

# What's in your Kombucha extract?

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## INTRODUCTION

Flavor profiles can make or break a good Kombucha or any fermented drink. Slight differences in the strain of yeast, feedstock, or added sugars have the potential to dramatically change the final flavor. When experimenting with different yeast strains or ingredient suppliers, identification of the flavor profile can be useful to improve taste, leading to increased sales. Typically, flavor profiles are determined using chromatography techniques such as gas chromatography mass spectrometry (GCMS). In addition to flavor information, GCMS can also be used to detect potentially harmful chemicals such as pesticides and endocrine mimickers such as bisphenol A (BPA). Similarly, it is vital to ensure that heavy metals are not present in the extracts used to make fermented beverages. X-ray fluorescence (XRF) spectroscopy is often used for the detection of trace metals down to parts per million concentration.

In this study, we employed a combination of GCMS, XRF and Fourier transform infrared (FTIR) spectroscopy to determine the composition of a Kombucha extract. The results demonstrate the synergy between the three techniques in understanding the structures of various organic compounds and possible harmful contaminants that may be present.

## EXPERIMENTAL

### GCMS

Two samples of the Kombucha extract concentrate were taken (for extraction and neat injection). The extraction was completed by extracting 5 mL of Kombucha with 5 mL of dichloromethane (DCM). The DCM was concentrated to 1 mL and analyzed. The sample was also injected as received with no dilution (neat). An instrument blank was measured by running the GCMS cycle with extraction solvent only (i.e., no "injection") prior to running any samples. All area values were calculated as the total integrated area under the curve. Samples were analyzed on a HP 6890A Gas Chromatograph/ HP 5973 Mass Spectrometer equipped with a 30 m X 0.25 mm DB-5MS column (J&W Scientific).

### FTIR

FTIR was performed directly on the liquid sample. Some evaporation of liquid was observed during the analysis. A small portion of the sample was transferred to an infrared transmitting substrate and examined using a Thermo-Nicolet 6700 Fourier Transform Infrared spectrometer equipped with a Continuum

**Table 1. GCMS results**

Spectr. #	Average RT (minutes)	Extraction (Area%)	Neat (Area%)	Compound Identification
1-1	1.67	1.73	ND	Ethanol
1-2	2.14	ND	0.28	Formic acid
1-3	2.57-3.08	79.48	73.26	Acetic Acid
1-4	3.79-4.10	12.15	0.68	Acetoin
1-5	6.10	ND	0.58	2,3-Butanediol
1-6	7.01	ND	1.31	Furfural
1-7	7.99	ND	3.16	Methyltartronic acid
1-8	8.23	0.82	ND	Butyrolactone
1-9	10.48	0.40	0.07	Phenylethyl Alcohol
1-10	10.74	ND	0.61	Pyranone
1-11	11.38	ND	8.83	5-Hydroxy-methylfurfural
1-12	15.36	2.05	0.22	Caffeine

**\*The total is calculated based upon the area of all integrated peaks in the TIC, including peaks not represented in this table.**

microscope, operating in transmission mode. The analytical area was 100 x 100 µm. Omnic 8.0 was used to process the data.

### XRF

The liquid sample was analyzed neat (as-received) on a Rigaku Primus II WDXRF using a helium atmosphere. Quantification was performed using the Fundamental Parameters (FP) standardless quantification software associated with the system. The fundamental parameters approach uses x-ray physics coupled with established sensitivity factors for pure elements. Data were collected in both scanned and fixed angle modes. In normal scanning mode analysis, the detector is rotated through the full range of dispersion to collect all the diffracted x-ray signals coming from the sample and subsequently, spectra are generated. In a fixed angle analysis, the detector dwells at a specific angle for an extended period while collecting a signal only from the peak maximum and from the adjacent background. Fixed angle analysis significantly improves sensitivities. Since a fixed angle analysis collects intensities from only two points (peak maximum and baseline) no actual spectra are created.

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## RESULTS AND DISCUSSION

The GCMS results from the extraction and neat injections are summarized in Table 1. Figures 1 and 2 provide a graphical representation of the compounds detected in the neat and DCM samples, respectively.

When omitting water, both methods showed that the extract was primarily composed of acetic acid (vinegar) and acetoin (buttery odor). Other compounds of interest include furfural derivatives, caffeine and methyltartronic acid. Due to the difference in concentration and solubility, several of the peaks were identified in one sampling method but not the other. These included species that were likely masked by large peaks in the neat sample and thus were more apparent in the extract, including small organic molecules such as ethanol and caffeine. Alternatively, trace volatiles that were polar were not extracted into the dichloromethane and appear only in the neat sample such as 5-Hydroxymethylfurfural (buttery, caramellike) and 2,3-Butanediol.

Notably no pesticides or endocrine mimickers were detected using either sampling method.

FTIR was performed on the extract. As shown in FTIR Spectrum 1, the extract is primarily composed of water and acetic acid. Specifically, the peaks at 3397 and 1644  $\text{cm}^{-1}$  are due to water, whereas the peaks at 1721, 1415 and 630  $\text{cm}^{-1}$  due to acetic acid.

