [A]	1.0076 (0.001)[B]
EtOH	4.54 (0.07) [A]	4.2 (0.07) [B]	4.41 (0.07) [A]	4.25 (0.07) [B]
AE%	83.8 (1.64) [A]	80.8 (1.64) [B]	83.8 (1.64) [A]	80.2 (1.64) [B]

N = 5; Average (Standard Deviation); OG: Original Gravity EtOH: Ethanol; FG: Final Gravity; AE: apparent extract**.** Values bearing different letters are statistically significant (P<0.05), G denotes there was no statistical differences between any of the malts

Total Nitrogen

	Malt TN (%wt.)[a]	Wort TN (mg/L)[b]	Beer TN (mg/L)[c]
Control	1.72±0.02	1054±38.51	866.7±11.95
Malt 1	1.53±0.0015	945±16.67	742.3±12.19
Malt 2	1.47±0.01	1027±23.81	778.9±2.57
Malt 3	1.61±0.045	1727±24.02	820.7±30.25

Free Amino Nitrogen

	Wort FAN (mg/L)[a]	Beer FAN(mg/L)[b]
Control	275.83±9.67	41.37±2.07
Malt 1	199.79±2.39	21.98±0.66
Malt 2	167.67±0.33	35.52±1.76
Malt 3	237.55±1.50	38.01±1.52

		Approximate Concentrations (mg/L)			
Compound	LRI Value	Control	Malt 1	Malt 2	Malt 3
Esters					
Ethyl Acetate	692	5.94	2.17	4.34	1.63
Ethyl isobutyrate	774	1.76	0.2	1.26	0.16
Ethyl butanoate	818	0.48	0.19	ND	ND
Ethyl 2-methylbutanoate	858	1.52	0.55	1.4	0.46
Ethyl isovalerate	862	3.54	0.9	1.88	0.58
Ethyl pentanoat	901	0.13	0.11	0.1	ND
Ethyl isohexanoate	959	0.08	0.05	0.09	ND
Ethyl hexanoate	999	8.3	4.37	2.39	2.06
Ethyl heptanoate	1093	0.13	0.12	0.28	0.12
Ethyl octanoate	1199	9.12	7.29	6.17	6.5
Ethyl benzene acetate	1237	0.5	0.13	0.24	ND
Ethyl nonanoate	1282	0.06	0.06	0.11	0.08
Ethyl 9-decenoate	1373	0.19	0.2	0.11	0.12
Ethyl decanoate	1382	6.04	6.07	4.61	4.91
Ethyl dodecanoate	1591	2.2	1.98	1.78	1.62
Ethyl myristate	1783	0.14	0.17	0.08	0.09
Ethyl hexadecanoate	1959	ND	0.09	0.07	ND
Total Esters		40.13	24.78	24.91	18.33
Phenols					
4-Ethylphenol (4-EP)	1161	0.177	0.13	0.2	0.14
4-Ethylguaiacol (4-EG)	1264	0.7	0.75	1.61	1.08
Total Phenols		0.877	0.88	1.81	1.22

N = 5 (mean). Values bearing different letters are statistically significant (P < 0.05). Letters denote statistical differences between the different fermentation concentrations, ND: not detected.