MALAYSIAN JOURNAL OF ANALYTICAL SCIENCES

Journal homepage: https://mjas.analis.com.my/

Research Article

Determination of trace ethanol levels in kombucha by GC–MS for halal verification

Wahyu Utami* and Maryam Dzakiyah Muthiatulloh

Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Sukoharjo, Jawa Tengah 57169, Indonesia

*Corresponding author: wahyu.utami@ums.ac.id

Received: 19 May 2025; Revised: 20 August 2025; Accepted: 28 September 2025; Published: 16 October 2025

Abstract

Ethanol, a by-product of the fermentation process in kombucha, is a crucial factor in assessing halal compliance, particularly in Muslim-majority countries. This study employed gas chromatography-mass spectrometry (GC–MS) with direct injection to quantify ethanol levels in kombucha, thereby providing high selectivity and specificity. The analysis employed a GC system featuring an Rtx-1MS column with a split ratio of 10:1, in conjunction with an MS system operating in scan mode across a mass range of 20.0 to 100.0 amu. Method validation was performed in accordance with the Association of Official Analytical Collaboration (AOAC) International guidelines, ensuring adherence to the Standard Method Performance Requirements (AOAC SMPR 2016.001) for ethanol determination in kombucha. The parameters assessed included selectivity, specificity, linearity, precision, accuracy, and the limits of detection and quantification (LOD/LOQ). The method demonstrated excellent linearity ($R^2 > 0.990$) over an ethanol concentration range of 0.05% to 3.2%. Furthermore, it exhibited a precision of 2.55% relative standard deviation (RSD), an accuracy ranging from 97% to 99%, and a limit of quantification (LOQ) of 0.001% alcohol by volume (ABV). The method met all the AOAC Standard Method Performance Requirements. Analysis of two kombucha products available on Indonesian e-commerce platforms revealed ethanol levels that exceeded halal standards. For instance, the Indonesian Council of Ulama (MUI) specifies a maximum limit of 0.5% ABV, while the Department of Islamic Development Malaysia (JAKIM) sets a threshold of 1% ABV.

Keywords: ethanol, kombucha, GC–MS, halal verification, method validation

Introduction

Kombucha, a traditional fermented drink, has gained considerable popularity among fermented foods and beverages. It is produced by fermenting a sweetened tea medium with a symbiotic culture of bacteria and yeast (SCOBY), which inhibits the growth of other microorganisms [1]. SCOBY, a cellulose-based biofilm containing diverse microbial communities, serves as the starter culture for the fermentation process [2]. Originating in northeastern China around 220 BCE, kombucha was introduced to Japan in 414 CE as a health beverage. Trade networks subsequently facilitated the spread of kombucha to other regions, particularly Eastern Europe and Russia. Its popularity has fluctuated over time, notably following World War II. In 2017, retail sales of kombucha and other fermented beverages increased by 37.4% [3]. Global kombucha sales that year were valued at approximately $1.5 billion and are projected to reach $5.45 billion by 2025 [4-5].

Kombucha is widely recognised for its numerous health benefits as a functional drink, in addition to its traditional role as a beverage [6, 7]. Its antioxidant properties are associated with various therapeutic advantages [1, 8], including the potential to inhibit cancer by reducing angiogenesis through the alteration of angiogenic stimulators [9, 10]. Additionally, several studies indicate that kombucha possesses hepatoprotective properties by mitigating oxidative stress, a significant factor in the pathogenesis of liver disease [6, 11, 12]. Furthermore, kombucha has been linked to lower cholesterol and blood pressure levels [15] and has been shown to reduce blood glucose levels in individuals with diabetes [13, 14].

The fermentation process in kombucha, as in other

fermented beverages, produces ethanol. In the United States, kombucha is classified as a non-alcoholic beverage if its alcohol by volume (ABV) is less than 0.5%. By contrast, the United Kingdom and the European Union have a higher limit of 1.2% ABV for non-alcoholic beverages [16]. Clear regulations defining kombucha as either an alcoholic or a non-alcoholic beverage, along with proper labelling, are crucial as the beverage's popularity grows [17]. The Indonesian Council of Ulama (MUI) is responsible for ensuring the halal status of products in Indonesia, a predominantly Muslim country. According to MUI Fatwa No. 10 of 2018, a beverage must not contain more than 0.5% ethanol to be considered halal. In contrast, Turkey has a more stringent limit of less than 0.3% ABV [18, 19]. However, the Department of Islamic Development Malaysia (JAKIM) permits natural fermentation to produce ethanol levels below 1% ABV, provided that the final product is not intoxicating. The Indonesian Council of Ulama (MUI) defines halal products as those manufactured in accordance with Islamic law and free from haram (prohibited) ingredients [21]. For Muslims, adhering to halal regulations is considered a fundamental religious duty that impacts not only their spiritual well-being but also their safety and quality of life [22]. From a religious perspective, maintaining halal compliance is also crucial for safeguarding public health and consumer interests [23].

