GC/MS Analysis of Aroma Active Compounds

"Application to the development of a novel dry hopping method"

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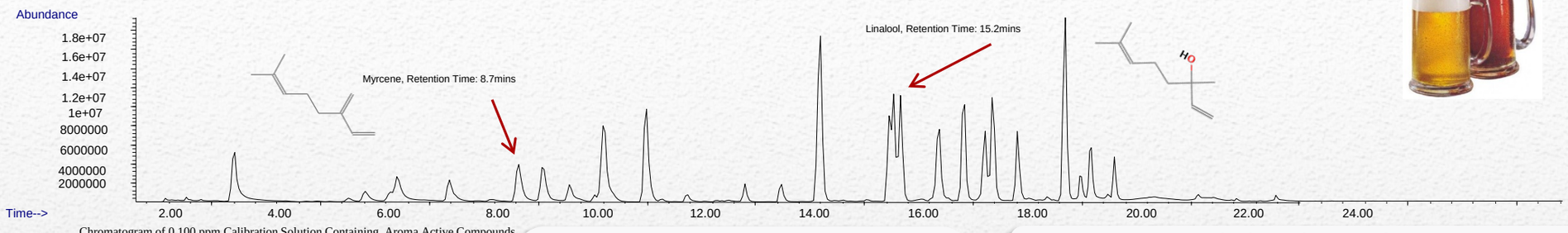

Chromatogram of 0.100 ppm Calibration Solution Containing Aroma Active Compounds

Goals

To provide 2nd year analytical chemistry students with the opportunity to apply their learning while collaborating with a local company.

To evaluate an automated GC/MS analysis method for quantifying flavor and aroma compounds commonly found in beer.

To measure aroma active compounds and evaluate the effect of sonication on dry hopping.

Table 1. Compounds of interest commonly found in beer that add to flavour/off-flavour, aroma, and spoiling.

Select Compounds	RT (min)	Ion (m/z)	Aroma
Off-flavour compounds			
DMS	1.94	47, 62	Canned corn
(E)-2-nonenal	15.1	41, 55, 70	Wet cardboard
DiAcetyl	4.86	43, 86	Microwave popcorn butter
Acetaldehyde	1.76	29, 22	Roasted nuts, sherri
Terpene/oil compounds			
alpha-terpinol	17.09	59, 93, 121	Floral lilac
3-carene	8.57	93, 77, 121	Sweet citrus
geraniol	18.01	69, 41	Floral hop
linalool	15.22	43, 71, 93	Floral citrus hop
Myrcene	8.70	93, 69, 41	Floral woody hop
(-)-trans-Caryophyllene	15.9	79, 133, 189	Spicy herbal hop
α-humulene	16.83	93, 121, 147	Woody
Farnesene	16.71	147	Magnolia flower
(+)-α-pinene	5.73	93, 136	Herbal, woody
R-(+)-limonene	9.73	68, 93, 136	Oranges
Acid/ester/alcohol compounds			
ethyl dodecanoate	18.68	88, 101, 183	Waxy
ethyl decanoate	16.38	88, 101, 155	Waxy
ethyl octanoate	14.00	88, 101, 155	Waxy
ethyl hexanoate	10.6	88, 43, 99	Red apple
phenylethyl acetate	18.465	104, 43, 91	Honey, floral
phenylethyl alcohol	19.47	91, 122, 65	Floral
dodecanoic acid	22.84	73, 60, 200	Fatty coconut
decanoic acid	21.95	60, 73, 129	Sour waxy fruit
octanoic acid	20.7	60, 73, 101	Fatty coconut
hexanoic acid	18.8	60, 73, 41	Cheese, coconut
isoamyl alcohol	10.121	55, 42	Oil, alcohol, banana
isoamyl acetate	8.146	43, 70	Banana, pear
ethyl butanoate	5.63	71, 88, 43	Apple, pineapple, fruity
butyl ethanoate	6.57	43, 56	Sweet fruity
ethyl acetate	-	-	Sweet fruit brandy
2-methyl-1-propanol	-	-	Sweet
2,3-butandiol	11.1, 11.4	57, 43	Mild fruity cream
1-hexanol	11.98	56, 43, 69	Mild sweet fruit oil
1-octanol	14.73	56, 41, 69	Orange, waxy, rose
2-methoxy-4-vinylphenol	20.9	150, 135, 77	Wood smoke

Applied Learning in 2nd Year Analytical Chemistry

Student groups meet with their instructor on two occasions outside of regular lab time. Firstly, to discuss the nature of the project, determine feasibility, and ordering. In the second meeting standard solutions are prepared and sample analysis is discussed. In the assigned lab time students prepare samples and carry out the analytical measurements. Reporting out is done using a google spread sheet so classmates can follow progress.

