Evaluation of EtG and EtS Alcohol Markers in Urine and Oral Fluid After Hard Kombucha Consumption

Sun Yi Li[1], Christina R. Smith[1], Sarah H. Bartock[2], Leland McClure[3], Les E. Edinboro[2], Madeleine J. Swortwood[1]

[1]*Department of Forensic Science, College of Criminal Justice, Sam Houston State University, Huntsville, TX, USA*

[2]*Quest Diagnostics Nichols Institute, Chantilly, VA, USA*

[3]*Drug Testing Consultations, LLC*

MEMBER THE TEXAS STATE UNIVERSITY SYSTEM

INTRODUCTION

Kombucha is a fermented beverage made from tea, sugar, yeast, and bacteria. Its consumption gained popularity over the past years due to suggested health benefits. Due to fermentation, kombucha naturally contains some alcohol content. In the market, two types of kombucha are available: 1) regular or non-alcoholic and 2) "hard" or alcoholic. It may be labeled as "non-alcoholic" only if the alcohol by volume content (ABV) of the finished product does not exceed 0.5%. A previous study determined that the alcohol markers ethyl glucuronide (EtG) and ethyl sulfate (EtS) were not detected in oral fluid but were present in some urine samples after consuming "non-alcoholic" kombucha. As a result, individuals may try to use consumption of kombucha as a general excuse for intoxication or impairment. As for hard kombucha, it has a %ABV similar to beer (4-7%). In this study, we aimed to evaluate EtG and EtS concentrations in urine and oral fluid after consuming hard kombucha for comparison purposes to the "non-alcoholic" type. Despite the increasing popularity of kombucha, limited studies exist examining alcohol markers in biological specimens following consumption of hard kombucha. The results of this study could provide insights on the effects of kombucha consumption to alcohol marker evaluation in abstinence-monitoring situations, such as workplace drug testing.

RESULTS & DISCUSSION

Table 1: Positive A) urine and B) oral fluid specimen detected by LC-MS/MS (Cutoff: EtS 100 & 25 ng/mL in urine & oral fluid).

A

Urine Void	EtS Positive Samples (100 ng/mL cutoff)	% Positive
Day 1: 0	2	4.1
1st	7	14.3
2nd	9	18.4
3rd	8	16.3
4th	7	14.3
5th	6	12.2
6th	4	8.2
7th	2	4.1
8th	1	2.0
Day 2: 1st	3	6.1
Day 3: 1st	0	0

B

Oral Fluid (time in h)	EtS Positive Samples (25 ng/mL cutoff)	% Positive
t_0	0	0
$t_{0.5}$	0	0
t_1	1	33.3
t_3	2	66.7
t_5	0	0
t_8	0	0
t_{10}	0	0
t_{24}	0	0
t_{32}	0	0
t_{48}	0	0

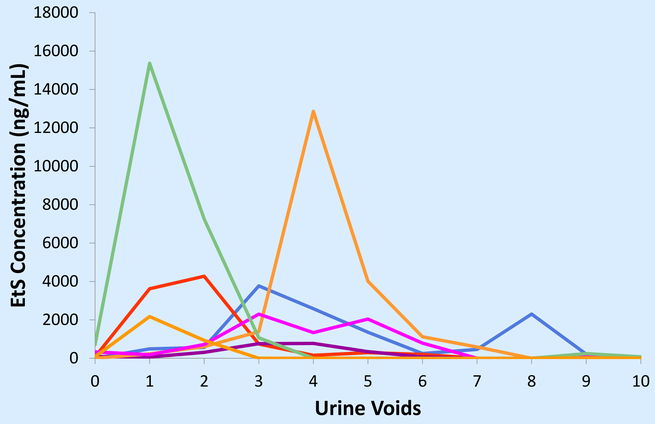

Figure 2: EtS concentrations (ng/mL) in urine voids by participant (n=9)

Table 2: Performance of immunoassay (EtG) vs LC-MS/MS confirmation for EtG or EtS in urine (n=75).