Multiple studies have demonstrated that kombucha frequently contains ethanol levels exceeding the permitted limits for non-alcoholic beverages [24]. For example, kombucha samples acquired in Carmel, USA, had ethanol concentrations ranging from 0.11% to 2.18% alcohol by volume (ABV) [25]. Other research has found that different flavours exhibited ABV levels from 0.03% to 1.63% [26]. In British Columbia, kombucha products often had more than 1% ABV, with the highest levels found in samples from farmers' markets, restaurants, and processors [27, 28]. Building upon these findings, the present study aims to determine whether kombucha products sold on Indonesian online marketplaces comply with the halal standards established by the Department of Islamic Development Malaysia (JAKIM) and the Indonesian Council of Ulama (MUI). This is a crucial issue in a country with a predominantly Muslim population.

Headspace sampling was predominantly used in earlier AOAC-compliant studies for ethanol quantification [25, 26]. The critical step of sample preparation significantly impacts the analytical accuracy of ethanol determination in kombucha [19]. To avoid the need for headspace instrumentation, this study utilised centrifugation and filtration as sample preparation methods, followed by direct injection for gas chromatography-mass spectrometry (GC–MS) analysis. Mass spectrometry was selected for its high sensitivity and selectivity in ion separation and detection, based on the mass-to-charge ratio (m/z) of ions. These techniques were chosen for their ease of use and sensitivity.

Materials and Methods

Materials and equipment

This study utilised an internal standard of 1-propanol (EMSURE®, Merck) and an external standard of ethanol (EMSURE®, Merck). Samples of distilled water and kombucha tea, specifically 'Sample A' (original flavour) and 'Sample B' (passion fruit flavour), were sourced from two Indonesian e-commerce platforms.

For this study, a gas chromatograph and a mass spectrometry detector (Shimadzu GC–MS-QP2010S, Serial No. C703844) with an Rtx-1MS column (30 m x 0.25 mm ID x 0.25 µm df) were employed. Other equipment included a centrifuge, volumetric flasks (10 mL and 50 mL), micropipettes (100 µL and 1000 µL), pipettes (5 mL and 10 mL), a vortex mixer, and a GC micro syringe (Model 801 NN 80135, Hamilton).

Preparation of ethanol stock solution

A 3.15 mL aliquot of the ethanol standard was measured using a 5.0-mL pipette and then diluted to a final volume of 50 mL with distilled water to prepare a 5% w/v ethanol stock solution.

Preparation of internal standard stock solution

A 3.10 mL volume of 1-propanol standard was added to a 50-mL volumetric flask using a 5.0-mL pipette. The flask was then filled to a capacity of 50 mL with pure water to prepare a stock solution of 5% w/v internal standard.

Preparation of calibration solution

Seven ethanol concentration levels ranging from 0.05% to 3.20% w/v were prepared through serial dilutions of the stock solution. A 1 mL solution of a 5% w/v internal standard was diluted by mixing it with distilled water in a 10-mL volumetric flask, then made up to volume, yielding a final concentration of 0.5% w/v for the internal standard.

Preparation of Kombucha samples for ethanol analysis

The kombucha sample was initially stored at room temperature prior to opening. A 10 mL sub-sample from each sample were then transferred into a 15-mL centrifuge tube and centrifuged at 5000 rpm for a duration of 10 minutes. Subsequently, 5.0 mL of the supernatant was transferred into a 10-mL volumetric flask, 1 mL of 5% w/v of the internal standard solution was used, and the mixture was diluted with distilled

water to a final concentration of 0.5% w/v. The flask was sealed and vortexed to ensure homogeneity. The solution was then filtered to eliminate the potential matrix and transferred into a vial [26].