In addition, as shown in FTIR Spectrum 2, a saccharide is a major component of the extract, with peaks at 1462, 1339, 1183, 1062, 981, 919, 869, 821, 782, 706 and 630  $\text{cm}^{-1}$  exhibiting an excellent match with D(-)-fructose. Note that fructose cannot be detected by GCMS due to its high boiling point although GCMS does confirm the presence of decomposition products of fructose (5-Hydroxymethylfurfural and furfural).

Finally, additional components detected by GCMS may be observed in the FTIR spectra, including butanediol (see FTIR Spectrum 3) and 5-hydroxymethylfurfural (See FTIR Spectrum 4).

The extract was analyzed by XRF and the results are shown in Table 2. The primary element in the solution was Oxygen (O), which was quantified as  $\text{H}_2\text{O}$  as H cannot be detected by XRF. Thus, approximately ~97% of the mixture was composed of water or similar oxygen-based molecules. Carbon at 2.7 wt% and Nitrogen at 0.64 wt% comprise all the organic molecules detected by FTIR and GCMS. Note that the GCMS analysis omitted water in the calculations or was run under specific conditions that minimize the presence of water (organic extraction).

Alkali and alkali earth metals such as K, Ca, Na and Mg were detected at trace levels, accounting for approximately 140 ppm. In addition, Chlorine (Cl) was detected at 40 ppm, Sulfur (S) was present at 20 ppm, whereas Phosphorus (P) was detected

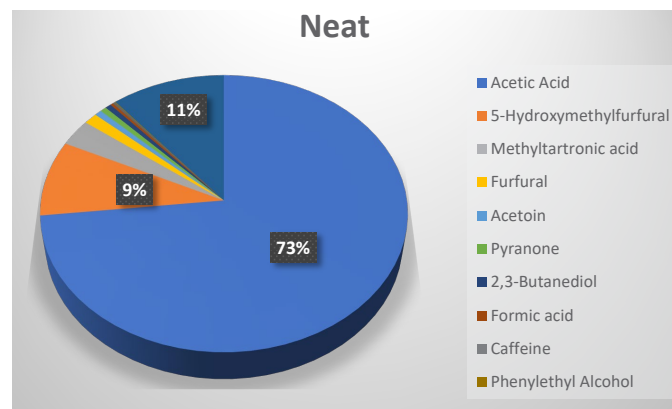


Figure 1. GCMS results neat

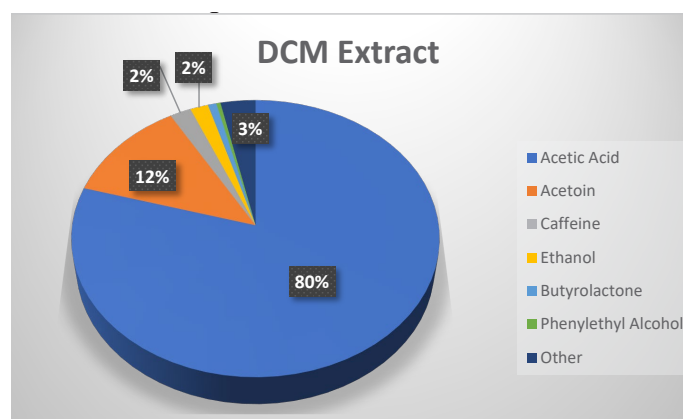


Figure 2. GCMS results DCM extract

Table 2. Sample Composition (in Wt%)<sup>a</sup>

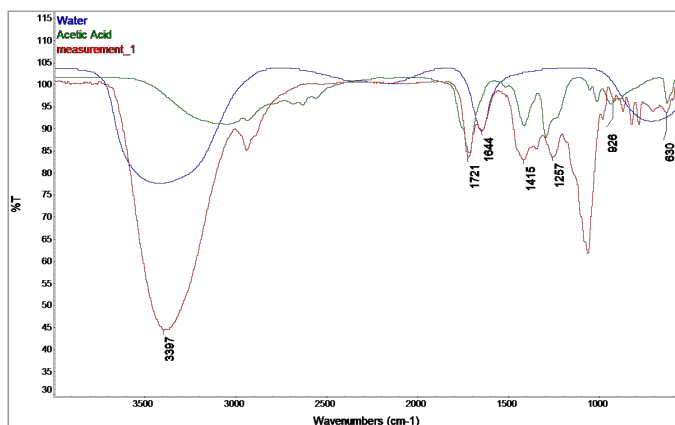
Component	Conc.	Component	Conc.
$\text{H}_2\text{O}$	96.6	Na	0.002
C	2.69	Mg	0.0009
N	0.64	Cr	0.0007
K	0.009	Fe	0.0007 <sup>b</sup>
Cl	0.004	Al	0.0004
Si	0.003	P	0.0004
Ca	0.003	Mn	0.0003
S	0.002	Ni	0.0003 <sup>b</sup>

a The results are normalized to 100% of the measured and detected elements  
Note: 1.0 wt%=10,000ppm

