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Research Article

Halal Authentication and Metabolite Mapping of Kombucha Products via Gas Chromatography-Mass Spectrometry (GC-MS) and Chemometric Analysis

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Abstract

The presence of ethanol in fermented beverages is a critical factor in the halal certification process. One of the key parameters for verifying the halal status of such products is the quantification of ethanol content. Kombucha tea, a fermented beverage produced from sugared tea and a Symbiotic Culture of Bacteria and Yeast (SCOBY), naturally contains ethanol as a byproduct of fermentation. This study aims to determine the ethanol content and differentiate the metabolite profiles of kombucha tea using a non-targeted metabolomics approach, based on variations in tea type, storage temperature, and duration. Ethanol levels were measured by gas chromatography, and metabolite profiling was conducted by gas chromatography–Mass Spectrometry (GC-MS), followed by Principal Component Analysis (PCA) to visualize compositional differences and identify characteristic compounds. The results indicated that tea type significantly influenced ethanol production. The ethanol content of kombucha prepared with black tea, green tea, and white tea was 0.1126% w/w ± 0.0003 v/v, 0.1708% w/w ± 0.0053 v/v, and 0.1301% w/w ± 0.0043 v/v, respectively. Green tea kombucha, which exhibited the highest ethanol content, was selected for storage analysis. During storage, ethanol levels increased slightly to 0.1789% w/w ± 0.0008 v/v in the first week, followed by a gradual decline to 0.1478% w/w ± 0.0071 v/v by the fourth week. Metabolomic profiling revealed distinct differences in secondary metabolite composition among the three tea variants, as evidenced by non-overlapping PCA groupings. Key discriminant compounds identified included ethyl acetate, ethyl octanoate, ethylamine, and (E)-2-decenal, which are proposed as characteristic markers for kombucha derived from black, green, and white teas. These findings contribute to understanding kombucha's biochemical diversity and support halal verification through ethanol quantification and metabolite-based authentication.

Keywords: Chemometric, ethanol, GC-MS, kombucha, metabolite profile

1. INTRODUCTION

The presence of alcohol in food and beverage products has become increasingly prevalent due to the widespread application of fermentation technologies. Products such as kombucha, kefir, yoghurt, and wine naturally generate ethanol as a byproduct of microbial metabolism. For Muslim consumers, the presence of alcohol—regardless of its origin or concentration—raises significant concerns regarding the halal status of such products. According to the OIC/SMIIC 1:2019 standard and MUI Fatwa No. 10 of 2018, ethanol derived from wine (khamr) is strictly prohibited (haram). However, ethanol present in non-wine-based fermented products is permissible up to a threshold of 0.5% (v/v), provided it is not harmful to health [1,2].

Kombucha is a fermented tea beverage produced through the metabolic activity of a Symbiotic Culture of Bacteria and Yeast (SCOBY) in

a sugared tea medium. In Indonesia, kombucha has gained popularity due to its perceived health benefits, including antioxidant, antimicrobial, and probiotic properties [3,4]. Despite its health-promoting potential, the ethanol content resulting from fermentation necessitates careful evaluation to ensure compliance with halal standards. Previous studies have shown that ethanol levels in kombucha vary depending on factors such as fermentation time, substrate composition, and microbial dynamics [56].

Beyond ethanol quantification, the metabolite profile of kombucha offers valuable insights into its functional properties. Metabolomics, the comprehensive analysis of small-molecule metabolites in biological systems, enables the identification of bioactive compounds and potential biomarkers. Non-targeted metabolomics, employing techniques such as Gas Chromatography–Mass Spectrometry (GC-MS) [7], Liquid Chromatography–Mass Spectrometry (LC-MS)[8], and Capillary Electrophoresis–Mass Spectrometry (CE-MS) [9], allows for the broad-spectrum detection of known and unknown metabolites. These analytical platforms, when combined with chemometric tools like Principal Component Analysis (PCA), facilitate the interpretation of complex datasets and the differentiation of samples based on their chemical composition [10].

In the study conducted by Djeni et al. (2020), [11] the metabolite profiles of palm wine derived from three different palm species collected from Ganding Beach were analyzed using Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS) [11]. The results revealed that palm wine was enriched with various metabolites, including organic acids (e.g., lactic acid), hexose deoxy sugars (e.g., fucose), sugar alcohols (e.g., sorbitol), and sugar acids (e.g., gluconic acid), with a total of 21 distinct metabolites identified across the three palm wine types. In a separate study, Kim et al. (2019) [12] investigated the metabolite profiles of fermented tomatoes inoculated with different lactic acid bacteria (LAB) strains using Gas Chromatography–Mass Spectrometry (GC-MS) combined with Principal Component Analysis (PCA). The PCA score plot, derived from 2,554 signal features, and the biplot of 18 identified metabolites demonstrated a clear separation into three distinct groups. Citric acid and malic acid were associated with the group inoculated with *Lactobacillus fermentum* (LF), *Bifidobacterium longum* (BL), and *Pediococcus pentosaceus* (PP), In contrast, lactic acid, succinic acid, and fructose were linked to *Lactobacillus plantarum* (LP) and *Leuconostoc mesenteroides* (LM). In contrast, *Lactobacillus brevis* (LB) was uniquely associated with erythritol [12].

In this study, we aim to determine the ethanol content and characterize the volatile metabolite profiles of kombucha beverages prepared using different types of tea. Ethanol analysis will be conducted using the SNI 8965:2021 gas chromatography method, while metabolite profiling will be performed using GC-MS followed by PCA-based chemometric analysis. The findings are expected to contribute to the halal authentication of kombucha products and provide a deeper understanding of their biochemical diversity.

2. RESEARCH METHODS

Instruments and Materials

The SCOBY starter was bought from Rumah Fermentasi (a kombucha fermentation company in South Tangerang City, Banten). The reagents used in this research were standard ethanol and 1-propanol of chromatographic quality (Merck), distilled water, and dried tea leaves (black, green, and white tea) from PT Perkebunan Nusantara, and sucrose obtained from the local market (**Figure 1**).