Measurement of ethanol levels

The GC–MS system was filled with a 1.0 µL aliquot of each sample and a standard solution. **Table 1** lists the operating parameters of the GC–MS system.

Optimisation and method validation

Method optimisation was conducted in accordance with the guidelines for single laboratory validation set by AOAC International (AOAC SMPR 2016.001) and the Indonesian National Standard (SNI) [25, 29].

Validation parameters

The analytical performance characteristics, such as selectivity, specificity, linearity, precision, accuracy, and the limits of detection (LOD) and quantification (LOQ), as outlined in the USP and ICH guidelines, provided the foundation for validation.

Selectivity and specificity

The chromatograms of samples and standards were evaluated for the distinct resolution of ethanol and 1-propanol peaks, devoid of any interfering signals. The method was considered selective and specific if no co-eluting peaks were detected at retention times associated with the analytes [25].

Linearity

Seven calibration curves were created using stock solutions with concentrations ranging from 0.05% to 3.2% w/v, and this process was repeated three times on different days. These calibration curves were generated by calculating the ratio of the signal response of the 1-propanol internal standard to that of ethanol. The calibration curves' linearity was examined using linear regression. An acceptable coefficient of determination (R^2) was one that was higher than 0.990 [29].

Precision

On two different days, samples were prepared in triplicate and analysed. The intra-day (repeatability), inter-day (intermediate precision), and overall precision of the analytical method were then statistically assessed. The precision of the method was assessed by computing the Horwitz Ratio (HorRat) and the relative standard deviation of repeatability (RSDr). HorRat values between 0.5 and 2.0 are acceptable according to the AOAC International guidelines. Additionally, the Standard Method Performance Requirements (SMPR 2016.001) for determining the amount of ethanol in kombucha state that samples with an ethanol content between 0.1 and 2.0% ABV can have an RSDr of 4% [25-26].

Table 1. Operational parameters of the GC–MS system used for trace ethanol detection in kombucha samples for halal verification

No.	GC Parameters	Conditioning
1	Column	Rtx-1MS (30 m x 0.25 mmID x 0.25 um 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 µL
7	Split ratio	10:1
8	Carrier gas	Helium
9	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

Accuracy

A common addition (spiking) protocol was used to assess the method's accuracy. An ethanol standard (>99.8% purity) was added to a blank kombucha matrix at three different concentrations: 0.10%, 0.50%, and 2.50% (w/v). The Indonesian Council of Ulama (MUI) set a limit of 0.5% ethanol for halal certification, and these concentrations were chosen to fall within that range. On two different days, the analyses were conducted twice. The accuracy was evaluated using the percentage recovery. According to the AOAC International guidelines, a recovery range of 97% - 102% is considered acceptable as per SMPR 2016.001 [26].

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were calculated from a series of low-concentration calibration curves (0.0125%–3.2% w/v). Using the standard deviation of the response and the slope of the linear regression line, the LOD and LOQ were statistically estimated [30, 31] as follows:

$$\text{LOD} = \frac{3.3\ x\ b}{SD} \qquad \text{(eq. 1)}$$

$$\text{LOQ} = \frac{10\ x\ b}{SD} \qquad \text{(eq. 2)}$$

b = slope of the calibration curve, and SD = standard deviation of the regression line

Results and Discussion

Validation of the analytical method

The chromatogram showed that the method was specific because there was no interference from other matrix components [32]. Ethanol and 1-propanol (internal standard) exhibited distinct peaks without overlapping signals. This shows that the method can accurately identify and measure ethanol levels even when other compounds are present. The chromatograms of samples A (**Figure 1**) and B (**Figure 2**) show clear separations, indicating the analyte identification method's selectiveness and specificity.

The linearity was determined by analysing the correlation between the response ratio of the detector and the ethanol concentration ratio within the tested range [33]. The calibration curve produced a coefficient of determination (R^2) of 0.9970 (**Figure 3**), exceeding the minimum acceptable criterion of $R^2 \geq 0.990$ as specified in SNI 8965:2021 [29]. This R^2 shows that the method is reliable for measuring ethanol concentrations at different concentrations.

