

# Determination of Trace Ethanol Levels in Kombucha by GC–MS for Halal Verification

Final Project Presentation

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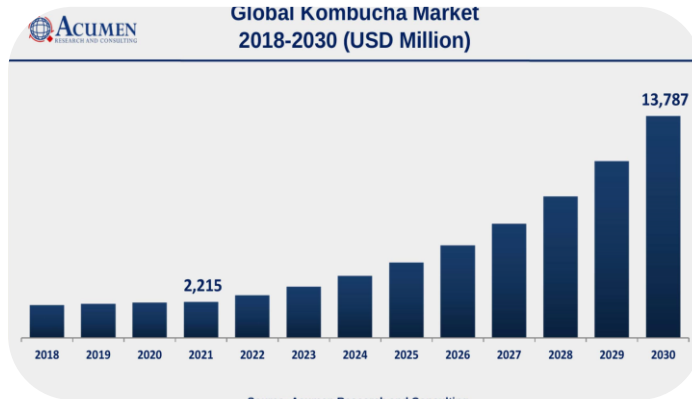
**Senin, 30 Juni 2025**

**Supervisor : Wahyu Utami, M. Si.,Ph.D., Apt**

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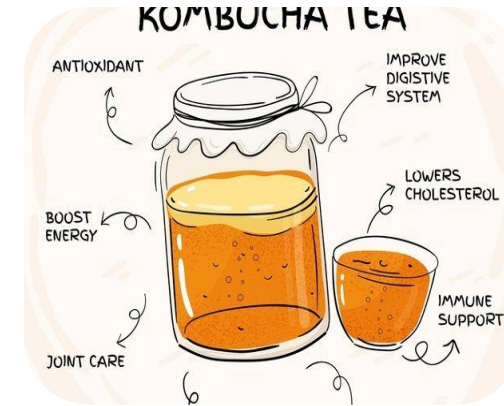
# Background of the Research