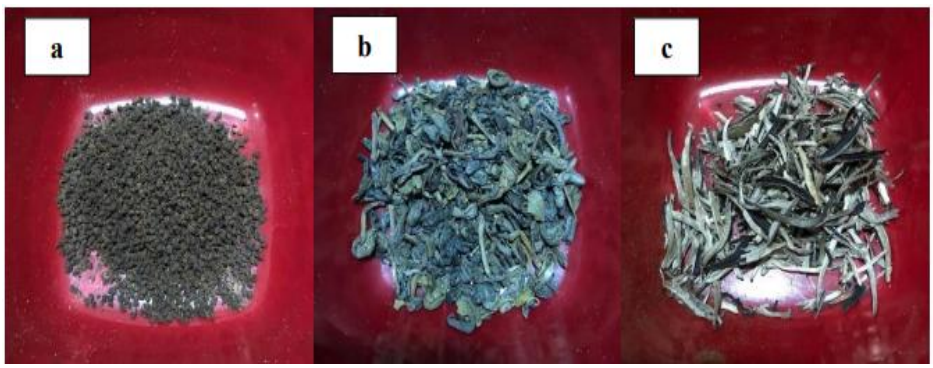

Figure 1. Types of tea sample (a) Black Tea (b) Green Tea (c) White Tea

Fermenting the kombucha

A sucrose medium (sucrose and distilled water in a ratio of 1:10) was formulated for kombucha fermentation. Kombucha fermentation is done by mixing 3 grams of tea leaves (1 tablespoon) into 1000 mL of boiled water heated to 100 °C for 15 minutes,

then filtering and cooling to room temperature. The starter culture, SCOBY (Symbiotic Culture of Bacteria and Yeast), is placed in the tea solution and covered with a sterile cloth. This process is carried out under sterile conditions. The tea and SCOBY mixture was fermented for 10 days at room temperature [6].

Measurement of Alcohol (ethanol) content by GC-FID

The ethanol content in the sample was quantified using gas chromatography equipped with a flame ionization detector (GC-FID) [13]. The analysis was conducted on a [specify GC-FID Trace 1310 (Scientific] fitted with a polar capillary column (e.g., DB-WAX, 30 m × 0.25 mm i.d., 0.25 µm film thickness). Nitrogen was employed as the carrier gas at a constant flow rate of 1.0 mL/min. The injector and detector temperatures were maintained at 250 °C and 280 °C, respectively. The oven temperature was initially set at 40 °C, held for 2 minutes, then ramped to 200 °C at 10 °C/min and held for 5 minutes.

Samples were prepared by diluting [1.0 mL] of the test solution with [9.0 mL] of deionized water. An internal standard (e.g., 1-propanol at 0.1% v/v) was added to each sample and calibration standard to ensure quantification accuracy. A 1.0 µL aliquot of each solution was injected in split mode (split ratio 20:1).

Quantification was performed using an external calibration curve constructed from ethanol standard solutions at concentrations ranging from [e.g., 0.1% to 10% v/v]. The peak area ratio of ethanol to the internal standard was plotted against the known ethanol concentrations. The ethanol content in the samples was calculated based on the linear regression equation derived from the calibration curve ($R^2 > 0.995$). All measurements were performed in triplicate, and the method was validated for linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ) in accordance with ICH guidelines.

Measurement of alcohol content was carried out using a GC-FID trace 1310 *Thermo Scientific* with gradient oven temperature program conditions where the initial temperature of 35 °C was held for 8 minutes, then increased to 150 °C, helium carrier gas with a flow rate of 1.4 mL/minute, injection volume 0.5 µL, split ratio 30 :1, HP5MS column (30 m; 0.25 µm; 0.250 mm), injector temperature 180 °C, detector temperature 260 °C, ion source temperature 230 °C with mass range scanning mode (m/z: 20.00 - 550.00)[14]. Determination of ethanol content in kombucha samples is calculated using the following formula (1):

$$[\text{Etanol}]\ (\%\text{b/b}) = \frac{(\frac{LA(ec)}{LA(pc)} - a)/b}{B\ (sampel)}\ x\ V(ld) \quad \ldots\ldots(1)$$

LA (ec) : The area of ethanol in the sample
LA (pc) : Area of 1-propanol in the sample
a : Intercept of the ethanol standard curve
b : Slope of the ethanol standard curve V (ld) : Final volume of distillate (25 mL)
B (sample) : Sample weight (g)
(SNI 8965:2021)[15]

Metabolite Profiling of Kombucha Tea by GC-MS

Metabolite profiling of kombucha tea samples was performed using a Shimadzu QP 2010 gas chromatography–mass spectrometry (GC-MS) system equipped with a headspace autosampler. The headspace method was employed to extract volatile compounds prior to injection. Separation was achieved using a [specify column type, e.g., DB-WAX, 30 m × 0.25 mm i.d., 0.25 µm film thickness], with helium as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature program was optimized to ensure resolution of key metabolites [12].

Chromatographic peaks and corresponding mass spectra were analyzed using the Wiley7 and NIST libraries. Compound identification was based on matching retention times, molecular structures, and spectral similarity scores. The total number of metabolites detected in each sample was recorded, and characteristic compounds were identified based on the highest relative peak areas. Compounds with area percentages significantly higher than those of others were considered dominant and representative of the kombucha tea metabolite profiles [11].

Chemometric Analysis by Principal Component Analysis (PCA)

Multivariate data analysis was performed using Principal Component Analysis (PCA) to evaluate metabolite distribution patterns across four kombucha tea samples. The input data consisted of compound profiles expressed as relative peak area percentages obtained from GC-MS analysis. PCA was conducted using Minitab version 19 software to simplify the multidimensional dataset and facilitate pattern recognition among the samples. The resulting score plot and biplot visualizations provided insights into the similarities and differences in metabolite composition among the kombucha samples prepared with different tea types [12,16].

3. RESULTS AND DISCUSSION

The Results of Kombucha Fermentation

Kombucha fermentation was conducted in sterile glass jars, selected for their inert properties and ability to minimize chemical interactions and microbial contamination, particularly from acidic compounds formed during fermentation. Although the typical fermentation period ranges from 7 to 14 days,

in this study, fermentation was terminated on the 10th day in accordance with the Fermentation House Company's standard operating procedures.

Three types of tea—black, green, and white—were used as substrates, each contributing distinct coloration to the resulting kombucha (**Figure 2**). Kombucha derived from black tea exhibited a darker hue due to its higher tannin content, which also influenced the appearance of the SCOBY [17]. The SCOBY surface developed brown or yellowish spots, attributed to yeast filaments adhering and hanging from the matrix.

Kombucha made from black tea is darker than kombucha from green or white tea. This is because black tea has a higher tannin content, which can give the kombucha a darker color [17]. Apart from that, the color of the SCOBY turns dark because the yeast sticks and hangs like threads, thus forming brown or yellowish spots on the surface of the SCOBY. The microbial composition of the SCOBY culture utilized in this study is detailed in **Table 1**.

