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Analytical Characterization of Hot Steeped Malt Flavor and Fragrance Volatiles by GC-MS

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Introduction

Malted barley, one of the main brewing ingredients, provides complex carbohydrates and sugars that are crucial for fermentation and also imparts flavor, body, and color to the beer. The flavor contribution depends largely on the style of malt used. Base, caramel, and roasted malts are all common and each lends unique flavor notes and characteristics. A chew test of whole kernels has traditionally been used as a check for flavor and freshness. While this test provides insight and has the benefit of being very fast, it does not fully reflect the flavors that are extracted during the brewing process that would be anticipated in the final product. A Hot Steep Method has been developed to prepare malt extracts for sensory analysis that are more representative of the malt contribution to the beer. In this work, we further investigate a variety of these types of malt extracts (including base, caramel, and dark roast specialty) with a non-targeted chemical analysis, using gas chromatography coupled to mass spectrometry (GC-MS).

Methods

Sample Preparation: Six varieties of malted barley were prepared for analysis based on the American Society of Brewing Chemist's (ASBC), "Hot Steep Malt Sensory Method". The method was scaled to 20% due to the reduced volume required for analytical analysis compared to sensory analysis. For each variety, 10 g of ground malt were added to 80 mL of 65 °C water in an insulated thermos and mixed with shaking. After 15 minutes of extraction, the contents were swirled and filtered, resulting in the malt extract/wort samples. As advised in the protocol, base malts were analyzed at 100%, specialty malts were mixed with base malt at 50%, and dark roasted specialty malts were mixed with base malt at 15%. All analytical data for a given sample was acquired within 4 hours of filtration.

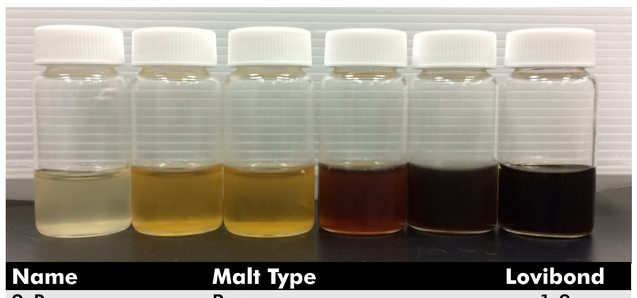
Analytical procedure: The extracts were sampled with head space solid phase micro-extraction (HS-SPME) to collect the volatile and semi-volatile aroma and flavor analytes. For each sample, 5 mL of wort were pipet into a 20 mL glass vial with a septum cap. The samples were incubated for 5 minutes at 35 °C and then extracted with a DVB/CAR/PDMS fiber (Supelco) for 10 minutes at the same temperature. Each sample was then analyzed by GC-TOFMS with the Pegasus BT (LECO). Instrument conditions are listed in Table 1.

Table 1. GC-TOFMS (Pegasus® BT)

GC	Agilent 7890 with LECO L-PAL3 Autosampler
Injection	SPME, 3 min desorption in 250 °C inlet
Carrier Gas	He @ 1.4 ml/min, Constant Flow
Column	Stabilwax, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Oven Program	Hold 3 min at 40 °C, ramp 10 °C/min to 250 °C, and hold 1 min
Transfer Line	250 °C
MS	**LECO Pegasus BT**
Ion Source Temp	250 °C
Mass Range	33-500 m/z
Acquisition Rate	10 spectra/s

Samples

Six varieties of malted barley were analyzed and compared, as described in Figure 1. Different malt styles are produced by altering the temperature and duration of kiln heating and/or roasting of the barley, with darker roasts generally heated to higher temperatures for greater lengths of time. These conditions drive various reactions, including caramelization and the Maillard reaction. The by-products of these reactions, which are often GC-amenable, are anticipated and can have important flavor and odor contributions.



Name	Malt Type	Lovibond
2-Row	Base	1.8
Aromatic	Specialty	20
Caramel 20L	Specialty	20
Caramel 120L	Specialty	120
Chocolate	Dark Roast Specialty	350
Black	Dark Roast Specialty	500

Figure 1. Malt details and photo of extract samples.

GC-MS Data

Each type of the extracts were analyzed with GC-TOFMS, resulting in the separation of hundreds of individual analytes. Example TIC chromatograms, that represent the aroma profiles, are shown in Figure 2. Some similarities and many differences are apparent.