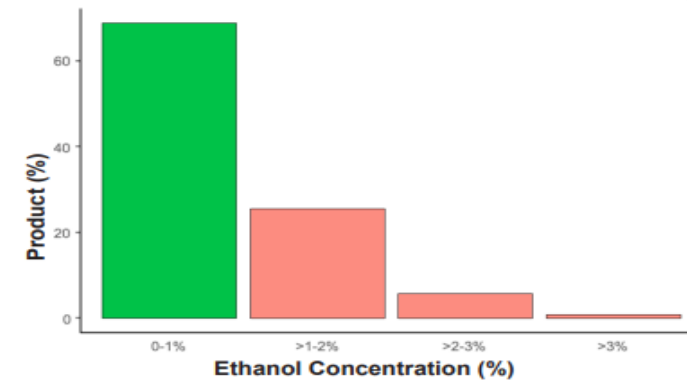
The popularity of kombucha



Halal and non-alcoholic requirements of kombucha



Kombucha as functional drink



Findings of the exceeding ethanol levels of kombucha

# Research Objectives

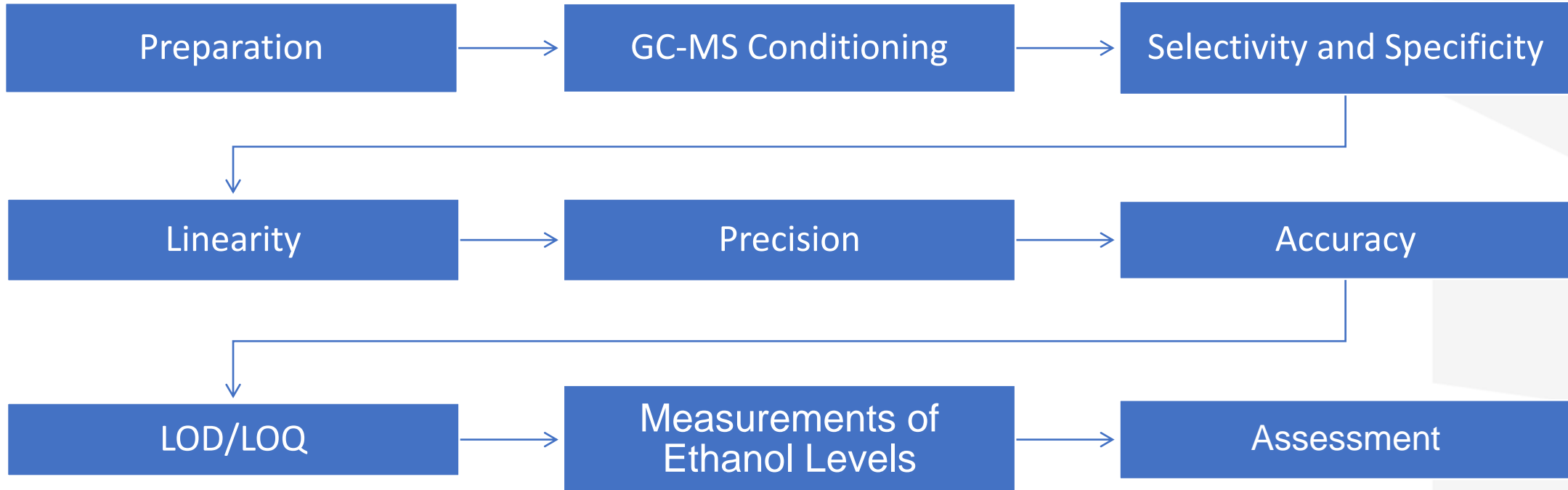


to provide a sensitive, rapid, and feasible method for halal verification in determination of ethanol in kombucha using gas-chromatography-mass spectrometry.



to assess the trace ethanol levels in kombucha products sold online in Indonesia.

# Methodology



# Methodology

GC-MS Conditioning can be seen in Table 1

No.	GC Parameters	Conditioning
1	Column	Rtx-1MS (30 m x 0.24 mmID x 0.25 $\mu$ m df)
2	Oven starting temperature	35 °C
3	Oven temperature program	Hold at 35°C for 4 minutes, then ramp at 30°C/minute to 215°C, hold for 2 minutes.
4	Run time	12 minutes
5	Injector temperature	220 °C
6	Injection volume	1.0 $\mu$ L
7	Carrier gas	Helium
8	Flow Rate	1.4 ml/min (constant flow)
No	MS Parameters	Conditioning
1	Source Temperature	230 °C
2	Quad temperature	150 °C
3	Acquisition mode	Scan
4	Scan Range	20.0 – 100.0 amu

# Result and Discussion

## Validation of the Analytical Method

Method optimization followed AOAC International's single laboratory validation guidelines (AOAC SMPR 2016.001) and the Indonesian National Standard (SNI). Method validation results can be seen in Table 2

No.	Parameter	Result	Acceptance Term	Reference
1	Linearity	$R^2 = 0,9970$	$R^2 \geq 0,990$	SNI 8965: 2021
2	Precision	% RSD = 2.55 HorRat = 1.3	% RSD $\leq 4$ 0.5 - 2.0	AOAC (SMPR 2016.001; 19)
3	Accuracy	Spike 0.1%: 97.27 % : 91.12 % Spike 0.5%: 98.37 % : 91.80 % Spike 2.5% : 99.21 % : 90.84 %	97 - 102%  97 - 102%  97 - 102%	AOAC (SMPR 2016.001; 19)
4	LOQ	0.01 %ABV	$\leq 0.05$ ABV	AOAC (SMPR 2016.001; 19)