Figure 2. The result of kombucha fermentation for various types of tea

Table 1. Composition of bacteria and yeast contained in SCOBY cultures [18]

Bacteria	Yeast
Acetobacter xylinum	*Saccharomyces cerevisiae*
Acetobacter pasteurianus	*Schizosaccharomyces pombe*
Acetobacter sp	*Brettanomyces bruxellensis*
Acetobacter aceti	*Zygosaccharomyces rouxi*
Gluconobacter	*Zygosaccharomyces bailii*

Black tea undergoes a fermentation process in which most of its catechins are oxidized at 24–27 °C over an extended period. This oxidation leads to the formation of theaflavins and thearubigins, compounds responsible for the characteristic strong aroma and dark brown coloration of black tea. In contrast, green tea is produced without fermentation. It undergoes a withering stage at approximately 100 °C, inactivating the heat-resistant polyphenol oxidase enzyme and thereby minimizing oxidation. White tea, similarly non-fermented, is derived exclusively from young shoots and the first two leaves. Its withering and drying are conducted at lower temperatures (20–25 °C) for a shorter duration to preserve its delicate profile [19].

During kombucha fermentation, a consortium of microorganisms—primarily bacteria and yeast—produces ethanol and organic acids, including acetic acid, glucuronic acid, and lactic acid [20]. Prolonged fermentation enhances the development of the daughter SCOBY (Symbiotic Culture of Bacteria and Yeast), which becomes increasingly thick and compact. SCOBY growth is closely linked to the nutrient availability in the fermentation medium; higher nutrient concentrations facilitate robust SCOBY formation with desirable physical characteristics.

Dutta and Paul (2019) [21] demonstrated that tea, when combined with sugar, serves as an effective substrate for SCOBY cultivation. This combination supports the proliferation of SCOBYs with diverse morphologies and sizes. Upon completion of the fermentation period, the mature kombucha culture—comprising the original SCOBY and its offspring—is removed from the medium [21]. The fermented liquid is then filtered to eliminate residual yeast filaments and

transferred into sterile glass bottles, which are sealed tightly. The final product is stored under refrigeration for subsequent analysis of ethanol concentration and metabolite profiling.

Ethanol Content in Kombucha Samples

Quantitative analysis of ethanol content was conducted using ethanol standard solutions to construct a calibration curve. Four of the resulting standard curves met the acceptance criteria, with coefficients of determination (r^2) ≥ 0.99. This r^2 value indicates a strong linear correlation between ethanol concentration and chromatogram peak area, validating the use of the derived linear regression equation for calculating ethanol levels in kombucha samples. Ethanol concentrations measured across different tea-based kombucha variants are presented in **Figure 3**.

Quantitative analysis revealed that the green tea kombucha sample exhibited the highest ethanol concentration, measured at 0.1708% w/w ± 0.0053. The white tea kombucha sample contained 0.1301% w/w ± 0.0043 ethanol, while the black tea kombucha sample showed the lowest concentration at 0.1126% w/w ± 0.0003. Chromatographic analysis indicated that the average retention time for the ethanol peak across all three kombucha variants was 2.45 minutes (**Figure 4**).

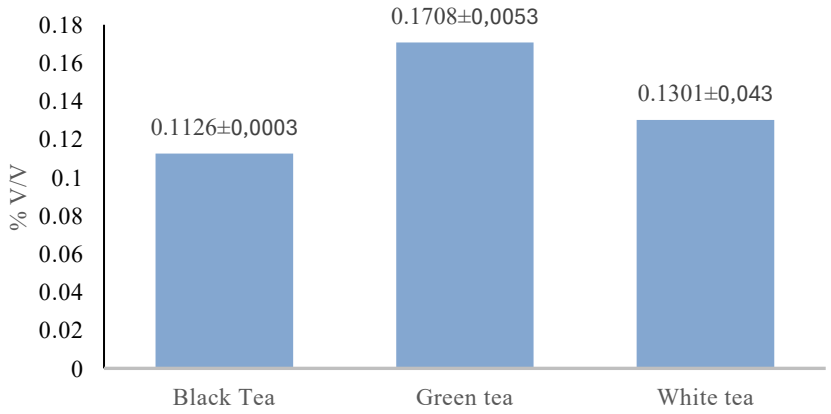

Figure 3. Ethanol content of kombucha based on various types of tea

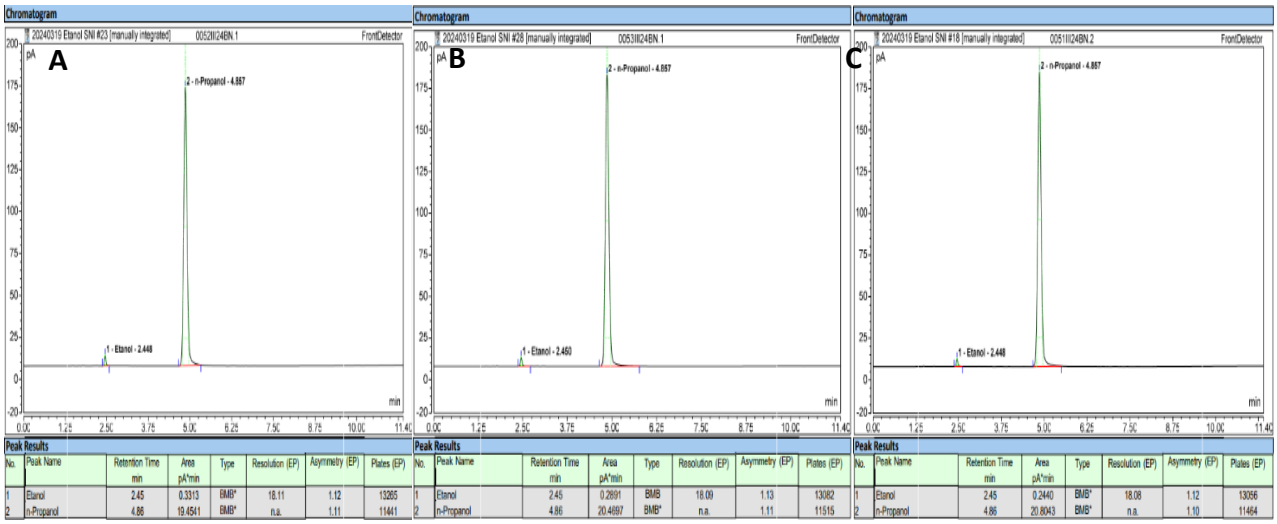

Figure 4. Peak of ethanol from the three variations of kombucha (A. green tea, B. White tea, C. Black tea) (Analyzed by GC-FID trace 1310 *Thermo Scientific*)

Ethanol Compliance and Storage Effects in Kombucha Samples

The ethanol content of kombucha samples produced from black tea, green tea, and white tea remained below the 0.5% w/w threshold, thereby meeting the minimum ethanol limit requirements as stipulated by the Indonesian Council of Ulama (Majelis Ulama Indonesia, MUI) [2]. Variations in ethanol concentration among the three tea-based kombucha samples are attributed to differences in microbial activity, which are influenced by the chemical composition of each tea type. Tea leaves contain varying levels of catechins, polyphenols, caffeine, flavonols, and tannins, all of which affect the fermentation dynamics and subsequent ethanol production. This observation aligns with findings by

Purnami et al. (2018),[17] who reported that different processing methods applied to the same tea plant species result in distinct physicochemical characteristics.