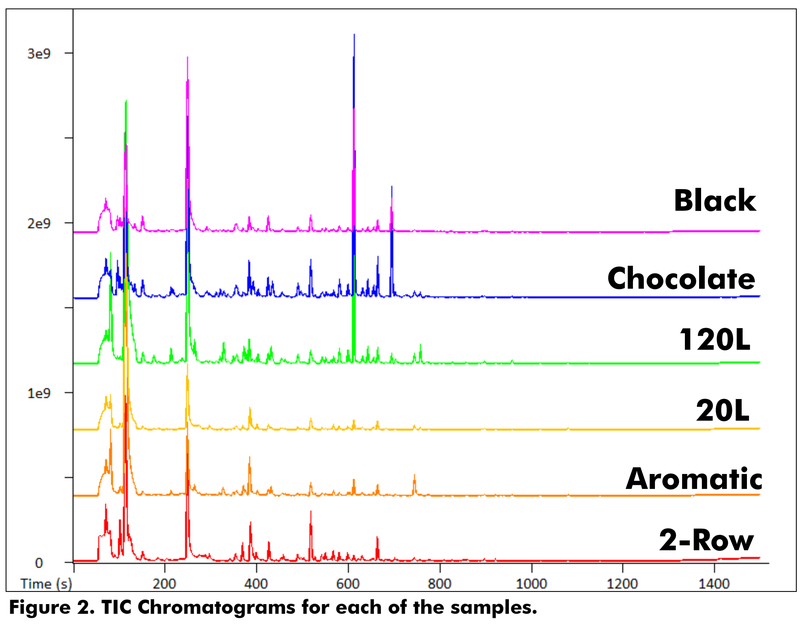


Figure 2. TIC Chromatograms for each of the samples.

Sample Comparisons

For general sample characterization, Principle Component Analysis (PCA) was calculated in MATLAB for the TIC traces. The scores plot, where each sample is represented as a data point, is shown in Figure 3. The extracts from each malted barley variety distinctly cluster, and the dark specialty roast and caramel malts are generally distinguished on PC1.

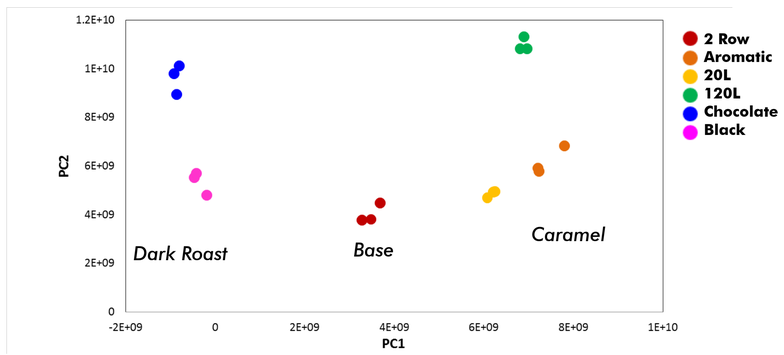


Figure 3. PCA Scores plot. The malt extract samples cluster based on the malt type, with dark roast specialty malts having lower PC1 scores and caramel malts having higher PC1 scores.

Individual Analytes

Even more insight can be gained by comparing trends of individual analytes across the samples. Individual analytes can be separated from each other chromatographically and mathematically with GC-TOFMS. Chromatographic coelutions can still occur with complex samples, but mathematical tools, like LECO's Non-Target Deconvolution® (NTD®), provide additional separating power of the full mass range TOFMS data. This capability is demonstrated in Figure 4. It appears that there are 2 peaks based on the TIC, but spectral patterns indicate the coelution of 4 analytes. By plotting m/z unique to each, the peak shapes can be observed. Pure spectral information for each leads to identification. With this capability, the relative trends of all four analytes were determined. Of note, 4-ethyl-2-methoxy-phenol that is observed at highest levels in the chocolate dark roasted malt, has smoky and spicy odor characteristics, while furaneol, observed at highest levels in a caramel malt, has odor descriptors of caramel and sweet. These trends were confounded in the TIC and difficult to discern without deconvolution.

