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Detection of storage-dependent hop oxidation by direct thermal desorption of hop solids for use with GC-FID and GC-MS

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Abstract

The current accepted method for the analysis of hop essential oils involves the time and energy consuming process of steam distillation of hop matter. While steam distillation is an effective method of hop oil extraction, removal of the added processing step would considerably increase sampling efficiency, especially when running multiple samples. Expanding on previous work, this study utilizes direct application of hop solids to a clean, glass GC liner packed with silanized glass wool with subsequent thermal desorption. Direct thermal desorption (DTD) requires small sample size, no sample processing, and can be performed on any standard GC without need for additional accessories. Initial results suggest that DTD agrees with results utilizing traditional steam distillation.

The development of this method will allow for rapid sampling with accurate results, which will aid in the study of hop aging as it relates to storage conditions. Previous studies have investigated oxidation of various hop essential oils and oxidation mechanisms have been described, however a broad study of the formation of oxidation products over time in varying storage conditions of several hop varietals has yet to be conducted. This study will lead to a more comprehensive understanding of how hop flavor profile changes during storage. This information can be used to determine optimal storage time and conditions depending on hop varietal and beer style.

Experimental

Hop samples

Hop samples were provided by Sixpoint Brewery. All samples were CY 2015 T-90 pellets in vacuum sealed packages. Samples consisted of eight varietals: Cascade, Centennial, Chinook, Citra, Comet, El Dorado, Glacier, and Simcoe. Pellets were blended into a fine powder before measurement. To examine oxidation over time, hop samples were left at ambient temperature in amber vials with limited light exposure.

GC method

GC analyses were performed on an Agilent 6850 Series II GC-FID using a DB Wax column (Agilent, 30m, 0.25μm film). The GC method used was adapted from ASBC's Hops-17 method. This method was adjusted to reduce runtime and increase sample throughput. Injection split was increased to 1:100 to account for higher oil concentrations. Initial oven temperature was set to 70 ºC (1 min hold), increased by 3 ºC/min to 190 ºC (1 min hold) and finally increased 30 ºC/min to 250 ºC (10 min hold). Myrcene, 2-octanol, linalool, farnesene, humulene, caryophyllene, and geraniol standards were run individually to determine respective retention times using this method.

Sample measurements

Steam distillation samples were prepared and measured according to ASBC's Hops-13 method. For direct thermal desorption DTD, each hop varietal was weighed on silanized glass wool and inserted directly into a clean glass GC liner. A 2% octanol in hexanes solution was used as an internal standard (IS) and introduced through liquid injection (1 μL) autosampling.

Standard addition calibration

A standard addition curve was created using humulene standard (Sigma Aldrich, CAS: 6753-98-6, Lot: 2298167) with the Simcoe hop varietal. A 1000 ppm stock solution was used to create standards of 0.01, 0.05, 0.10, 0.20, and 0.50% humulene in hexanes. Hop matter (5.0, 5.0, 4.9, 5.2, and 5.3 mg, respectively) was weighed on tared glass wool and inserted in to a clean GC liner. Standard solutions, each including a 2% octanol internal standard, were added to the liner through liquid injection autosampling (1 μL) to ensure uniform additions throughout trials. GC liners with fresh hop matter were introduced after each GC run. For added QA/QC, an external calibration curve was created using the same concentration of standards but without hop matter in the GC liner. Figure 3 displays the resulting calibration curves. After a Grubbs outlier test, the first point (0.01% addition) of the standard addition curve was determined to be an outlier and omitted ($G = 5.341$, $G_{table} = 1.672$).

Experimental

Figure 1 is an example of a sample preparation of hop material for direct thermal desorption (DTD). Hop material was weighed on a tared sample of silanized glass wool and inserted directly into a clean GC liner which was subsequently inserted directly into the GC inlet. In general, less than 10 mg of hop material was used per run, with sample preparation taking less than 5 minutes.

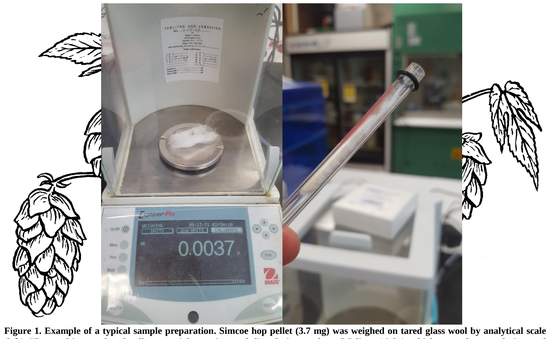

Figure 1. Example of a typical sample preparation. Simcoe hop pellet (3.7 mg) was weighed on tared glass wool by analytical scale (left). The resulting wool and pellet material were inserted directly into a clean GC liner (right), which was subsequently inserted into the GC-FID inlet.