# Result and Discussion

## Validation of the Analytical Method

Selectivity and specificity

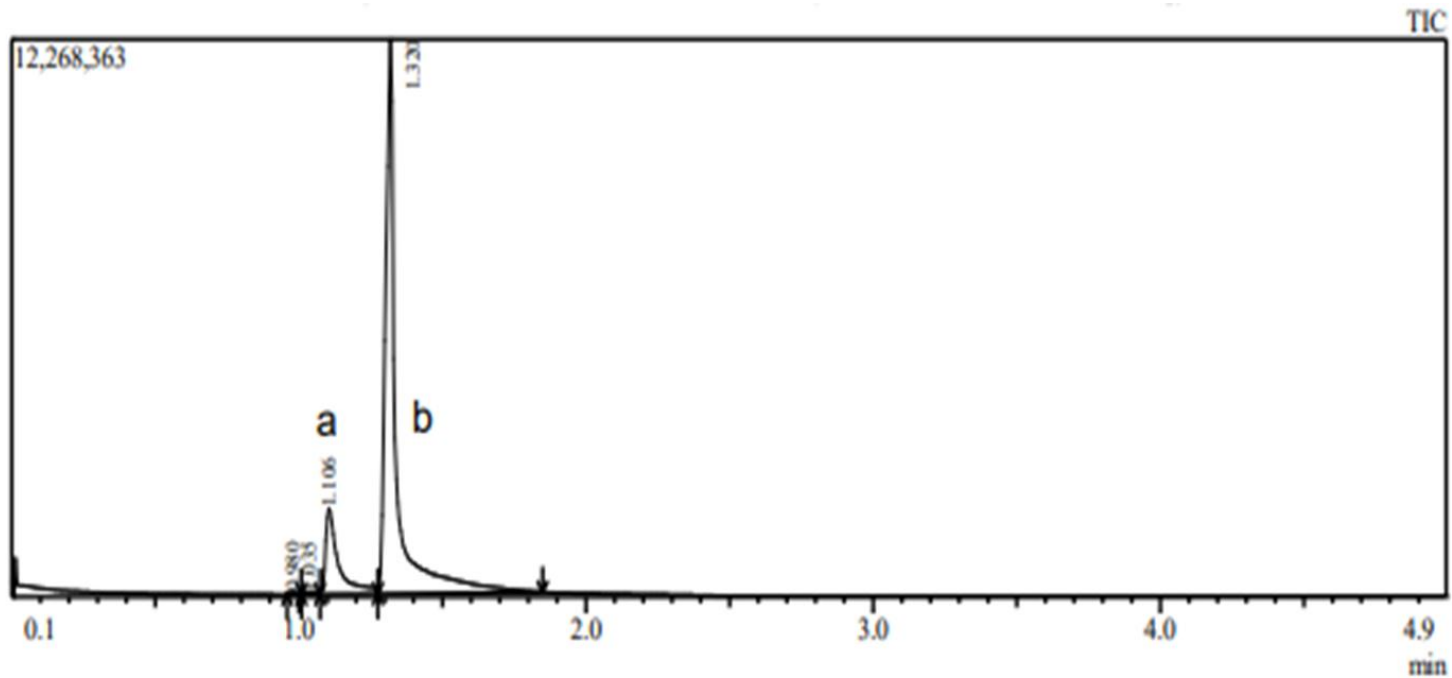


Figure 1. GC–MS chromatogram showing clear separation of ethanol (a) and 1-propanol (b) (internal standard) peaks for halal-compliance analysis

# Result and Discussion

## Comparison in Preparation Methods

The comparison between two preparation methods can be seen in Table 4

<b>Replicate</b>	<b>Sample (%ABV)</b>	<b>A</b>	<b>Sample (%ABV)</b>	<b>B</b>
1	1.239585		0.584173	
2	1.260995		0.613691	
3	1.198978		0.622745	
<b>average</b>	1.233186		0.60687	
<b>SD</b>	0.0315		0.020171	
<b>% RSD</b>	2.554346		3.323769	

# Result and Discussion

## Comparison in Preparation Methods

The comparison between two preparation methods can be seen in Table 5

Preparation Methods	Spike	Recovery (%)
Centrifugation	0.10%	97.2733
	0.10%	91.1222
	0.50%	98.3792
	0.50%	91.8098
	2.50%	99.2127
	2.50%	90.8458
	<b>average</b>	94.77383333
Distillation	0.10%	100.12
	0.10%	99.16
	0.50%	99.16
	0.50%	97.41
	2.50%	101.37
	2.50%	104.41
	<b>average</b>	100.2716667

t-Test: Two-Sample Assuming Equal Variance		
	Variable 1	Variable 2
Mean	94.77383	100.2717
Variance	15.29978	5.803177
Observations	6	6
Pooled Variance	10.55148	
Hypothesized Mean Diff	0	
df	10	
t Stat	-2.93154	
P(T<=t) one-tail	0.007501	
t Critical one-tail	1.812461	
P(T<=t) two-tail	0.015001	
t Critical two-tail	2.228139	

This study also doing the comparison between the two methods with distillation showed better result in precision and accuracy.