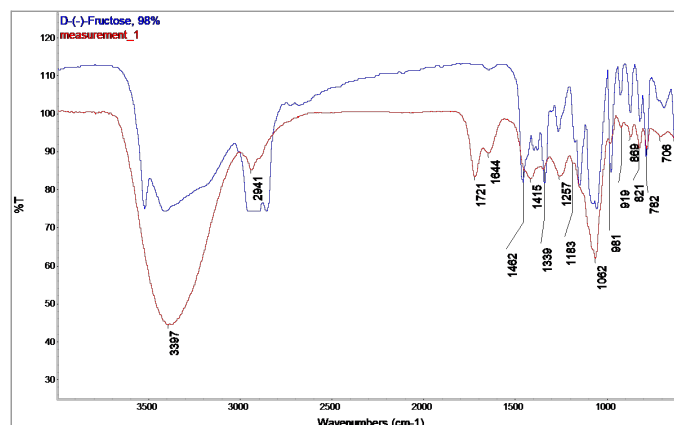
b At the levels observed, these signals may be largely or solely from the instrument background

at only 4 ppm. Heavier metals such as Iron (Fe) and Nickel (Ni) were observed at 7 and 4 ppm, respectively but those are most likely due to the instrument background. In contrast, 4 ppm of Aluminum, 7 ppm of Chromium and 3 ppm of Manganese may be trace metal contaminants in the Kombucha extract.

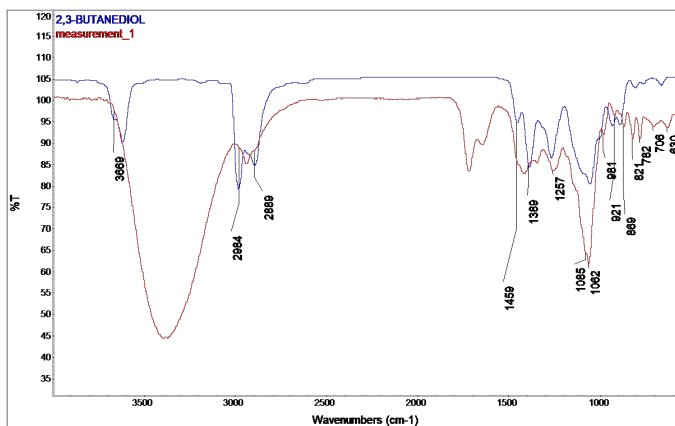
# What's in your Kombucha extract?



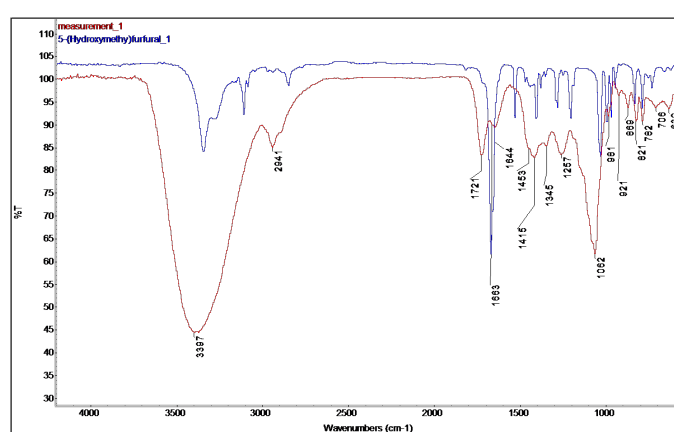
FTIR Spectrum 1



FTIR Spectrum 2



FTIR Spectrum 3



FTIR Spectrum 4

## CONCLUSION

GCMS, FTIR and XRF were used to investigate the composition of a Kombucha extract. According to GCMS, the main components include acetic acid and acetoin. Several flavor compounds were detected, including acetic acid (vinegar), acetoin (cream, buttery taste), furfural (woody, bready), butyrolactone (creamy, lactose), phenylethyl alcohol (floral bouquet), pyranone (herbal), and 5-Hydroxymethylfurfural (buttery, caramel-like). Overall, more than 12 primary compounds were detected by GCMS.

FTIR confirmed the presence of acetic acid as a major component along with water. A saccharide similar to D(-)-fructose was also detected, which is not volatile enough to be observed by GCMS. GCMS detected butanediol and 5-hydroxymethylfurfural, which may be observed in the FTIR spectra, although those compounds are close to the detection limit of the technique.

Notably, no pesticides or endocrine mimickers were detected by either technique.

XRF revealed that the sample is primarily composed of water with low levels of C and N. Heavy metals such as Mn and Cr were detected at 3 and 7 ppm, respectively. Other elements such as K, Cl, S, Ca and Na were detected at 20-90 ppm, whereas other metals such as lead, mercury or cadmium were not observed above the detection limit of 10-50 ppm.

Overall, this study demonstrates the efficacy of three complementary techniques in elucidating the structure of organic molecules and the detection of low-level metals in a complex matrix.

## REFERENCES

Carnavali, D., et al., "Gas-phase Fructose Conversion to Furfural in a Microfluidized Bed Reactor" ACS Sustainable Chem. Eng. 2018, 6, 4, p. 5580-5587.