Results

Figure 2 shows a comparison between a distillate sample and DTD of Cascade hops using the GC-FID method provided by ASBC's Hops-17 method and a comparison of DTD of Chinook hops using the adjusted GC method. Compounds of interest and their respective retention times were examined, including myrcene (13 min), octanol (23 min), linalool (27 min), caryophyllene (31 min), farnesene (32, 35-38 min), humulene (36 min), and geraniol (39 min). This figure helps to demonstrate that DTD is comparable to steam distillation with similar retention times and that no peaks were lost with a similar correlation of retention times between Hops-17 and the adjusted GC methods.

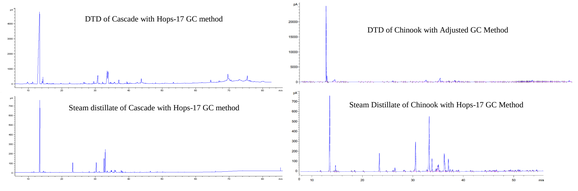

Figure 2. A comparison of DTD (top left) and steam distillation (bottom left) extraction methods of Cascade hops run according to ASBC's Hops-17 GC-FID separation and detection method as well as a comparison of DTD of Chinook hops using the adjusted GC method and steam distillation of Chinook hops using the Hops-17 method. Comparable retention times were observed with peaks of interest, including myrcene (13 min), octanol (23 min), linalool (27 min), caryophyllene (31 min), farnesene (32, 35-38 min), humulene (36 min), and geraniol (39 min), found to correlate closely between the two methods.

Results and Discussion

Once correlations were determined between DTD and steam distillation extraction methods, as well as between the Hops-17 and adjusted GC methods, a standard addition calibration curve with humulene was used in an attempt to quantify hop oil levels. An external calibration was run using the same standards with a clean GC liner to determine precision of the standards themselves. The standards were found to have an R^2 of 0.9993, suggesting high reliability, whereas the standard addition curve was found to have an R^2 of 0.6144 (Figure 3), suggesting less reliability. It is hard to determine what the complication in the standard addition method was and therefore further investigation is suggested before quantification of oils can be considered reliable.

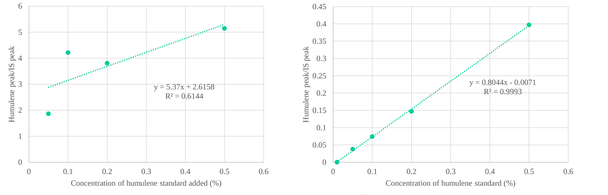

Figure 3. Results from the standard addition of humulene to Simcoe hops (left, n = 4, R^2 = 0.6144) and external calibration of humulene standard (right, n = 5, R^2 = 0.9993). Standard addition and external calibration solutions were made at 0.01, 0.05, 0.1, 0.2, and 0.5% humulene in hexanes. The initial data point (0.01%) of the standard addition curve was omitted as an outlier determined by the Grubbs test (G = 5.341, G_{table} = 1.672).

To further investigate the similarities between the two extraction methods, correlations between detector responses of the various hop oils were examined. Strong correlations were found in most varietals with the major exceptions being Comet and Citra, which were noted to have irregular spectra. It was further noted that with the exception of Simcoe and Centennial varietals, all the correlations improved when not accounting for myrcene responses, which were generally greater for the steam distillation method. While it is difficult to determine why this is, it is possible that the detector was becoming saturated at high myrcene concentrations. Another factor could be that myrcene is oxidizing during DTD extraction. Figure 4 shows a few examples of these correlations.