# Result and Discussion

## Comparison in Preparation Methods

The comparison between two preparation methods can be seen in Table 6

Metode Preparasi	Spike	Recovery (%)
Sentrifugasi	0.10%	97.2733
	0.10%	91.1222
	0.50%	98.3792
	0.50%	91.8098
	2.50%	99.2127
	2.50%	90.8458
	<b>average</b>	<b>94.77383333</b>
	Destilasi	0.10%
0.10%		99.16
0.50%		99.16
0.50%		97.41
2.50%		101.37
2.50%		104.41
<b>average</b>		<b>100.2716667</b>

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# Result and Discussion

## Implications for Halal Certification and Consumer Safety

a. Knowing that ethanol content found in kombucha products could be varied, the reliable ethanol determination methods in the production and regulation of kombucha and similar fermented products become a necessity.

b. Ethanol content labelling also crucial in public health considering that ethanol consumption can be particularly restrictive in some demographics such as pregnant and breast feeding women. Ethanol also poses higher risk in children.

# Conclusion



a. This study revealed that ethanol levels in some kombucha products commercially sold in Indonesia exceeded the halal thresholds set by both the Indonesian Council of Ulama (MUI) and the Department of Islamic Development Malaysia (JAKIM).



b. This study successfully validated a GC-MS method for ethanol detection in kombucha.



c. Given that ethanol content in kombucha may increase over time due to ongoing fermentation, this study underscores the importance of stringent production control and accurate labelling.

# References

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# Appendix



Figure 1. Sample kombucha tea (A)



Figure 2. Sample kombucha tea (B)