	Confirmed w/ EtG >500 ng/mL	Confirmed w/ EtS >100 ng/mL
True Positive	48	49
True Negative	24	22
False Positive	2	1
False Negative	1	3
Sensitivity	98.0%	94.2%
Specificity	92.3%	95.7%

- In urine, the median (range) concentrations of alcohol markers after hard kombucha consumption were:
  → EtS: 770 (104 – 15364) ng/mL
  → EtG: 3381 (510 – 7025) ng/mL
  → Alcohol markers could still be detected in some specimen 24h after hard kombucha consumption
  → All specimens were negative by 48h after drinking
- Hard kombucha bottles contained 4.0 – 4.5% ABV, which was significantly lower than the bottle advertised at 6.5%
- In oral fluid, 28 specimens had EtS >LOD (10 mg/mL)
  → C_{max} were 27.1, 29.8, and 50.6 ng/mL occurring at 1, 3, and 3h after start of drinking
- Confirmatory urine testing for EtS is crucial in alcohol marker confirmation in addition to immunoassay for EtG

MATERIALS AND METHODS

IRB approval

This study was approved by SHSU Institutional Review Board (#IRB-2019-249) with written informed consent.

Participants

Nine (9) subjects participated in this study: five (5) males and four (4) non-pregnant females. Participants were asked to avoid using alcohol-containing mouthwash and abstain from all alcohol consumption the evening before administration and during the three-day period of the study. Participants were asked to provide urine voids (V_0) and oral fluid specimens (t_0) before consuming a single 16 oz. bottle of hard kombucha (6.5% ABV) within 20 min. A breathalyzer result of 0.000 was required for the dismissal of participants by the end of day 1.

Specimen collection

All oral fluid specimens were collected using Oral-Eze™ devices. Specimen collection started 10 min after the hard kombucha was consumed ($t_{0.5h}$) and at 1, 3, 5, 8, 10, 24, 32, and 48h relative to the start of drinking (Fig. 1). As for urine specimens, participants provided all urine voids for the first day and then the first urine void on days 2 and 3. All urine and oral fluid specimen were stored under refrigeration.

Aliquots of the hard kombucha beverage (~1 mL) were collected for %ABV evaluation by headspace gas chromatography.

Specimen analysis

Oral fluid specimens were screened and confirmed when positive for EtS (25 ng/mL cutoff) by liquid chromatography – tandem mass spectrometry (LC-MS/MS). Urine specimens were analyzed by immunoassay (IA) (500 ng/mL EtG cutoff) and LC-MS/MS for EtG (500 ng/mL cutoff) and EtS (100 ng/mL cutoff). Urine concentrations were not normalized for creatinine. Urine specimens were considered positive if EtS was present with or without EtG above these limits; urine EtG-only specimen were not considered positive.

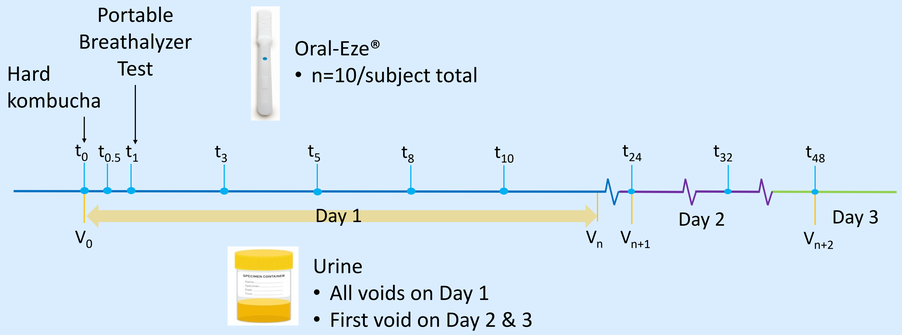

Fig. 1: Specimen collection timeline relative to the start of drinking.

CONCLUSIONS

- %ABV of hard kombucha were lower than advertised.
- Positive results for alcohol markers were obtained in both oral fluid and urine: EtS detected for up to 8h in oral fluid and EtG/EtS detected for up to 24h in urine.
- Confirmatory urine testing, especially to include EtS is crucial.
- Hard kombucha consumption might interfere with results in abstinence-monitoring testing situations.

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