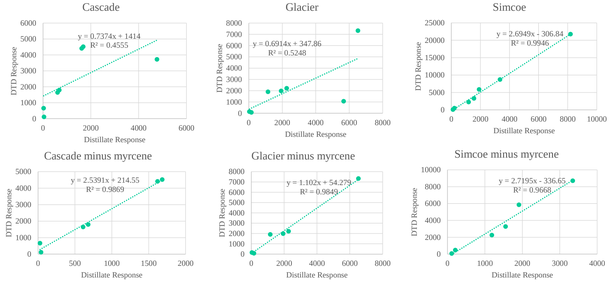

Figure 4. Example correlation curves of signal responses for myrcene, 2-octanol, linalool, caryophyllene, farnesene, humulene, and geraniol between steam distillation and DTD extraction methods. Strong correlations were found in nearly every varietal with the exception of Comet and Citra. Correlations were found to improve with the removal of myrcene response, with the exception of Simcoe and Centennial. The strong correlation between extraction methods suggests that DTD is comparable to steam distillation in that relative responses strongly correlate with the exception of myrcene. This anomaly could be the result of detector saturation due to the fact that myrcene is found in the highest concentration of all essential oils, or that myrcene is oxidizing during the DTD method.

The relatively high R^2 values of these curves, especially when not accounting for myrcene, suggest that the extraction methods result in comparable relative essential oil content. Further study is recommended to determine whether one or both methods can be adjusted to properly account for myrcene.

Results and Discussion

Table 1 displays a comparison in relative abundance of hop essential oils in eight different hop varietals as determined by both methods.

Table 1. Comparison of relative abundance of hop essential oils between the ASBC Hops-13 distillate with Hops-17 method and DTD with modified GC-FID method. While most varietals (Chinook, Centennial, Simcoe, El Dorado, and Cascade) were found to have similar compositions between the two methods, it should be noted that the remaining varietals (Glacier, Comet, and Citra) varied widely.

	Myrcene (%)		Linalool (%)		Caryophyllene (%)		Farnesene (%)		Humulene (%)		Geraniol (%)	
Hops	DTD	HOPS17	DTD	HOPS17	DTD	HOPS17	DTD	HOPS17	DTD	HOPS17	DTD	HOPS17
Cascade	28.29	54.05	0.86	0.48	13.62	7.72	33.51	18.36	18.72	19.09	5.00	0.30
Comet	16.29	61.30	1.22	1.01	32.10	10.16	43.18	11.40	5.91	15.35	1.31	0.78
Glacier	8.33	34.15	0.58	0.97	17.31	13.67	15.44	11.67	57.21	39.30	1.13	0.23
Citra	3.21	39.33	3.43	1.22	33.07	21.87	18.95	32.91	40.85	3.96	0.49	0.70
El dorado	25.91	43.08	1.21	0.95	21.35	14.04	20.92	20.48	28.63	20.76	1.98	0.69
Simcoe	54.21	53.44	1.18	1.37	14.57	12.47	8.17	10.14	21.70	21.85	0.16	0.74
Centennial	48.71	60.68	1.39	1.31	11.01	8.13	18.79	11.76	19.43	16.69	0.68	1.43
Chinook	21.75	27.94	1.09	0.82	17.37	12.95	26.62	29.66	32.45	28.06	0.73	0.56

Table 2 displays data collected (percent weight of hop matter) from hop analysis using the modified DTD extraction and separation methods collected over time. Aging was induced by leaving hop pellets at ambient temperature and oxygen conditions while limiting exposure to light. Whereas it can be noted that, in general, hop oil composition decreased over time, it cannot be determined whether differences are within a margin of error.

Table 2. Examination of hop oil composition (percent weight of total hop matter) over time in simulated oxidative conditions. Data were obtained using DTD with modified GC-FID method. While it can be noted that, in general, hop oil composition decreased over time, it cannot be determined if the change in composition is within a margin of error. Data from El Dorado hops on the second day of analysis could not be determined due to a corrupted data file and therefore are omitted.

	Myrcene (%)		Linalool (%)		Caryophyllene (%)		Farnesene (%)		Humulene (%)		Geraniol (%)	
Hops	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
Cascade	1.55	<MDL	0.05	0.02	0.75	2.04	1.83	0.12	1.02	5.21	0.27	0.85
Comet	1.31	0.35	0.10	0.05	2.58	1.75	3.46	0.03	0.47	0.46	0.10	0.14
Glacier	0.38	<MDL	0.03	0.04	0.79	0.53	0.71	0.01	2.63	1.68	0.05	0.02
Citra	0.74	1.98	0.79	0.20	7.59	2.26	4.35	0.02	9.38	3.68	0.11	0.04
Simcoe	6.60	31.98	0.14	0.07	1.77	1.61	0.99	0.01	2.64	3.38	0.02	0.04
Centennial	11.13	0.28	0.32	0.07	2.52	0.61	4.29	0.01	4.44	1.17	0.15	0.03
Chinook	3.03	0.79	0.15	0.05	2.42	1.31	3.71	0.02	4.53	2.42	0.10	<MDL

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