# Appendix

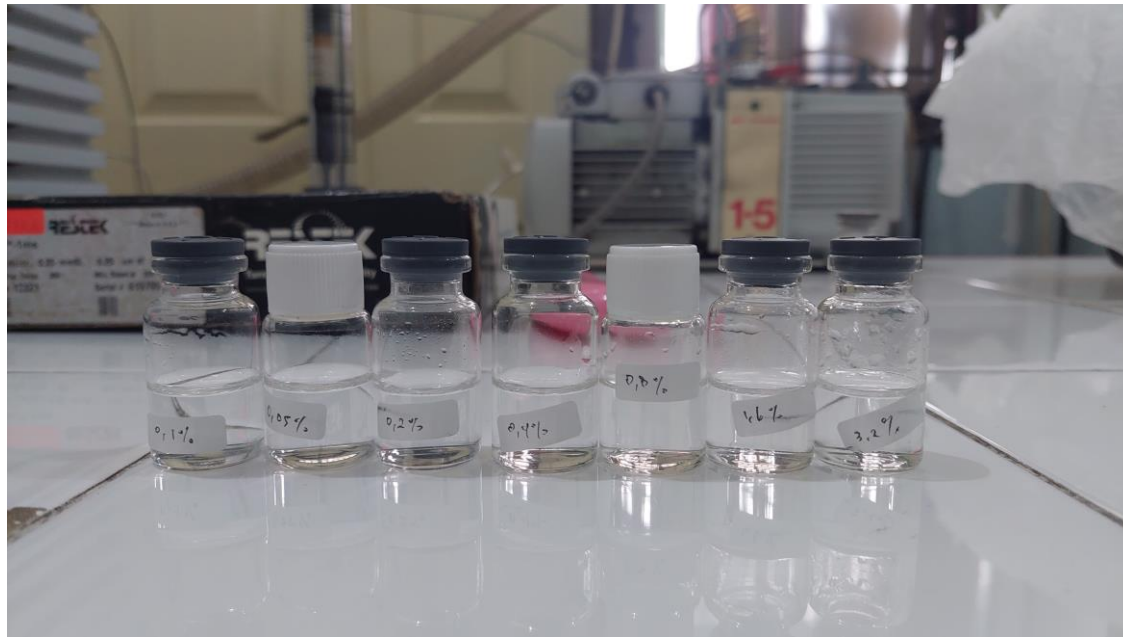


Figure 3. Calibration solution

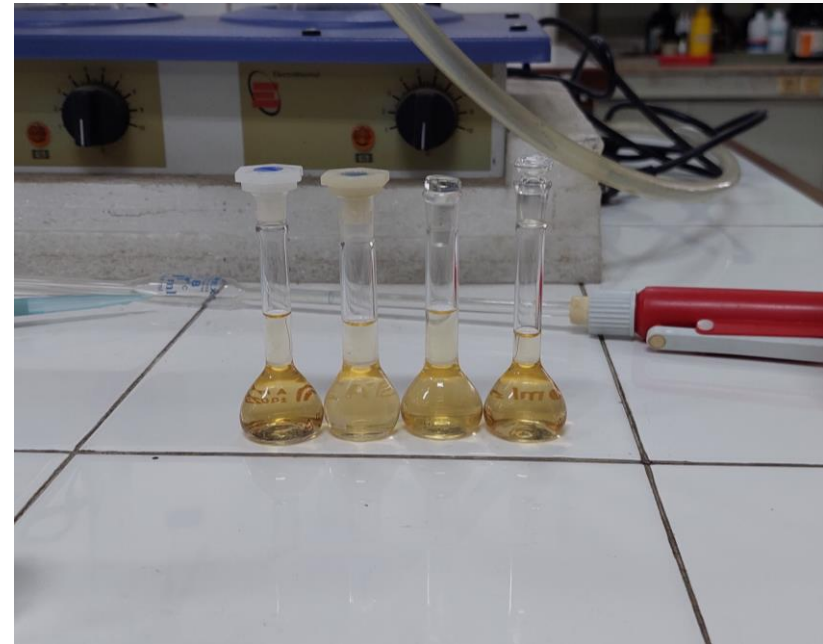


Figure 4. Sample solution

# Appendix

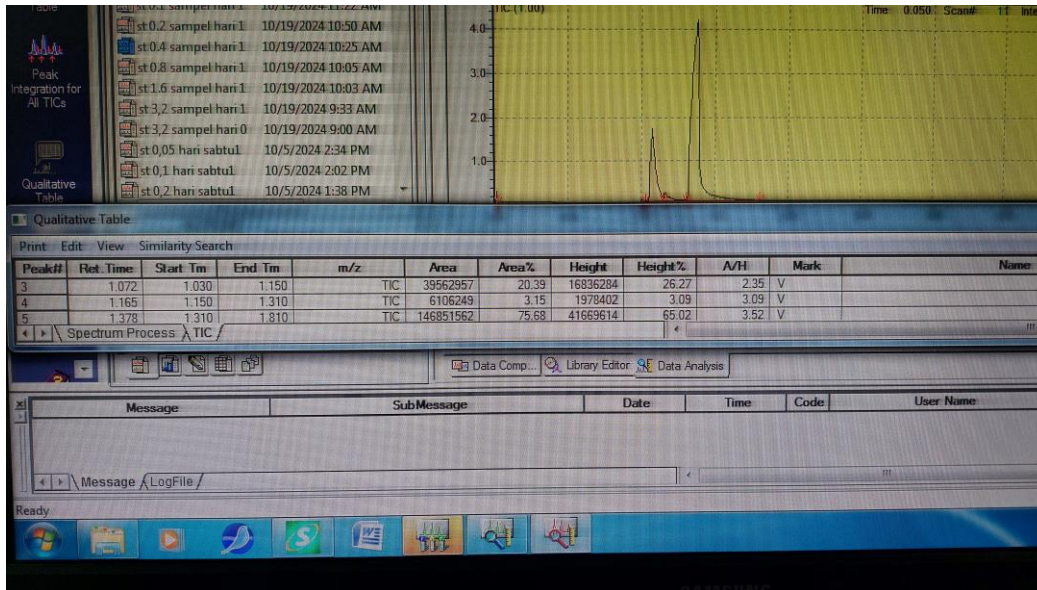