The type of tea used in kombucha fermentation plays a critical role in determining the beverage's taste, color, aroma, metabolite profile, and ethanol content. In addition to tea type, storage duration is a key variable influencing ethanol accumulation. Among the samples analyzed, green tea kombucha exhibited the highest ethanol concentration. To evaluate the impact of storage time, green tea kombucha was stored at 4 °C for four weeks, with ethanol measurements taken weekly. This storage protocol followed consumer guidance provided by the Fermentation House Company. The objective was to determine whether ethanol levels exceeded the permissible limit (>0.5%) during refrigerated storage. The ethanol content of green tea kombucha over the four weeks is presented in **Figure 5**.

The research results showed that kombucha samples made from black, green, and white tea still had <0.5% ethanol content and met the minimum ethanol limit set by the MUI Fatwa [22]. The differences in ethanol levels of the three kombucha samples were due to the activity of different microorganisms in each medium. It is known that each type of tea contains catechins, polyphenols, caffeine, flavonols, and tannins at different levels; therefore, differences in ethanol levels can also vary depending on the type of tea used during kombucha fermentation. This is also in line with research by Purnami et al. (2018), [17] which found that teas from a single tea plant processed differently exhibit distinct characteristics

The fermentation process of kombucha with different types of tea is the main factor influencing differences in taste, color, aroma, metabolite composition, and ethanol content. Apart from that, storage time is a fermentation variable in kombucha products that affects the ethanol content. Kombucha is a type of tea with the highest ethanol content, obtained from the green tea variety. Green tea kombucha was stored for 4 weeks at 4 °C after 10 days of fermentation, and its ethanol content was analyzed weekly. This condition time follows the information on how to store kombucha provided by the Fermentation House Company to consumers. This was done to determine whether, during storage, the ethanol content exceeded the required limit (>0.5%). The results of measuring the ethanol content of green tea kombucha stored for four weeks are shown in **Figure 5.**

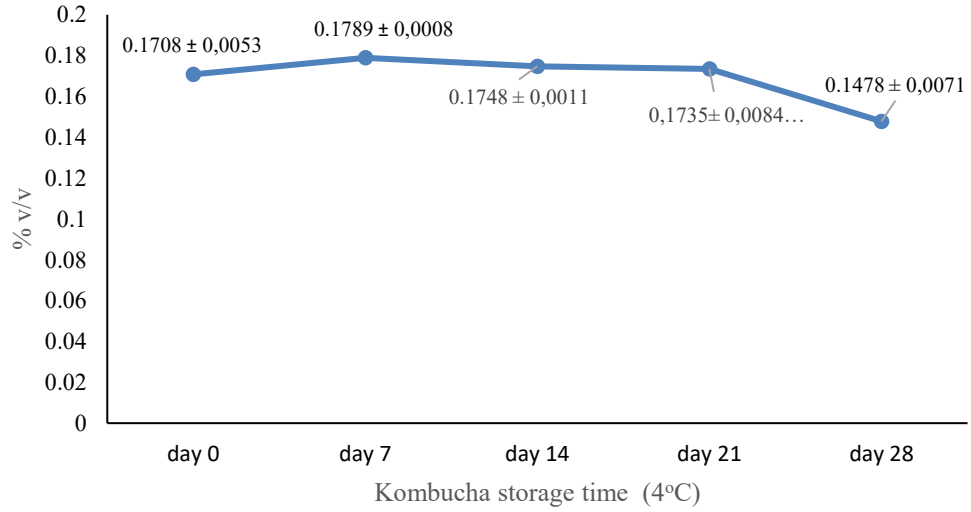

Figure 5. Results of measuring the ethanol content of green tea kombucha based on variations in storage time

Ethanol content analysis of green tea kombucha stored at 4 °C for four weeks demonstrated that the concentration remained below the safety threshold established by the Indonesian Council of Ulama (MUI) Fatwa, with a final value of 0.1478% w/w ± 0.0071. During the first week of storage, a slight increase in ethanol concentration was observed. This rise is attributed to the availability of residual sugar, which serves as a carbohydrate and energy source for yeast metabolism, thereby promoting continued ethanol production.

However, ethanol levels declined progressively from the second to the fourth week of storage. This reduction is likely due to the depletion of fermentable sugars, leading to cessation of active ethanol synthesis. Despite the removal and filtration of the SCOBY, residual microorganisms remain in the kombucha matrix and may continue to influence ethanol levels. Over time, microbial activity diminishes, contributing to the gradual decrease in ethanol concentration during refrigerated storage [23].

The measured ethanol content of green tea kombucha after storage for 4 weeks at 4 °C was still below the safe limit set by the MUI Fatwa (0.1478 + 0.0071%). Green tea kombucha stored in the first week of storage showed a slight increase, likely due to the sugar content, which served as a source of carbohydrates and energy at that time and remained high enough for yeast to consume, leading to a slight increase in ethanol production. Ethanol levels in the second to fourth week tend to decrease as yeast gradually consumes the sugar. Once ethanol production stops, the ethanol content in the kombucha decreases over time, even though the SCOBY has been removed and filtered. Microorganism residue remains in the kombucha. The presence of residual microorganism activity can affect the ethanol content in the kombucha product during storage time; the longer it is stored, the more the ethanol content will decrease [21,23]

Metabolite Profile of Kombucha from Various Tea Types

Metabolite profiling of kombucha samples derived from black tea, green tea, and white tea was conducted using Headspace Gas Chromatography–Mass Spectrometry (HS-GCMS). The analysis generated chromatograms and mass spectra, which included detailed information on detected compounds, chemical structures, mass-to-charge ratios (m/z), retention times, and peak areas of interest. Data acquisition and processing were performed using PostRun Shimadzu software. The HS-GCMS results revealed the presence of nine distinct compounds in black tea kombucha and 14 in each of the green tea and white tea kombucha samples (**Figure 6**).

Based on mass spectra identified using the Wiley7 and National Institute of Standards and Technology (NIST) libraries, the metabolite composition of kombucha, particularly its volatile organic compounds, across various tea types is presented in Table 2.

Volatile Metabolite Profile of Kombucha from Various Tea Types

Based on the data presented in Table 2, only a limited number of volatile organic compounds were detected in kombucha samples using Headspace Gas Chromatography–Mass Spectrometry (HS-GC-MS). This limitation is attributed to the nature of kombucha as a water-based fermented beverage. Water, as a polar, non-volatile solvent, poses analytical challenges in HS-GC-MS. As noted by Jacq et al. (2008), the presence of water in injected samples can lead to backflash—where water rapidly evaporates upon injection, disrupting the GC column and reducing the efficiency of volatile compound separation. This phenomenon can significantly reduce the instrument's sensitivity, resulting in the detection of only a few volatile components [24].

