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Kinetic modeling of terpenes in packaged beers

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OBJECTIVES

- To observe and draw correlations between (beer) chemical (HS-SPME) data and sensory data.
- To understand how terpenes behave in a model system.
- To model the change, (including first-ordered declines) of terpene concentrations in heavily hopped beers.

Background

In modern brewing, it is well understood that aromatic compounds in freshly dry-hopped beer are not in equilibrium and change rapidly. However, it is still unclear as to how and to what extent dry-hopped beer aroma declines. The essential oil fraction, making up only 1% of a dried hop cone, is responsible for the 'raw' or 'green-hopped' aroma. The most prevalent compounds in 'green hop' aroma are terpenes, terpene alcohols, and sulphur compounds- each of which is inherently unstable.

This study describes the dynamic change of monoterpenes and monoterpene alcohols as dry-hopped beer ages. This study can help to serve as a 'best practice' guideline for brewers seeking to understand the rate of hop aroma decline and inform shelf life strategies.

Sensory Descriptor
Catty Hop (p-menthane-8-thiol-3-one)
Citrus Hop
Earthy Hop
Floral Hop
Fresh Grass
Passionfruit
Peach Hop
Pine Hop
Raw Hop

Table 1A. Terms used in sensory analysis by descriptive profiling.

GC/MS-SPME Targeted Compounds
Myrcene
Linalool Oxide
Linalool
Citronellol
Geraniol
β-caryophyllene
α-humulene
Caryophyllene Oxide

Table 1B. Compounds monitored by SIM in GC/MS-SPME analysis.

METHODS

Beer samples were aged for up to 12 weeks at 4°C and 20°C. Four bottles were taken for sensory analysis. All panellists had undergone intensive sensory training on various hop and beer flavour attributes/taints. Each sample was descriptively profiled and upon intensity ratings, ranked from 1-10 on sensory attributes found in Table 1A. Two bottles were degassed and frozen for HS-SPME. Samples were prepared according to internal methods for utilising a 65µm, PMDS/DVB fibre and damascone as an internal standard. Calibration curves were made and concentrations were calculated for monoterpenes/ monoterpene alcohols as displayed in Table 1B.

Beer production

An IPA was brewed at BrewDog in Ellon, Scotland (Aberdeenshire, U.K.) with 95% Extra Pale, 5% caramalt (Muntons, U.K). T90 pellets of Chinook, Ahtanum, Simcoe, Nelson Sauvin, Cascade, and Amarillo were added at various points throughout the brewing and conditioning processes. The beer was fermented using Wyeast (Mt. Hood, OR) American Ale yeast to a percentage of 5.6% alcohol by volume (ABV) and International Bitterness Units (IBU) of 40.

Statistical Analysis

All statistical analysis was performed using SYSTAT software, version 11 (Chicago, IL). Pearson correlation tables were prepared correlating each terpene/ temperature treatment with sensory data. Substantial variation was noted between sensory descriptors and hop volatiles. However, significant correlations between sensory descriptors and terpenes were observed as noted in Table 2.

EXPERIMENTAL DESIGN

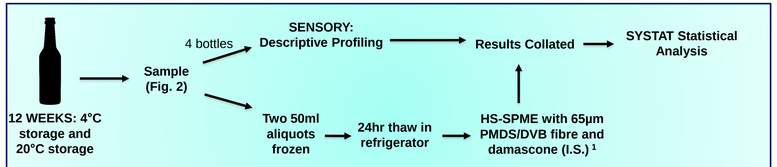

Figure 1. Schematic of experimental set up, sampling and testing.
[1]Internal Standard

Sample	Weeks Aged	Days Aged
0	0	0
1	0	2
2	1	7
3	2	14
4	4	28
5	6	48
6	8	56
7	10	70
8	12	84
9	14	98
10	16	112

Figure 2. Sampling schedule.

GC/MS-SPME Analysis

To a 20ml, screw topped vial, 10mls of beer (diluted to 4% ABV with distilled water) and 50ul of internal standard was added. The vial was immediately crimp sealed with a magnetic cap and mixed. The samples were pre-incubated at 45°C for 10 minutes using the AOC 5000 autosampler. After the incubation, a SPME fibre (65um, PDMS/DVB) was introduced to the headspace of the vial and incubated at 45°C for 1 hour. The SPME fibre was inserted into the injection port of the GC and desorbed for 5 minutes. All samples underwent a targeted analysis in selected ion mode (SIM). All GC analysis was performed in splitless mode (flow rate: 4.6mL/min) with a helium carrier gas at 4.0 bar with a temperature programme of 40°C for 5 minutes, raised to 240°C at a rate of 3°C/min and held at 240°C for 10 minutes.