Figure 5. Chromatogram reading of calibration standard

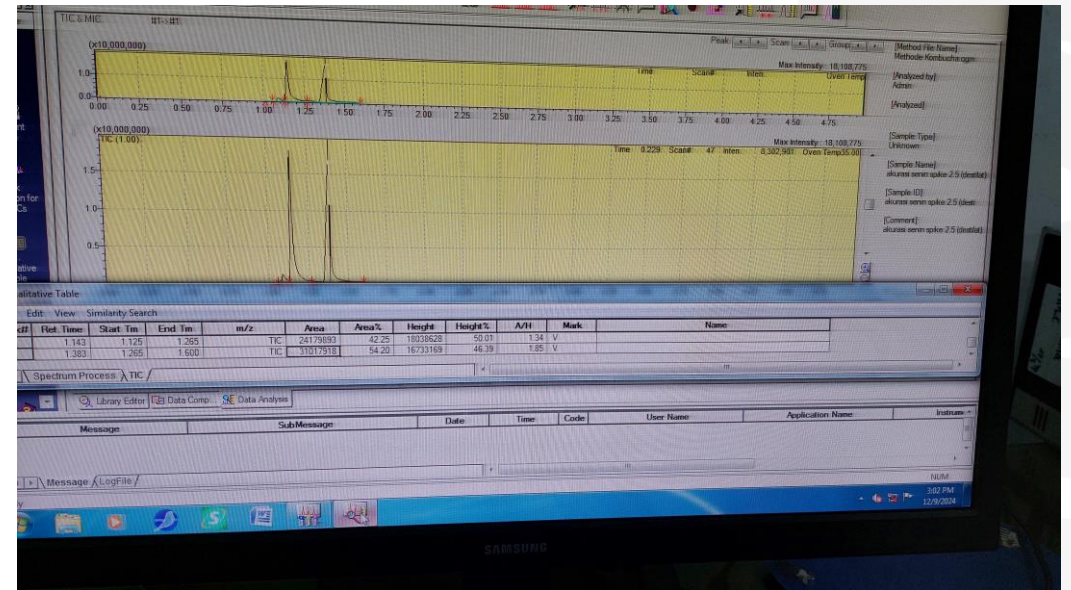


Figure 6. Chromatogram reading of spiking solution