According to Wang et al. (2023), [25] volatile compounds in kombucha include alcohols and organic acids, which contribute to its sour taste and serve as precursors to aroma compounds. In the early stages of fermentation, aromatic compounds primarily originate from the tea itself. These compounds diminish or disappear as fermentation progresses. During the middle and final stages, alcohols, aldehydes, and other volatiles are gradually converted into esters, making esters the most abundant volatile compounds in mature kombucha [25]. These esters play a key role in shaping the organoleptic properties of kombucha, including its tart aroma and refreshing character. The composition and concentration of volatile compounds are influenced by the type of tea used, the microbial community (yeast and bacteria), and fermentation parameters.

Metabolite profiling of kombucha samples made from black, green, and white tea revealed distinct differences in compound types and concentrations (**Table 2**). Notably, three ester compounds—ethyl acetate, ethyl caprate, and diethyl phthalate—were consistently detected across all tea variants. According to Phung et al. (2023), [26] higher concentrations of ester compounds indicate superior beverage quality. Ethyl acetate, the most abundant short-chain ester, was present in all kombucha samples [26]. It is primarily formed through the esterification of acetic acid with ethanol, catalyzed by lipase, or via yeast-mediated esterification of acyl-CoA with ethanol through alcohol acetyltransferase. Ethyl acetate is commonly found not only in kombucha but also in alcoholic beverages such as wine and distilled spirits [27]. Beyond its sensory properties, ethyl acetate exhibits several bioactive effects, including anti-inflammatory, analgesic, antipyretic, antifungal, and antioxidant activities. Diethyl phthalate was detected as an impurity compound in the resulting kombucha product. Diethyl phthalate (DEP) is *not* a natural component of kombucha or other fermented beverages, but it can be detected as a contaminant from packaging or environmental sources [28]. Phenol, 3,5-bis(1,1-dimethylethyl) — also known as 3,5-di-tert-butylphenol — is *not* a naturally occurring compound in kombucha. However, it has been detected at trace levels in some commercial kombucha products, likely as a contaminant or a degradation byproduct [29].

Based on the data in **Table 2**, only a few volatile organic compounds in kombucha were detected by the HS-GC-MS instrument. This is because kombucha is a fermented drink, with water as the primary solvent. Water is polar and non-volatile. According to Jacq et al. (2008), analysis of HS-GC-MS samples containing water can cause backflash: water injected into the GC

column will evaporate, interfere with the GC column, reduce the efficiency of separating volatile compounds, and even reduce the sensitivity of the analysis. To detect volatile compounds, so that only a few volatile compound components in the sample are detected [27].

Wang et al. (2023) [30] stated that the volatile compounds in kombucha are alcohol and other organic acids that are formed. These volatile organic acids impart a sour taste and serve as precursors to aroma compounds in kombucha. The main aroma compounds in kombucha during the early stages of fermentation come from tea; they decrease over time and may even disappear during fermentation. Furthermore, alcohol, aldehydes, and other volatile components are gradually converted into esters during the middle and final stages of fermentation, making esters the most abundant compounds produced. These volatile components contribute to the overall organoleptic properties of kombucha, including its tart aroma and freshness [30]. The composition of volatile compounds depends on the type of tea used, the yeast and bacteria present in the kombucha, and the fermentation process parameters [26].

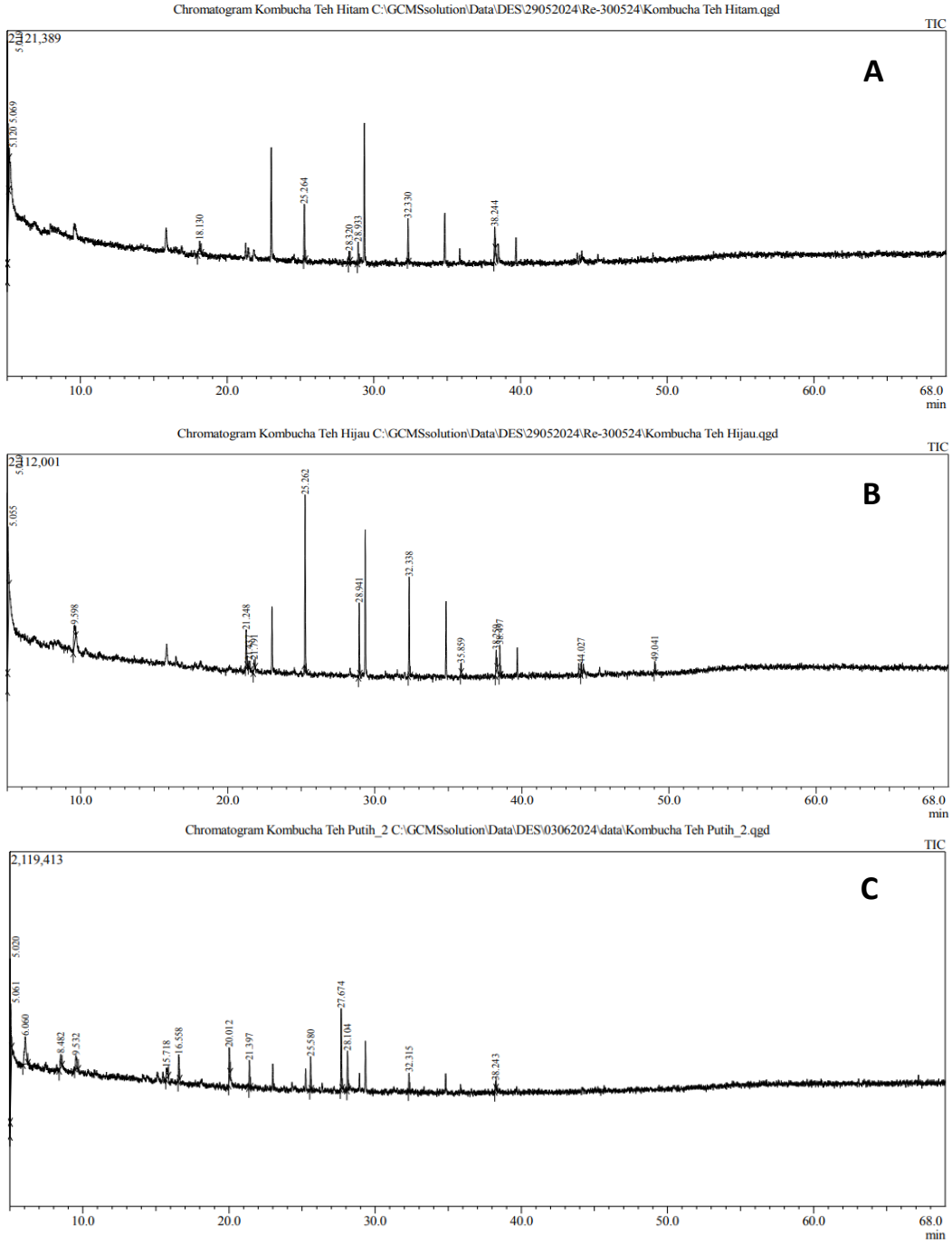

Figure 6. HS-GCMS Chromatogram of kombucha in various types of tea (A) black tea, (B) green tea, (C) white tea (GCMS Shimazu QP 2010, Column RTX-5MS (Restex)