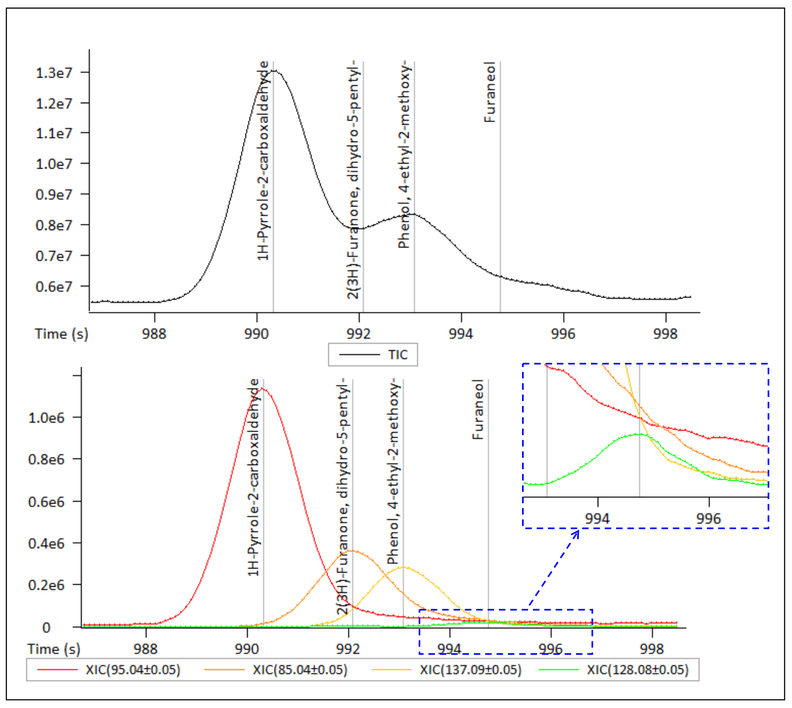


Figure 4. Deconvolution separates 4 individual analytes that appear as 2 peaks in the TIC. These analytes are present at different levels in each sample, likely contributing to some of the flavor differences related to malt variety used.

Analyte	Sim	Formula	CAS	R.T. (s)	RI	Lib RI	Odor Notes
1H-pyrrole-2-carboxaldehyde	920	C_5H_5NO	1003-29-8	990.3	2022	2030	coffee, musty, beefy
2(3H)-furanone, dihydro-5-pentyl-	851	$C_9H_{16}O_2$	104-61-0	992.1	2025	2024	sweet, buttery, coconut, creamy, waxy, oily
phenol, 4-ethyl-2-methoxy-	864	$C_9H_{12}O_2$	2785-89-9	993.1	2027	2032	smoky, spicy, bacon, phenolic, clove
furaneol	859	$C_6H_8O_3$	3658-77-3	994.7	2030	2031	caramel, cotton candy, sweet, strawberry, sugar

Analyte Trends

Almost 200 specific analytes were identified by mass spectral similarity (>800) and retention index (library vs observed <40 RI units). A wide range of analyte types were observed, including: alcohols, aldehydes, ketones, hydrocarbons, aromatics, esters, furans, pyranones, pyrazines, pyridines, pyrroles, sulfides, thiophenes, etc. The relative quantitation of these analytes was compiled to determine similarities and differences across the samples. Additional analytes of interest with distinct trends are shown in Figure 5 and a summary of the peak area trends for these analytes is shown in Figure 6. Many of the observed analytes have known flavor and odor properties which can be connected with the sensory attributes of the malts.

Analyte	Sim	Formula	CAS	R.T. (s)	RI	Lib RI	Odor Notes
dimethyl sulfide	976	C_2H_6S	75-18-3	70.5	794	754	sulfury, onion, sweet, corn, vegetable, green, cabbage, tomato, radish
methional	887	C_4H_8OS	3268-49-3	603.2	1453	1454	musty, potato, tomato, earthy, vegetable, creamy
2,6-dimethyl-pyrazine	882	$C_6H_8N_2$	108-50-9	490.7	1320	1328	cocoa, roasted, nuts, roast beef, coffee
maltol	916	$C_6H_6O_3$	118-71-8	955.9	1964	1969	sweet, caramel, cotton candy, jam, fruity, baked bread