# Appendix

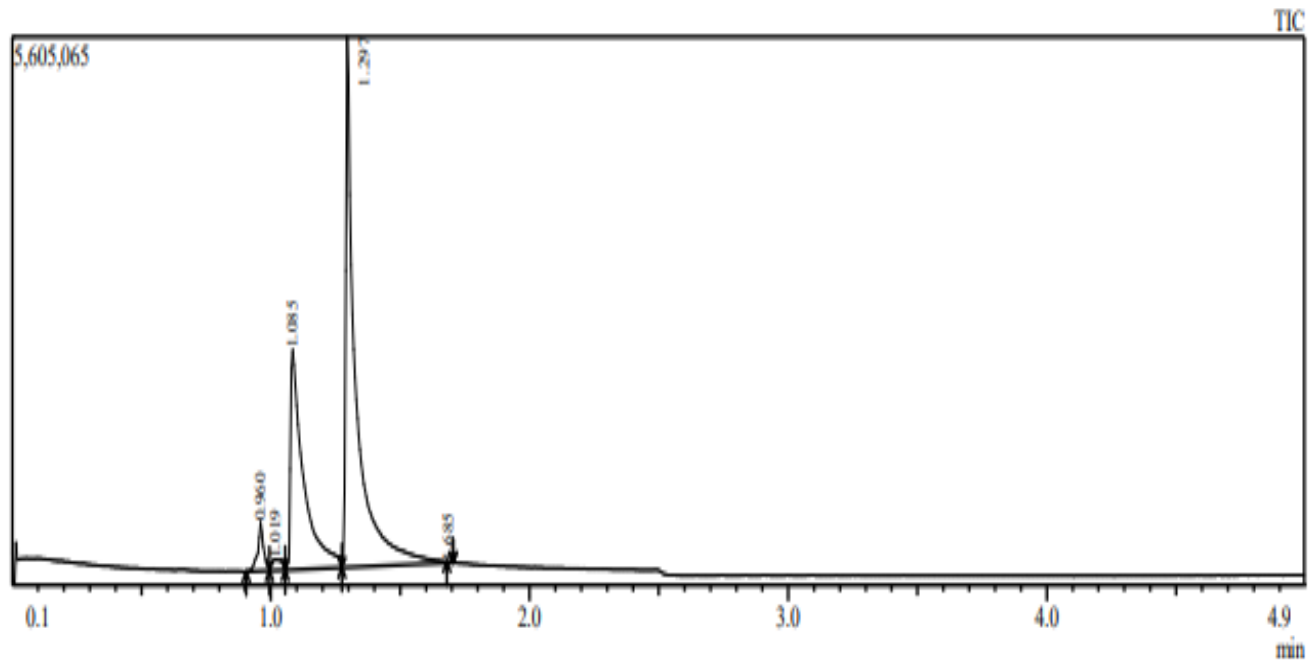


Figure 7. Chromatogram of Sample

# Appendix

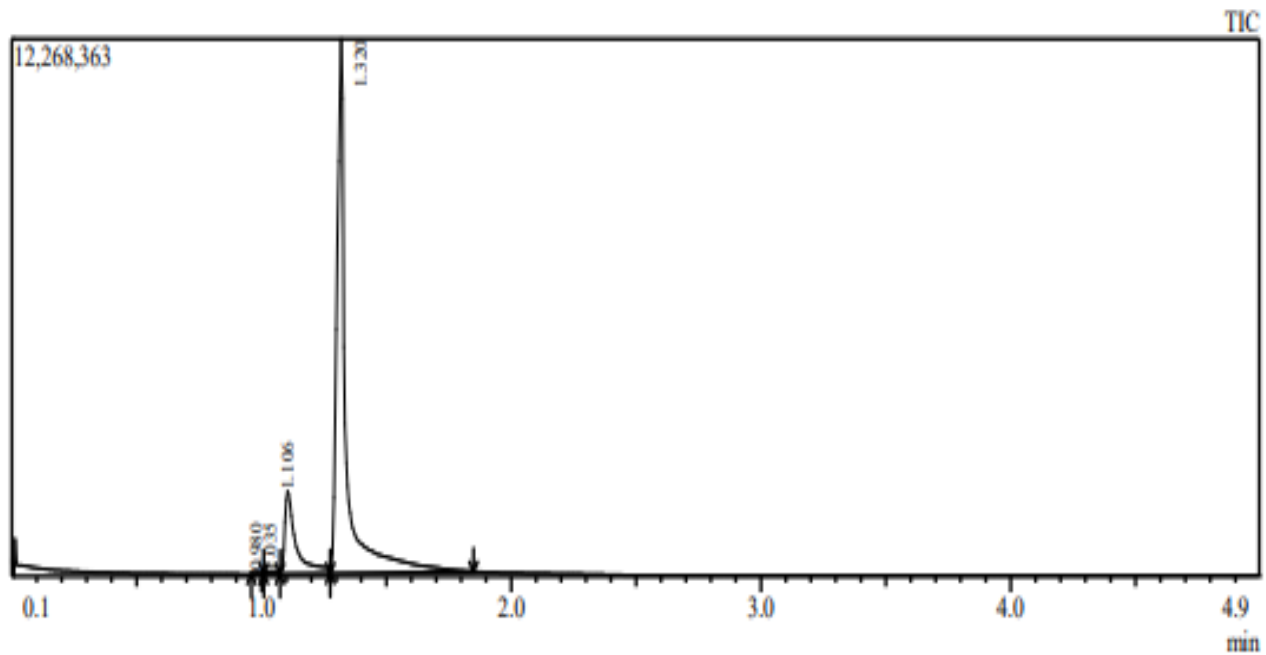


Figure 8. Chromatogram of standard (0.2% ethanol + 0.5% 1-propanol)