Tabel 2. Volatile organic compounds contained in Kombucha based on variations in types of tea

Black Tea Kombucha					
Type of compound	**Compound name**	**m/z**	**RT**	**%area**	**Formula**
Ester	ethyl acetate	43.00	5.030	36.36	$C_4H_8O_2$
	ethyl octanoate	88.05	25.210	13.40	$C_{10}H_{20}O_2$
	ethyl nonanoate	39.95	28.933	5.05	$C_{11}H_{22}O_2$
	3-acetoxy-p-menthan-1-ol	39.95	17.980	5.62	$C_{12}H_{22}O_3$
	ethyl caprate	88.05	32.330	8.83	$C_{12}H_{24}O_2$
	diethyl phthalate	149.00	38.244	7.81	$C_{12}H_{14}O_4$
Amina	ethylamine	39.95	5.019	16.98	$C_2H_5NH_2$
Amida	n-butyl-3-methylbutanamide	43.00	5.120	3.48	$C_9H_{19}NO$
Isocoumarin	2(1h)-naphthalenone, 3,4,4a,5,6,7-he	177.05	28.320	2.47	$C_{10}H_{14}O_4$
Green tea Kombucha					
Type of compound	**Compound name**	**m/z**	**RT**	**%area**	**Formula**
Ester	ethyl octanoate	88.05	25.262	23.06	$C_{10}H_{20}O_2$
	ethyl nonanoate	88.05	28.941	9.19	$C_{11}H_{22}O_2$
	ethyl dodecanoate	88.05	38.497	3.97	$C_{14}H_{28}O_2$
	ethyl hexadecanoate	88.05	44.027	1.04	$C_{18}H_{36}O_2$
	ethyl icosanoate	88.05	49.041	1.65	$C_{22}H_{44}O_2$
	ethyl acetate	43.00	5.055	18.90	$C_4H_8O_2$
	ethyl caprate	88.05	32.338	13.51	$C_{12}H_{24}O_2$
	1,3,5-triazine, 2,4,6-tris(cyanomethoxy)	39.95	21.437	0.95	$C_9H_6N_6O_3$
	diethyl phthalate	149.05	38.259	3.34	$C_{12}H_{14}O_4$
	1-butanol, 3-methyl-, acetate	39.95	9.598	6.09	$C_7H_{14}O_2$
Alcohol	phenylethyl alcohol	91.05	21.791	1.86	$C_8H_{10}O$
	3-butynyl alcohol	39.95	5.019	9.37	C_4H_6O
	linalool	93.10	21.248	6.09	$C_{10}H_{18}$
Fenol Cyclic Monoterpenoids Phenols	phenol, 3,5-bis(1,1-dimethylethyl)	191.10	35.859	0.98	$C_{14}H_{22}O$
White tea Kombucha					
Type of compound	**Compound name**	**m/z**	**RT**	**%area**	**Formula**
Ester	ethyl acetate	43.00	5.061	21.99	$C_4H_8O_2$
	heptadecafluorononanoic acid, pentyl ester	43.00	9.532	3.86	$C_{14}H_{11}F_{17}O_2$
	ethyl caprate	88.05	32.315	2.64	$C_{12}H_{24}O_2$
	diethyl phthalate	149.00	38.243	1.42	$C_{12}H_{14}O_4$
Alcohol	3-furanmethanol	39.95	8.482	3.43	$C_5H_6O_2$
	1-octanol	56.05	20.012	6.34	$C_8H_{18}O$
	3-butynyl alcohol	39.95	5.020	8.70	C_4H_6O
Alkanes	hexane, 2,4-dimethyl-	43.00	6.060	12.29	C_8H_{18}
	cyclopropane, nonyl-	55.05	28.104	7.47	$C_{12}H_{24}$
Ketones	3-octanone	57.05	15.718	2.87	$C_8H_{16}O$
Aldehyde	octanal	56.05	16.558	5.21	$C_8H_{16}O$
	nonanal	57.05	21.397	4.85	$C_9H_{18}O$
	decanal	57.10	25.580	5.29	$C_{10}H_{20}O$
	2-decenal, (e)-	55.05	27.674	13.64	$C_{10}H_{18}O$

Principal Component Analysis (PCA) of Kombucha Metabolite Profiles

Multivariate data analysis using Principal Component Analysis (PCA) was performed on chromatographic data comprising compound names and their corresponding percent area values from three kombucha sample variations. The PCA revealed a total explained variance of 100%, with the first principal component (PC1) accounting for 61.6% and the second principal component (PC2) accounting for

38.4%. The score plot (**Figure 7**) illustrates distinct clustering patterns among the kombucha samples derived from black tea, green tea, and white tea. The spatial separation of these clusters indicates significant differences in metabolite composition across the three tea types, suggesting that each tea base contributes uniquely to the fermentation profile of kombucha.

The PCA results confirm that the metabolite profiles of kombucha samples derived from black tea, green tea, and white tea are distinct, with no significant overlap in secondary metabolite composition. This separation indicates that each tea type contributes uniquely to the fermentation outcome. As noted by Song et al. (2021), [31] the scor e plot illustrates the proximity between samples—samples with similar metabolite profiles cluster closely, while those with divergent profiles are distributed farther apart. In this study, the wide spatial separation of sample points in the score plot reflects the compositional differences among the three kombucha variants.

The loading plot provides insight into the influence of individual metabolites on the principal components. Metabolites positioned furthest from the origin in the loading plot contribute most significantly to the variance between sample groups. This analysis highlights the key compounds that differentiate kombucha profiles and supports the conclusion that tea type plays a critical role in shaping the final product's metabolite landscape.

This indicates that the metabolite profiles of the three kombucha variations differ and are not similar, or that there is no similarity in the secondary metabolite composition produced by each of the three variations. A study conducted by Fahri and Happyana (2025) [32] showed that a multivariate analysis approach using OPLS-DA can differentiate several coffee bean varieties from the Cimulek and Cimenong coffee regions. Trigonelline, 5-chlorogenic acid, and citric acid were detected as discriminant compounds for Cimulek and Cimenong coffees, whereas lactic acid, lipids, and stearic acid were associated with Ganda Paraja coffee. Tran et al., (2022) [33] stated that, the score plot section shows the closeness between samples, if the samples have many similarities, they will be clustered and located at points that are close to each other, while the loading plot section shows how strong the relationship between variables is. Loading plots are usually used to analyze the contribution of each metabolite found to the Principal Component (PC), where the component furthest from the group is the component that contributes most to the differences between groups [33].