Precision was assessed in terms of repeatability within a single day and intermediate precision across two distinct days. The relative standard deviation for repeatability (RSDr) was 2.55%, and the Horwitz Ratio (HorRat) was 1.3 (**Table 2**). Both values fall within the acceptable limits stipulated by AOAC SMPR 2016.001 [25, 26], which are RSDr $\leqslant$ 4% and HorRat between 0.5 and 2.0. These results indicate that the proposed method exhibits good repeatability and reproducibility.

The accuracy of the method was assessed using spiked samples at different concentrations, as shown in **Table 3**. The initial recovery rates for each concentration ranged from 97% to 102%, satisfying the AOAC acceptance criteria (AOAC, 25-26). However, with an overall mean of 94.77%, the recovery values over several days were marginally below the threshold. Daily variability, which could be impacted by variables such as analyst performance, instrument stability, or sample degradation, could be the cause of this decline.

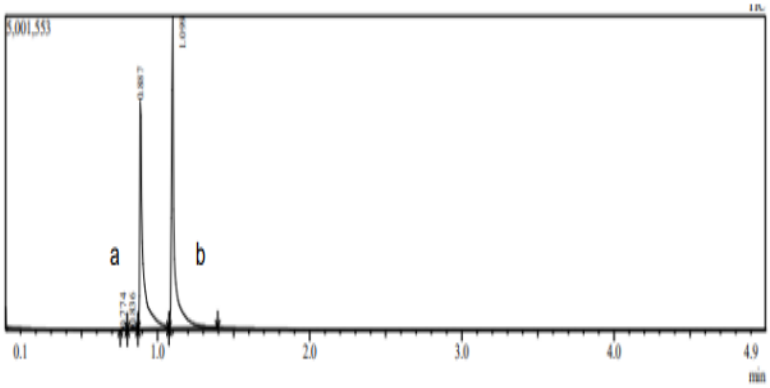

Figure 1. GC–MS chromatogram of sample A showing clear separation of ethanol (a) and 1-propanol (b) (internal standard) peaks for halal-compliance analysis

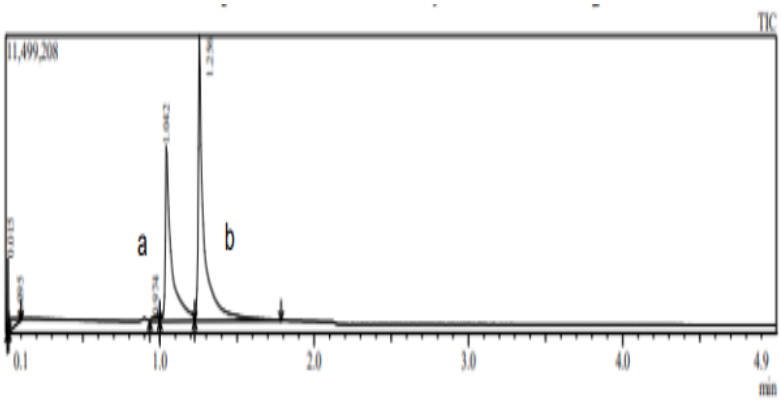

Figure 2. GC–MS chromatogram of sample B demonstrating the clear separation of the peaks for ethanol (a) and 1-propanol (b) (internal standard) for halal compliance analysis

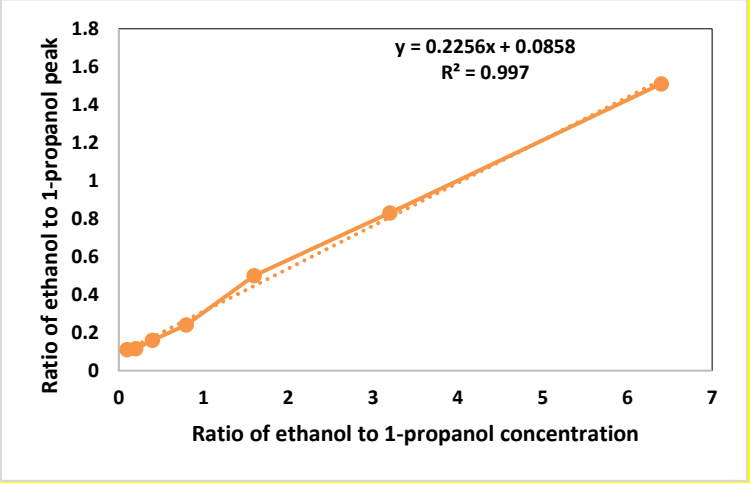

Figure 3. Calibration curve of ethanol-to-1-propanol peak area ratio versus concentration ratio, demonstrating method linearity for trace ethanol quantification