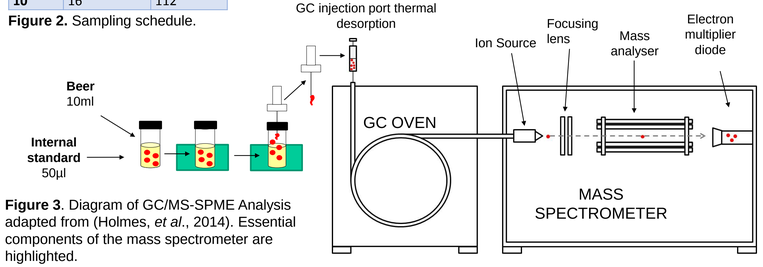

Figure 3. Diagram of GC/MS-SPME Analysis adapted from (Holmes, *et al.*, 2014). Essential components of the mass spectrometer are highlighted.

RESULTS

In addition to various correlations, the change of hop volatiles with time was also examined. Again, substantial variation was observed in most hop volatile compounds, however, some trends were clear. Notably, β-caryophyllene, geraniol, and citronellol all exhibited an exponential decline with time and could be modelled as a kinetic first order decline (Speers et al. 1987). Figure 4 demonstrates this fit using Prism software (Graphpad software, La Jolla, CA) to model the generalized equation, $B_t=B_e+(B_o-B_e)* e^{(-k*t)}$ where B_t is the product at time t, and B_o and B_e are the initial and equilibrium values, and k is the reaction rate using a non-linear regression technique.

4°C Treatment			
Sensory Descriptor	**Terpene**	**R-value**	**Correlation Significance**
Citrus	β-caryophyllene	0.49	<0.05
Fresh Grass	Citronellol	0.48	<0.05
20°C Treatment			
Sensory Descriptor	**Terpene(s)**	**R-value**	**Correlation Significance**
Citrus	Linalool Oxide	0.74	<0.001
	Myrcene	0.78	
	Citronellol	0.77	
	Geraniol	0.73	
	β-caryophyllene	0.76	
Passionfruit	Linalool Oxide	0.64	<0.01
	Myrcene	0.57	
	Geraniol	0.55	
	α-humulene	0.55	
Floral Hop	α-humulene	0.58	<0.01
Freshly Cut Grass	Geraniol	0.49	<0.05
Pine	Linalool Oxide	0.43	<0.1
Floral Hop	Linalool Oxide	0.42	<0.1

Table 2. Correlation of sensory descriptors to hop volatiles.

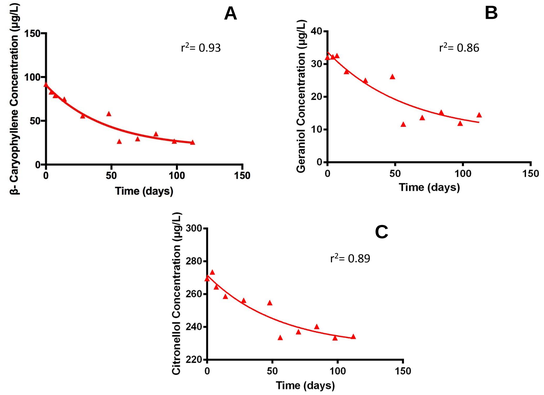

Figure 4. First-order fit of the decline in β-caryophyllene (A), geraniol (B), and citronellol (C).

CONCLUSIONS

It was interesting to note a decline in β-caryophyllene, as it is characteristically present in very low levels in raw hops. Substantial variation was observed in many of the hop volatiles tested. Synergistic reactions could account for some of the variation in the data. Geraniol and citronellol have been observed to significantly influence the 'citrusy' aroma of linalool (Biendl et al., 2014). In addition, excess levels of linalool in the presence of geraniol and β-caryophyllene have also been found to enhance citrus aroma perception (Takoi et al., 2012). This might account for the strong sensory response and correlation to the 'citrus' descriptor. Further investigation of this phenomena is ongoing.
Residual yeast cells have been found to convert geraniol to citronellol (Biendl et al., 2014). Although very few yeast cells remained in the package, warm temperatures may have intensified these otherwise quiescent reactions.
Finally, non-volatile metabolites may also be a causative agent of the significant variation that were observed (Heuberger et al., 2016).

FUTURE WORK

Due to the extensive variability in monoterpene/terpene alcohol levels, the experiment will be extended, utilising several sample sets, to build a more robust statistical model. Further data collection is required to understand how terpenes and like compounds change in packaged beers. We expect, continued modelling may inform brewers of shelf-life changes and thus develop 'best practice' guidelines.

Acknowledgements

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