The loading plot analysis in **Figure 8** highlights several characteristic metabolites that differentiate kombucha samples by tea type. Four dominant compounds were identified: ethyl acetate and ethyl octanoate (ester group), ethylamine (amine group), and 2-decenal (E)-(aldehyde group). These metabolites are proposed as potential biomarker compounds for kombucha derived from black tea, green tea, and white tea, respectively.

Dominant compounds are defined as those with the highest relative abundance, determined by percent area in the chromatographic data. Ethyl acetate was consistently detected across all kombucha samples and was the most abundant of the identified compounds. Its percent area varied by tea type: 36.36% in black tea kombucha, 18.90% in green tea kombucha, and 21.99% in white tea kombucha.

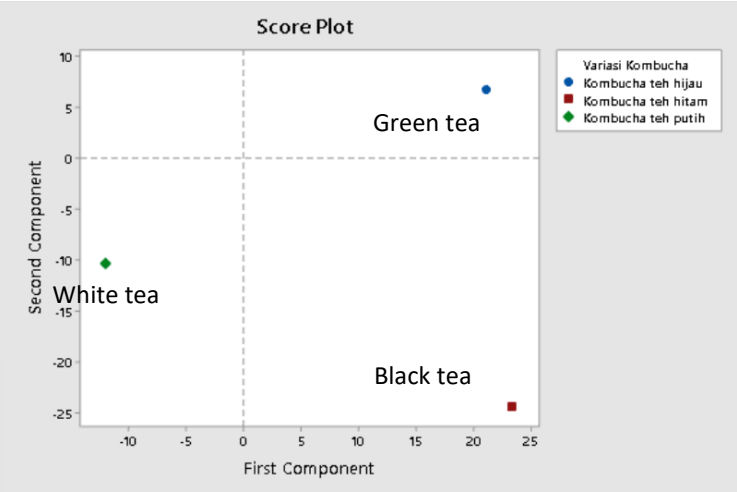

Figure 7. Score Plot of kombucha metabolites for various types of tea

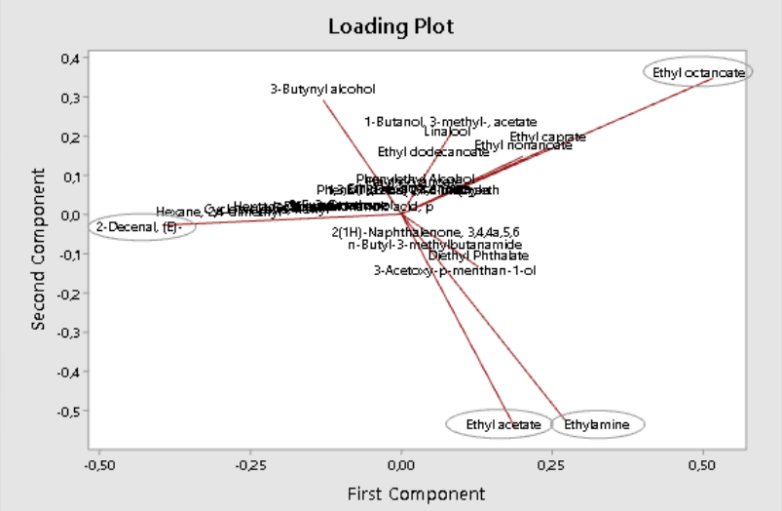

Figure 8. Loading Plot of Characteristic Compounds in Kombucha from Various Tea Types

In addition to ethyl acetate, each tea-based kombucha sample exhibited a unique dominant compound:

- Black tea kombucha: ethylamine (16.98%)
- Green tea kombucha: ethyl octanoate (23.06%)
- White tea kombucha: 2-decenal, (E)- (13.64%)

These findings suggest that specific volatile compounds are influenced by the type of tea used during fermentation and may serve as chemical markers for product differentiation and quality assessment.

4. CONCLUSIONS

Based on the research, it can be concluded that variations in the types of tea used in kombucha production (black, green, and white) influence the final product's ethanol content. Green tea kombucha produced the highest ethanol content, which decreased by the second week of storage, indicating that storage time affects ethanol levels. The dominant characteristic compound detected in all three kombucha variations was ethyl acetate. Additionally, other dominant characteristic compounds were identified: ethylamine in black tea kombucha, ethyl octanoate in green tea kombucha, and 2-decenal in white tea kombucha. Differences in metabolite profiles among kombucha products can serve as a means to identify authentic products and detect counterfeit products, which may impact the quality standards of functional food products in the future.

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REFERENCES

1. Standards and Metrology Institute for Islamic Countries (SMIIC). *OIC/SMIIC 1:2019 – General Requirements for Halal Food*. 2nd ed. Istanbul (TR): Organisation of Islamic Cooperation; 2019.
2. Majelis Ulama Indonesia. (MUI). Fatwa No. 10 of 2018 on Food and Beverage Containing Alcohol. Jakarta: MUI; 2018.
3. Arfa Yanti N, Ambardini S, Ode Leni Marlina W, Dwi Cahyanti Jurusan Biologi FMIPA Universitas Halu Oleo Kampus Hijau Bumi Tridharma K, Kendari Sulawesi Tenggara A, Korespondensi P. Aktivitas Antibakteri Kombucha Daun Sirsak (Annona muricata L.) Dengan Konsentrasi Gula Berbeda (Antibacterial Activity of Soursoup Leaves Kombucha (Annona muricata L.) With Different Sugar Concentration).
4. Chou YC, Lin HW, Wang CY, Hsieh CC, Santoso SP, Lin SP, et al. Enhancing Antioxidant Benefits of Kombucha Through Optimized Glucuronic Acid by Selected Symbiotic Fermentation Culture. Antioxidants. 2024 Nov 1;13(11).
5. Wang B, Rutherfurd-Markwick K, Zhang XX, Mutukumira AN. Kombucha: Production and Microbiological Research. Foods [Internet]. 2022;11(21). Available from: https://www.mdpi.com/2304-8158/11/21/3456
6. Khaerah A, Nawir N, Nurhilmi H, Abstrak N. Perbandingan Total Mikroba Kombucha