Table 2. The precision of the method showed good repeatability

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

The lowest concentration of a material that can be detected using a particular analysis technique is the LOD. The LOD and LOQ were calculated using the standard deviation of the response and the calibration curve slope. According to the data, the calculated LOD was 0.00259 w/v, or 0.00328% ABV, and the LOQ was 0.00864 w/v, or 0.0109% ABV. Neither of these values meets the AOAC SMPR 2016.001 criterion of LOQ $\leqslant$ 0.05% ABV. This indicates that the technique is sufficiently sensitive to identify trace ethanol levels, particularly when the ethanol threshold is less than 0.5% ABV, which is relevant to halal certification. The LOD and LOQ calibration curves are shown in **Table 4**.

Trace ethanol levels in Kombucha products

Two kombucha products, Sample A (original flavour) and Sample B (passion fruit flavour), were purchased online for analysis. As presented in **Table 5**, the trace ethanol levels in each product were evaluated. Sample A demonstrated an ethanol concentration exceeding 1% ABV, which surpasses the permissible limits set by both MUI ($\leqslant$0.5% ABV) and JAKIM (<1% ABV). Conversely, Sample B contained an ethanol concentration of <1% ABV, thereby meeting the requirements stipulated by JAKIM but exceeding the more stringent limits established by MUI.

The discovery of elevated ethanol levels in online kombucha products has raised concerns about their halal compliance. Continuous fermentation in kombucha can lead to increased ethanol levels over time, particularly when the products are not stored under refrigerated conditions. Talebi et al. reported similar findings, observing an increase in ethanol levels in kombucha stored at both room temperature (22°C) and elevated temperature (48°C) over a period of 60 days. Ethanol content exceeded 0.5% ABV even when stored under cold conditions [37].

Table 3. Accuracy of the GC–MS method for ethanol determination in kombucha based on spike recovery at halal-relevant concentration levels

	Spike Concentration	**% Recovery**
Day 1	0.10%	97.27
Day 2	0.10%	91.12
Day 1	0.50%	98.38
Day 2	0.50%	91.81
Day 1	2.50%	99.21
Day 2	2.50%	90.85
Average		94.77

Table 4. LOD and LOQ results confirming the method's sensitivity for detecting trace ethanol in compliance with halal thresholds

Concentration (%w/v)	**Peak 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
r^2	0.9963	
LOD	0.00259 % w/v	0.00328 % ABV
LOQ	0.00864 % w/v	0.0109 % ABV

Table 5. Quantified ethanol levels in two commercial kombucha samples from the Indonesian market

Replicate	Sample A (%ABV)	Sample B (%ABV)
1	1.24	0.58
2	1.26	0.61
3	1.20	0.62
Average	1.23	0.60
SD	0.03	0.02

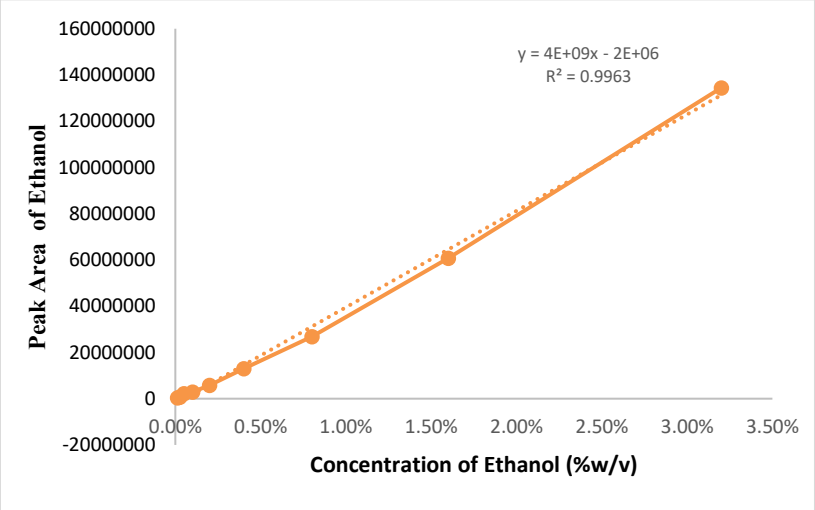

Figure 4. A linear calibration curve of the peak area of ethanol vs the concentration, for the determination of LOD and LOQ

Implications for halal certification and consumer safety

The variability in ethanol content underscores the need for reliable ethanol determination methods in the manufacture and regulation of kombucha and other fermented beverages. For Muslim consumers, the halal status of a product constitutes a fundamental religious consideration. Therefore, regular monitoring and accurate labelling are essential for supporting informed consumer choices and maintaining religious compliance [38].