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Automation of GC/MS Analysis

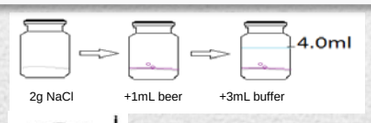
GC : Agilent 7890 with CTC CombiPal including SPME/Headspace

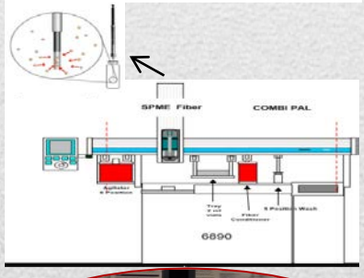
Column: Stabilwax-MS,
30m x 0.25mm x 250mm (Restek)
Injection volume: N/A
Inlet Conditions: Splitless at 250ºC

Mass Spec : Agilent 5975C

Ionization mode: EI (+) ion
Source Temp: 230ºC
Acquisition: SIM (3 segments)
mass scan: 25-300 m/z

Buffer: 0.1 mM NaH_2PO_4 (aq) pH = 3





Automation in Chemical Analysis

The instrument shown above is completely programmable and capable of solvent less extraction of organic compounds using an inert fiber from an aqueous sample.
Perfect for the testing of Beer!

Sonication and Dry Hopping

Dry hopping: The process of adding hops after fermenting and at a lower temperature

Sonication Study: Beer samples were dry hopped with and without sonication treatment. Sonication adds energy in hopes of increasing the extraction of aroma compounds.

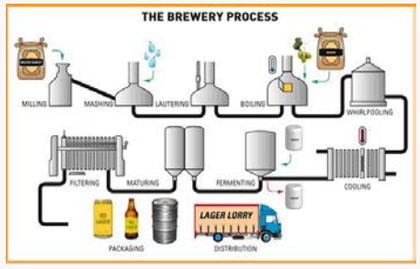
Table 2. Summary of Concentrations (ppm) of Select Hop Terpenes

Hop Aroma Compounds	RT (min)	Undryhopped (ppm)	Dryhopped No Sonication (ppm)	Dryhopped Sonication (ppm)	% Difference
3-carene	8.57	0.008	0.21	0.19	-9.5
linalool	15.22	0.166	0.268	0.263	-1.9
Myrcene	8.70	0.019	0.107	0.391	+264.4
(-)-trans-Caryophyllene	15.9	0.006	0.032	0.151	+371.5
α-humulene	16.83	0.002	0.020	0.139	+600.5
(+)-α-terpeneol	16.31	0.032	0.041	0.042	+3.4
R-(+)-limonene	9.73	0.005	0.012	0.017	+40.0

The Bigger Pint... Ahem Picture

Through analytical chemistry and microbiology many stages of the brewing process are being investigated.

Recently, NSERC funding was obtained to investigate yeast genetics in order to produce a non-alcoholic craft lager. But first, let's have a brief look at how beer is brewed, and the role of yeast.



The role of yeast is to ferment sugars from the wort into alcohol, flavor, and aroma compounds. Non-alcoholic beer though, ideally, follows the same procedure but due to the fact that alcohol is not wanted as a product, fermentation is passed through very quickly or skipped entirely before moving on. While this succeeds in keeping the alcohol below the required limits, it also keeps the flavor and aroma from developing. So, the idea is to create a yeast strain that utilizes different metabolic pathways so that there is a different product to replace alcohol during fermentation. This way fermentation can be used for a full duration as normal so a large flavor and aroma are acquired while staying under the 0.5% alcohol by volume limit, to be classified 'non-alcoholic'.

*note that there are other methods for making non-alcoholic beer but the standard method of brewing has the best potential for all the flavors and smells of alcoholic beer

Future Work

This particular analysis is but one of a few small projects that will contribute to the development of a non-alcoholic fermenting yeast strain. It is a key part in determining what has been changed in the initial yeast sample, and if the changes are the ones desired. Some other contributing projects to look forward to are; Sugar Analysis, Pilot Brews, and the key portion CRISPR-Cas9 Mutagenesis.