dengan Variasi Jenis Teh dan Lama Fermentasi.
7. Tangapo AM, Veralyn Maabuat P, Kolondam B, Wahyudi L, Mambu SM, Pasassa M. Metabolite Profiling of Kombucha Fermented Beverage with Substrate Variation. J Bios Logos. 2025 Aug 29;15(2):163–73.
8. Jansen CA, Zanzarin DM, Março PH, Porto C, do Prado RM, Carvalhaes F, et al. Metabolomic kinetics investigation of Camellia sinensis kombucha using mass spectrometry and bioinformatics approaches. Heliyon. 2024 Apr 15;10(7).
9. Ramautar R. New Developments in Mass Spectrometry No. 6 Capillary Electrophoresis-Mass Spectrometry for Metabolomics Edited by Rawi Ramautar 1.1 Introduction Capillary Electrophoresis-Mass Spectrometry for Metabolomics-From Metabolite Analysis to Metabolic Profiling [Internet]. 2018. Available from: www.rsc.org
10. Farag MA, Mohamed TA, El-Hawary EA, Abdelwareth A. Metabolite Profiling of Premium Civet Luwak Bio-Transformed Coffee Compared with Conventional Coffee Types, as Analyzed Using Chemometric Tools. Metabolites. 2023 Feb 1;13(2).
11. Djeni TN, Kouame KH, Ake FDM, Amoikon LST, Dje MK, Jeyaram K. Microbial Diversity and Metabolite Profiles of Palm Wine Produced From Three Different Palm Tree Species in Côte d'Ivoire. Sci Rep [Internet]. 2020;10(1):1715. Available from: https://doi.org/10.1038/s41598-020-58587-2
12. Kim EJ, Park SE, Seo SH, Kweon OC, Son HS. A GC–MS based metabolic profiling of fermented tomato by lactic acid bacteria. Appl Biol Chem [Internet]. 2019;62(1):2. Available from: https://doi.org/10.1186/s13765-019-0418-4
13. Yoram Gerchman. A simple rapid gas-chromatography flame-ionization-detector (GC-FID) method for the determination of ethanol from fermentation processes. Afr J Biotechnol. 2012 Feb 21;11(15).
14. Kim EJ, Park SE, Seo SH, Kweon OC, Son HS. A GC–MS based metabolic profiling of fermented tomato by lactic acid bacteria. Applied Biological Chemistry. 2019;62(1).
15. Azzahra MF, Nofita, Tutik. Analisis Kehalalan Produk Pembersih Wajah Berdasarkan Kandungan Etanol Menggunakan Metode Kromatografi Gas. 2024;10(13):508–15.
16. Permatasari FI, Nazir N, Anggraini T, Hellyward J, Techavuthiporn C, Syukri D. GC-MS Metabolite Profiling and Chemometric Analysis of Robusta Green Beans (Bantjah Coffee). Journal of Global Innovations in Agricultural Sciences. 2025;13(3):919–24.
17. Purnami I.miK, Anom Jambe A, Wayan Wisaniyasa N, Jurusan Ilmu dan Teknologi Pangan M, Teknologi Pertanian F, Udayana U, et al. Pengaruh Jenis Teh Terhadap Karaktersitik Teh Kombucha. Vol. 7. 2018.
18. Santiago-Santiago RM, Michel MR, Rodríguez-Herrera R, Aguilar-Zárate P, Ascacio-Valdés JA, Flores-Gallegos AC. Symbiotic Culture of Bacteria and Yeast (SCOBY) in the Food Sector as a Source of Polysaccharides and Other Applications in the Food Sector. Polysaccharides [Internet]. 2025 Oct 21;6(4):97. Available from: https://www.mdpi.com/2673-4176/6/4/97
19. Abudureheman B, Yu X, Fang D, Zhang H. Enzymatic Oxidation of Tea Catechins and Its Mechanism. Vol. 27, Molecules. MDPI; 2022.
20. Kitwetcharoen H, Phung LT, Klanrit P, Thanonkeo S, Tippayawat P, Yamada M, et al. Kombucha Healthy Drink—Recent Advances in Production, Chemical Composition and Health Benefits. Vol. 9, Fermentation. MDPI; 2023.
21. Dutta H, Paul SK. Kombucha Drink: Production, Quality, and Safety Aspects. In 2019. p. 259–88.
22. MUI. Fatwa Majelis Ulama No10 Tahun 2018. 2019. Fatwa Majelis Ulama Indinesia No 10 Tahun 2018 tentang Makanan/Minuman yang mengandung Alkohol. Available from: https://mui.or.id/baca/fatwa/produk-makanan-dan-minuman-yang-mengandung-alkohol-etanol
23. Abstracts of the International Halal Science Conference 2023. Abstracts of the International Halal Science Conference 2023. AIJR Publisher; 2023.
24. Water Injections in Capillary GC "How Wet Can You Get?"
25. Meng Y, Wang X, Li Y, Chen J, Chen X. Microbial interactions and dynamic changes of volatile flavor compounds during the fermentation of traditional kombucha. Food Chem [Internet]. 2024;430:137060. Available from: https://www.sciencedirect.com/science/article/pii/S0308814623016783
26. Phung LT, Kitwetcharoen H, Chamnipa N, Boonchot N, Thanonkeo S, Tippayawat P, et al. Changes in the chemical compositions and biological properties of kombucha beverages made from black teas and pineapple peels and cores. Sci Rep [Internet]. 2023;13(1):7859. Available from: https://doi.org/10.1038/s41598-023-34954-7

27. Mozzon M, Rinaldi L, Ahmed AEM, Kovács B, Foligni R. Investigating the Volatiles of Kombucha During Storage Under Refrigerated Conditions. Beverages. 2025 Oct 1;11(5):143.
28. Iannone A, Di Fiore C, Carriera F, Avino P, Stillittano V. Phthalates: The Main Issue in Quality Control in the Beverage Industry. Vol. 11, Separations. Multidisciplinary Digital Publishing Institute (MDPI); 2024.
29. Hoang NMH, Park K. Applications of Tert-Butyl-Phenolic Antioxidants in Consumer Products and Their Potential Toxicities in Humans. Vol. 12, Toxics. Multidisciplinary Digital Publishing Institute (MDPI); 2024.
30. Meng Y, Wang X, Li Y, Chen J, Chen X. Microbial interactions and dynamic changes of volatile flavor compounds during the fermentation of traditional kombucha. Food Chem [Internet]. 2024;430:137060. Available from: https://www.sciencedirect.com/science/article/pii/S0308814623016783
31. Song X, Canellas E, Nerin C. Screening of volatile decay markers of minced pork by headspace-solid phase microextraction-gas chromatography-mass spectrometry and chemometrics. Food Chem. 2021 Apr;342:128341.
32. Fahmi SS, Happyana N. Discriminating Green Beans of Puntang Arabica Coffees with 1H NMR Based- Metabolomics. Jurnal Kimia Valensi. 2025 May 1;11(1):1–8.
33. Tran T, Romanet R, Roullier-Gall C, Verdier F, Martin A, Schmitt-Kopplin P, et al. Non-Targeted Metabolomic Analysis of the Kombucha Production Process. Metabolites. 2022 Feb 1;12(2).