From a public health perspective, it is also essential to label ethanol content. Even in small amounts, ethanol can pose risks to vulnerable populations, such as pregnant or breastfeeding women and infants, who may lack the liver enzymes necessary to metabolise alcohol [39]. Exposure to ethanol levels of >2% ABV can result in toxic effects in infants or children with low body weight, as well as in those with underdeveloped alcohol metabolism [40].

A GC–MS method for detecting ethanol in kombucha was successfully validated in this study. The validated method is selective, precise, accurate, and sensitive, and it does not require advanced sample preparation or specialised equipment, making it suitable for routine analysis in both laboratory and production settings. The findings suggest that some online kombucha products may contain ethanol levels that exceed halal alcohol limits, highlighting the need for ongoing monitoring, proper storage, and clear labelling. This research contributes to a deeper understanding of halal verification in fermented products and underscores the importance of analytical methods in ensuring religious compliance and public health safety.

Conclusion

This study aimed to establish and verify a gas chromatography–mass spectrometry (GC–MS) method for detecting ethanol trace levels in kombucha products. This method offers a practical and uncomplicated analytical approach that does not require the use of headspace equipment. The technique demonstrated satisfactory levels of specificity, linearity, precision, accuracy, and sensitivity, meeting the validation requirements of the

Association of Official Analytical Chemists (AOAC) and national standards.

The application of the validated method to commercially available kombucha products in the Indonesian online market revealed that the ethanol levels in the tested samples exceeded the halal thresholds set by both the Indonesian Council of Ulama (MUI) and the Department of Islamic Development Malaysia (JAKIM). Notably, one sample contained more than 1% ABV, whereas another sample exceeded the MUI limit of 0.5% ABV. These findings indicate that some Indonesian kombucha products do not consistently meet established halal standards, which raises concerns for Muslim consumers.

This study found that the ethanol level of kombucha increases with continuous fermentation. Manufacturers should closely monitor production and clearly label their products to ensure safety. The study also highlights the need for simple and trustworthy testing methods to verify whether fermented drinks meet halal standards. In countries like Indonesia, where most people are Muslim, it is not only a matter of following Islamic rules but also of public trust and a consumer's right to know if a product is halal.

Acknowledgement

This research was supported by Universitas Muhammadiyah Surakarta (UMS) through the Doctoral Grant Programme of the Institute for Research and Innovation (LRI) UMS, under contract number 95.26/A.3-III/LRI/IV/2025. The authors acknowledge the financial assistance provided by the institution.