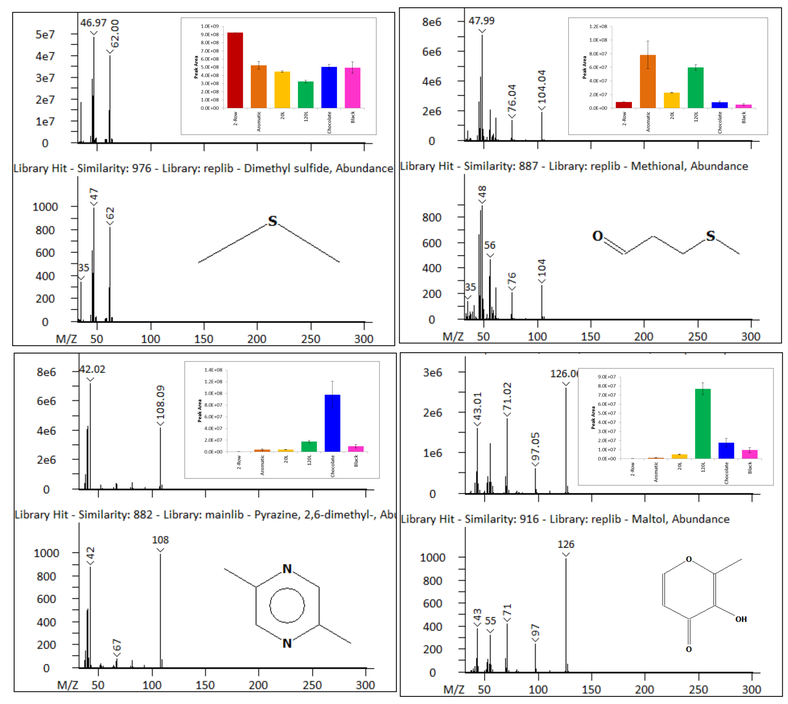


Figure 5. Representative analytes with distinct trends related to the malt varieties are shown.

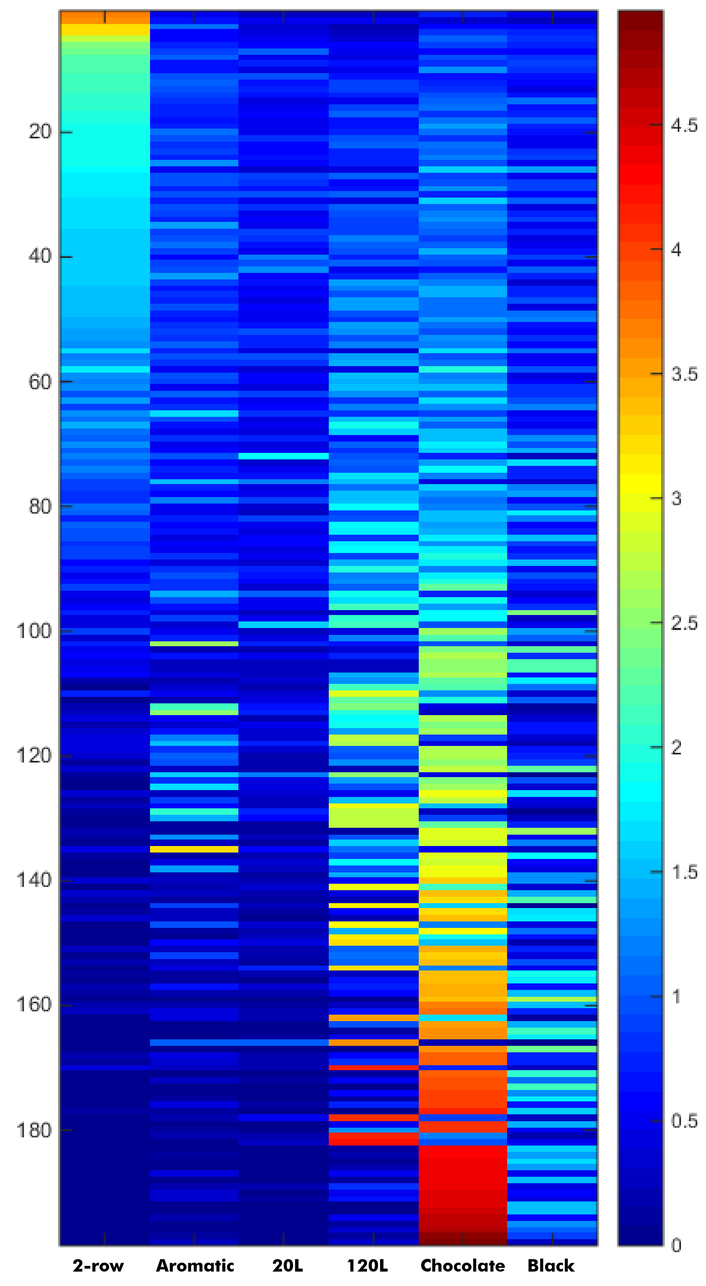


Figure 6. Peak area trends by malt variety.

Conclusions

In this work we demonstrate an analytical method for the chemical analysis of malt extracts, prepared with the ASBC's Hot Steep Method for sensory analysis. The malt extracts were sampled with HS-SPME and analyzed with GC-TOFMS. This analytical approach can provide valuable complementary data on individual analytes. Hundreds of analytes were identified and compared, with many malt specific trends observed. Many caramelization and Maillard reaction products were observed at elevated levels in the samples roasted at higher temperatures.