Spring 4-2022

Analysis of the Headspace Gases Formed from the Fermentation of Barley

Ken Overway
koverway@bridgewater.edu

Cassie Taylor
Bridgewater College

Analysis of the Headspace Gases Formed from the Fermentation of Barley

Cassie Taylor and Dr. Kenneth Overway

Chemistry Department, Bridgewater College, Bridgewater VA 22812, USA

Introduction

Volatile compounds contribute to the aromas that are associated with mixtures. The analysis of these gases can be completed using a gas chromatograph to provide a sort of fingerprint identification. The analysis of the volatile compounds for barley fermentation samples was completed by collecting the gas above the fermenting solution, the headspace gas, and injecting this gas into the gas chromatograph. These fermentation samples showed predominantly the formation of carbon dioxide for a significant length of time before finally ethanol was detected. A subsequent investigation of the limit of detection for ethanol was made. This analysis method was extended to a variety of household liquids including peppermint oil, almond extract, vanilla extract, Festivus Beer, and red wine. Notable volatile compounds of each sample were not detected so various types of sample preparation were completed in hopes of gaining a higher concentration sample of these volatile compounds.

Experimental

Fermentation

The fermentation process was started by combining barley and DI water. The mixture heated at approximately 60-63 ºC for thirty minutes and then heated at approximately 66-72 ºC for an additional thirty minutes. The temperature was then raised to approximately 90-100 ºC and held within that range for an hour. Once the hour was complete, the mix was cooled, filtered, and oxygenated. Yeast was added, mixed and then a bubbler was added, allowing for gases to escape but not allow air into the bottle.

Figure 1. *The image on the left shows the set up used to prepare the fermentation. The image on the right shows two bottles with bubblers that held the fermenting solution and the headspace gas*

The solution was allowed to ferment and samples of the headspace above the solution were collected over time. These samples were injected into the ThermoFisher Scientific TRACE 1310 GC/MS.

Experimental Cont.

Household Alcohol Testing

Household alcohols were tested to get a better understand of the head-space gas of alcoholic samples. The samples that were tested included peppermint oil, almond extract, vanilla extract, red wine, and Festivus Beer. The head-space gas of these samples were injected into the TRACE 1310 GC/MS. While some of the volatile compounds that were expected were seen, many were not so various preparation methods were attempted. The first two included syringing out head-space gas, allowing the volatile compounds to replace the space, and heating the sample vials. When these methods proved unsuccessful, SPE chromatography was attempted, in which a SPE cartridge was used along with a vacuum pump. This also proved to be unsuccessful, and preparation was moved to a bubbling method, in which it was attempted to bubble the head-space gas of the household alcohols into an organic solvent.

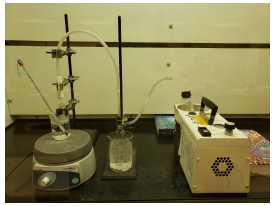

Figure 2. *One of the latest bubbler set ups, including a vacuum pump, an ice bath, and a hot water bath.*

Results & Discussion

Fermentation Results

Between the two fermentation trials that were run, CO_2 (highlighted in the left plot) and ethanol (highlighted in the right plot) were able to be detected.

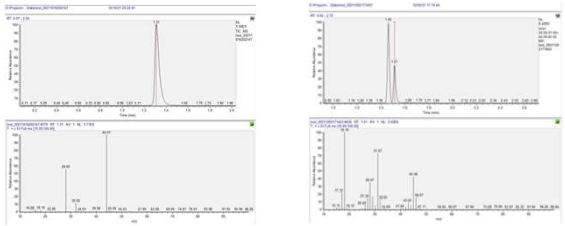

Figure 3*. The left plot shows the chromatogram and mass spectrum of an injection of the head-space gas from the first fermentation, completed a week after the start of the fermentation process. The right plot shows the chromatogram and mass spectrum of an injection of the head-space gas from the second fermentation, completed just over a month after the start of the fermentation process.*

Results & Discussion Cont.

Household Alcohol Testing

For some of the samples several compounds were able to be detected and identified such as peppermint oil, in which eucalyptol, menthol, and menthyl acetate were identified. For others, such as vanilla extract, only ethanol was able to be identified. The search for other known volatile compounds in these samples is ongoing.

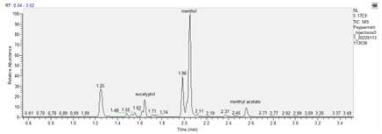

Figure 4. *Chromatogram of the head-space gas of peppermint oil*

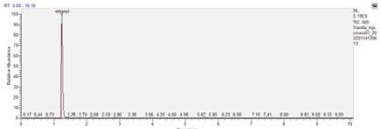

Figure 5. *Chromatogram of the head-space gas of vanilla extract*

Conclusions

The following conclusions are based on the instrumentation that was available to BC's Chemistry Department:

- When fermenting barley, given the adequate amount of time, the presence of CO_2 and ethanol can be detected.
- Volatile compounds of household alcoholic products can be seen in some case but why it is only works for some compounds and under some conditions requires further investigation.

Acknowledgments

Thank you to Dr. Overway for his advising throughout this process as well as to the rest of the chemistry department faculty for their support and assistance.

References

1.) Ballast Point, All-Grain Brewing Instructions, *Home Brew Mart white paper,* **2018,** https://www.ballastpoint.com/ (accessed 2022 -04 -03).

2.) Analysis of Fermentation Gas from Home-Brewed Beer by Solid-Phase Microextraction (SPME) and GC/MS*, JEOL Resource,* **2005**, https://www.jeolusa.com/ (accessed 2022 -04 -03).

3.) Ridgway, K. The Challenges of Flavour Analysis Comparison and Choices of Extraction Techniques, *rssl white paper*, **2016**, https://www.rssl.com/ (accessed 2022 -04 -03).