References

1. Vitas, J., Malbasa, R., Grahovac, J., and Loncar, E. (2013). The antioxidant activity of Kombucha fermented milk products with stinging nettle and winter savoury. *Chemical Industry and Chemical Engineering Quarterly*, 19(1): 129-139.
2. Harrison K. and Curtin C. (2021). Microbial composition of scoby starter cultures used by commercial kombucha brewers in North America. *Microorganisms,* 9(5): 1-21.
3. Kapp J.M. and Sumner W. (2019). Kombucha: a systematic review of the empirical evidence of human health benefit. *Annals of Epidemiology,* 30: 66–70.
4. Grand View Research. (2018). Kombucha market size, share & trends analysis report by flavor (original, flavored), by distribution channel (supermarkets, health stores, online stores), by region, and cement forecasts 2018–2025. Grand View Research Inc., San Francisco, CA.
5. Fields, D. (2018, July 9). Interest is brewing in kombucha as a healthy alternative to beer and soda. Forbes. Retrieved July 9, 2018, from https://www.forbes.com/sites/mergermarket/2018/07/09/interest-brewing-in-kombucha-as-healthy-beer-soda-alternative/#7dc3599643f2/ [Accessed April 10, 2024].
6. Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S., and Sathishkumar, M. (2014). A review on kombucha tea–microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Food Science and Food Safety,* 13(4): 538-550.
7. Hafsari, A. R., and Farida, W. N. (2021). Karakteristik pH kultur kombucha teh hitam dengan jenis gula berbeda pada fermentasi batch-culture. Prosiding Seminar Nasional Biologi (SEMABIO), 6: 228-232.
8. Jayabalan R., Subathradevi P., Marimuthu S., Sathishkumar M., and Swaminathan K. (2008). Changes in free radical scavenging ability of kombucha tea during fermentation. *Food Chemistry,* 109: 227-234.
9. Jayabalan R., Chen P.N., Hsieh Y.S., Prabhakaran K., Pitchai P., Marimuthu S., Thangaraj P., Swaminathan K., and Yun S.E. (2011). Effect of solvent fractions of kombucha tea on viability and invasiveness of cancer cells characterization of dimethyl 2-(2-hydroxy-2-methoxypropylidine) malonate and vitexin. *Indian Journal of Biotechnology*, 10: 75–82.
10. Srihari T., Arunkumar R., Arunakaran J., and Satyanarayana U. (2013). Downregulation of signalling molecules involved in angiogenesis of prostate cancer cell line (PC-3) by kombucha (lyophilized). *Biomedicine Prev Nutrition*, 3: 53–58.
11. Bhattacharya S., Gachhui R., and Sil P.C. (2011). Hepatoprotective properties of kombucha tea against TBHP-induced oxidative stress via suppression of mitochondria-dependent apoptosis. *Pathophysiology,* 18: 221–234.
12. Wang Y., Ji B., Wu W., Wang R., Yang Z., Zhang D., Tian W. (2014). Hepatoprotective effects of kombucha tea: identification of functional strains and quantification of functional components. *Journal Sci Food Agriculture,* 94: 265–272.
13. Hiremath U.S., Vaidehi M.P., Mushtari B.J. (2002). Effect of fermented tea on the blood sugar levels of NIDDM subjects. *Indian Practice*, 55(7): 1-10.
14. Mendelson, C., Sparkes, S., Merenstein, D. J., Christensen, C., Sharma, V., Desale, S., Auchtung, J. M., Kok, C. R., Hallen-Adams, H. E., and Hutkins, R. (2023). Kombucha tea as an antihyperglycemic agent in humans with diabetes – a randomized controlled pilot