# Appendix

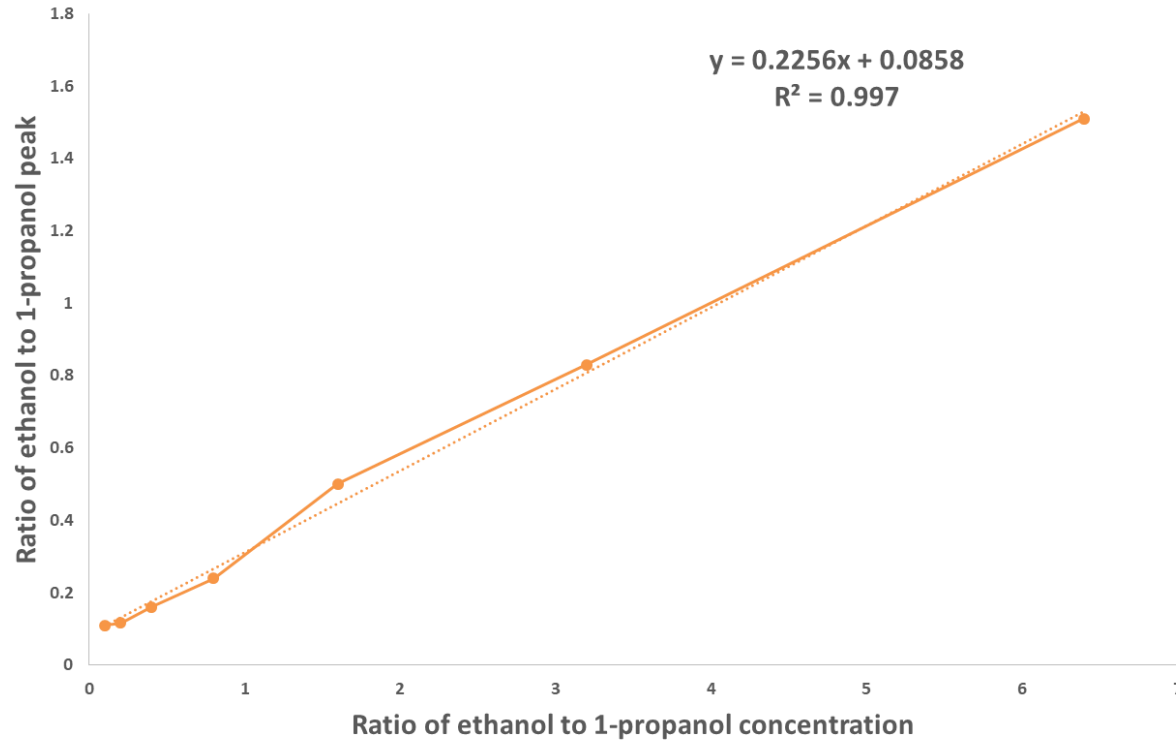


Figure 8. Calibration curve of ethanol-to-1-propanol peak area ratio versus concentration ratio, demonstrating method linearity for trace ethanol quantification

# Appendix

<b>Replication</b>	<b>Content (% w/v)</b>
<b>1</b>	1.23958
<b>2</b>	1.26099
<b>3</b>	1.19897
<b>Average</b>	1.23318
<b>SD</b>	0.0315
<b>% RSD</b>	2.5543
<b>CV Horwitz</b>	1.9378
<b>Horwitz Ratio</b>	1.3

Figure 9. Precision of the method showed good repeatability

# Appendix

<b>Spike Concentration</b>	<b>% Recovery</b>
0.10%	97.2733
	91.1222
0.50%	98.3792
	91.8098
2.50%	99.2127
	90.8458
<b>Average</b>	94.7738

Figure 10. Accuracy of the GC–MS method for ethanol determination in kombucha based on spike recovery at halal-relevant concentration levels

# Appendix

Concentration (%b/v)	Area	Status
3.20%	134425562	Detected
1.60%	60705237	Detected
0.80%	26812651	Detected
0.40%	12953636	Detected
0.20%	5786695	Detected
0.10%	2824572	Detected
0.05%	2134208	Detected
0.0250%	678151	Detected
0.0125%	412463	Detected
<b>R<sup>2</sup></b>	0.9963	
<b>LOD</b>	0.0025 % b/v	0.0032 % ABV
<b>LOQ</b>	0.0086 % b/v	0.0109 % ABV

Figure 11. LOD and LOQ results confirming the method's sensitivity for detecting trace ethanol in compliance with halal thresholds





# Thank you