investigation. *Frontiers in Nutrition,* 10(1): 190–248.
15. Martínez Leal, J., Valenzuela Suárez, L., Jayabalan, R., Huerta Oros, J., and Escalante-Aburto, A. (2018). A review of the health benefits of kombucha nutritional compounds and metabolites. *CyTA – Journal of Food,* 16(1): 390–399.
16. Liu Y., Chan M., Ebersole B., Sy H., and Brown P.N. (2019). Determination of ethanol content in kombucha products by gas chromatography with flame ionization detection: a multilaboratory study. *Journal of AOAC International,* 102(3): 878–882.
17. International Alliance for Responsible Drinking (IARD). (2019). Beverage alcohol labeling requirements. Retrieved January 5, 2019. https://iard.org/science-resources/detail/Beverage-Alcohol-Labeling-Requirements/[Accessed June 25, 2024].
18. Majelis Ulama Indonesia. (2018). Fatwa Majelis Ulama Indonesia Nomor 10 Tahun 2018 tentang Makanan dan Minuman yang Mengandung Alkohol. https://halalmui.org/[Accessed April 4, 2025].
19. Majidah L., Gadizza C., and Gunawan S. (2022). Analysis of halal product development of kombucha beverages. *Halal Research Journal,* 2(1): 36-51.
20. Jabatan Kemajuan Islam Malaysia. (2011). Fatwa tentang alkohol dalam makanan, minuman, parfum dan ubat-ubatan. Muzakarah Jawatankuasa Fatwa Majlis Kebangsaan Bagi Hal Ehwal Ugama Islam Malaysia. https://e-smaf.islam.gov.my/e-smaf/index.php/main/mainv1/fatwa/pr/10281/[Accessed April 10, 2025].
21. Majelis Ulama Indonesia. (2003). Fatwa Majelis Ulama Indonesia Nomor 4 Tahun 2003 tentang Standarisasi Fatwa Produk Halal. Jakarta: Majelis Ulama Indonesia. https://halalmui.org/ [Accessed April 14, 2025].
22. Alzeer, J., & Abou Hadeed, K. (2016). Ethanol and its halal status in food industries. Trends in *Food Science & Technology,* 58: 14–20.
23. Roslan, R., and Tukiran, N. A. (2024). Analytical methods to authenticate fats and oils: Principles, comparison, and application in halal food products. *Malaysian Journal of Analytical Sciences,* 28(5): 1032–1047.
24. Chen C., and Liu B.Y. (2000). Changes in significant components of tea fungus metabolites during prolonged fermentation. *Journal Applied Microbiology*, 89: 834–839.
25. Ebersole B., Liu Y., Schmidt R., Eckert M., and Brown P.N. (2017). Determination of ethanol in kombucha products: single-laboratory validation, first action 2016.12. *Journal of AOAC International,* 100(3): 732–736.
26. Chan M., Sy H., Finley J., Robertson J., and Brown P.N. (2021). Determination of ethanol content in kombucha using headspace gas chromatography with mass spectrometry detection: single-laboratory validation. *Journal of AOAC International,* 104(1): 122–128.
27. McIntyre, L., and Jang, S. S. (2020). A study of alcohol levels in kombucha products in British Columbia. Environmental Health Services, BC Centre for Disease Control. Vancouver, BC.
28. Jang S.S.I.K., McIntyre L., Chan M., Brown P.N., Finley J., and Chen S.X. (2021). Ethanol concentration of kombucha teas in British Columbia, Canada. *Journal of Food Protection*, 84(11): 1878–1883.
29. Badan Standardisasi Nasional. (2021). SNI 8965:2021 Metode Deteksi dan Kuantifikasi Etanol pada Produk Minuman. Badan Standardisasi Nasional, Jakarta.
30. Badan Pengawas Obat dan Makanan Republik Indonesia (BPOM). (2013). Operational Guidelines for the Implementation of Good Manufacturing Practice 2012. https://www.pom.go.id/[Accessed April 20, 2025].
31. Utami W., Araiz F., Hanwar D. (2017). Validation of the method for determination of acetosal and dipiridamol levels by reverse phase densitometric KLT. *University Research Colloquium*, 6: 483–488.
32. Yuwono, M., Ayuningtyas, R., and Primaharinastiti, R. (2021). The HPLC methods for identification and determination of nitrofuran metabolite levels in shrimp meatballs. *Indonesian Journal of Pharmacy and Pharmaceutical Sciences*, 8(2): 117–124.
33. Wenclawiak, B., and Hadjicostas, E. (2010). Validation of analytical methods – to be fit for the purpose. In Wenclawiak, B. W., Koch, M., & Evsevios, H. (Eds.), *Quality Assurance in Analytical Chemistry*, 215–245.
34. Hanwar, D., Handayani, V. R., and Suhendi, A. (2020). Validasi metode HPLC untuk analisis kurkumin pada ekstrak rimpang temulawak (Curcuma xanthorrhiza Roxb.). Prosiding *University Research Colloquium*, 243–248.
35. Andi Suhendi. (2016). Validation of high-performance liquid chromatography method for the determination of levonorgestrel and ethinyl estradiol in tablets. *Journal Pharmaceutical Sciences & Research*, 8(1): 6–9.
36. Sulistiawaty, L., and Solihat, I. (2022). Kombucha: fisikokimia dan studi kritis tingkat kehalalan. *Warta Akab*, 46(1): 21–27.
37. Talebi, M., Frink, L. A., Patil, R. A., and Armstrong, D. W. (2017). Examination of the varied and changing ethanol content of

commercial kombucha products. *Food Analytical Methods,* 10: 4062-4067.
38. Riaz, M. N., and Chaudry, M. M. (2004). Halal Food Production. CRC Press.
39. Mamluk, L., Edwards, H. B., Savović, J., Leach, V., Jones, T., Moore, T. H. M., Ijaz, S., Lewis, S. J., Donovan, J. L., Lawlor, D., Smith, G. D., Fraser, A., and Zuccolo, L. (2017). Low alcohol consumption and pregnancy and childhood outcomes: time to change guidelines indicating apparently 'safe' levels of alcohol during pregnancy? *BMJ Open*, 7(7): e015410.
40. Denny, L., Coles, S., and Blitz, R. (2017). Fetal alcohol syndrome and fetal alcohol spectrum disorders. *American Family Physician,* 96: 515–522.