Research Article

Sciences of Phytochemistry

Identification and Characterization of Volatile Compounds in *Antidesma bunius* (Buni) Fruit Kombucha During Fermentation

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[The author informations are in the declarations section. This article is published by ETFLIN in Sciences of Phytochemistry, Volume 5, Issue 2, 2026, Page 214-220. DOI: 10.58920/sciphy0501676]

Received: 04 May 2026
Revised: 22 May 2026
Accepted: 10 June 2026
Published: 04 July 2026

Editor: Khafit Wiradimafan

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Keywords: buni fruit, fermentation, GC-MS, kombucha, volatile compound.

Abstract: Buni fruit (*Antidesma bunius*) is a fruit commonly found in tropical countries such as Indonesia. This fruit has the potential to be developed into kombucha tea because it contains beneficial bioactive compounds. However, the use of buni fruit as kombucha tea remains very limited and has not been studied in depth. The objective of this study was to identify and characterize the volatile compounds in buni fruit kombucha using GC-MS. This study employed a descriptive experimental design to analyze the volatile compounds in buni fruit kombucha after 8 days of fermentation using GC-MS. Buni fruit kombucha fermented for 8 days produced a dark purple color. Based on the analysis of volatile compounds, buni fruit kombucha was identified as containing 30 volatile compounds. The dominant compounds were hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, 9-octadecenoic acid methyl ester, (E)-9-octadecenoic acid ethyl ester, octadecanoic acid ethyl ester, eicosanoic acid ester, and docosahexaenoic acid methyl ester, which are classified as fatty acid esters and lipids. Additionally, the identified alcohol compounds are patchouli alcohol and 13-tetradecen-11-yn-1-ol. Buni fruit kombucha tea undergoes chemical transformations characterized by the formation of volatile compounds such as esters, alcohols, and fatty acid derivatives as a result of microbial metabolic activity. The profile of the resulting compounds highlights the important role of biotransformation during fermentation in shaping the sensory characteristics and bioactive potential of kombucha tea.

Introduction

Kombucha is a traditional fermented beverage produced by the fermentation of sweetened tea by a symbiotic consortium of microorganisms known as the Symbiotic Culture of Bacteria and Yeast (SCOBY). Fermentation is one of the oldest methods used in food production and preservation and plays an important role in the development of functional quality and beneficial properties in kombucha tea (1, 2). Innovations based on local ingredients, such as the addition of fruits, are key strategies for enhancing the functional and sensory value of kombucha products. The kombucha fermentation process involves complex interactions between microorganisms and the substrate, producing various metabolites, including organic acids, alcohols, and volatile compounds. Volatile compounds play a role in determining the aroma and flavor characteristics of kombucha, making them key parameters in product quality. Recent research shows that kombucha fermentation can produce various groups of volatile compounds, such as esters, alcohols, carboxylic acids, phenols, and aldehydes, which contribute to the beverage's sensory profile (3). In addition, the dynamics of volatile compound formation are strongly influenced by microbial activity and fermentation conditions, including fermentation time and the type of raw materials used (4).

Current research is still dominated by studies on conventional tea based kombucha, while exploration of the use of local fruits as fermentation substrates remains limited. One promising fruit is buni (*Antidesma bunius*). *A. bunius* is a tropical fruit commonly found in Southeast Asia and is known to be rich in bioactive compounds, particularly phenolic compounds, flavonoids, and anthocyanins. These compounds act as natural antioxidants that may offer health benefits, such as anti-inflammatory and antimicrobial activity. In addition, buni fruit possesses a naturally balanced sweet sour taste,

attractive dark purple red pigmentation, and high phenolic content, making it highly suitable as a kombucha substrate compared to many other tropical fruits. Its rich anthocyanin composition not only enhances the functional properties of kombucha but also contributes to the development of distinctive color, aroma, and flavor characteristics during fermentation. Furthermore, the availability of natural sugars and organic acids in buni fruit can support microbial metabolism during SCOBY fermentation, potentially promoting the formation of diverse volatile compounds and improving sensory acceptance of the final product. Previous studies have reported that buni fruit contains bioactive compounds with significant anti-AGEs activity, which are associated with potential health benefits, including the prevention of oxidative stress and metabolic disorders (5). Furthermore, the fruit extract has been reported to inhibit carbohydrate digesting enzymes and protein glycation, indicating its potential in managing hyperglycemia and preventing metabolic disorders (6).

In addition, the buni fruit has a distinctive profile of volatile compounds that contribute to its aroma and flavor. Fruits that are rich in anthocyanins, one of which is buni, contain various volatile compounds such as esters, alcohols, and aldehydes that contribute to their sensory characteristics (7). Moreover, compared to commonly used

Table 1. Volatile compounds identified in buni fruit kombucha after 8 days of fermentation.

No.	Retention Time (RT) in minutes	Compounds	Molecular Formula*	Relative abundance (%)
1	14.051	Azulene 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-	C15H24	0.129
2	16.401	Patchouli alcohol	C15H26O	0.288
3	19.088	Hexadecanoic acid, methyl ester	C17H34O2	1.384
4	19.797	Hexadecanoic acid, ethyl ester	C18H36O2	7.303
5	20.889	9-Octadecenoic acid, methyl ester	C19H26O2	2.780
6	21.475	9,12-Octadecadienoic acid	C18H32O2	0.950
7	21.535	(E)-9-Octadecenoic acid, ethyl ester	C20H38O2	5.046
8	21.960	Octadecanamide	C18H37NO	0.009
9	21.778	Octadecanoic acid, ethyl ester	C20H40O2	0.973
10	22.332	1,1,1-Trifluoroheptadecan-2-one	C17H31F30	0.747
11	22.814	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	C35H68O5	11.321
12	22.967	Eicosanoic acid ester	C24H48O2	1.020
13	23.023	3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris	C18H52O7Si7	0.941
14	23.572	9-Octadecenamide	C18H35NO	3.620
15	24.975	1,2-Octadecadienoyl chloride	C18H31ClO	6.081
16	25.055	Di-(9-octadecenoyl)-glycerol	C39H72O5	5.591
17	25.189	13-Tetradecen-11-yn-1-ol	C14H24O	0.408
18	25.283	Naphthalene derivative	C20H32	0.720
19	25.454	Benzene, (3-methyl-2-butenyl)	C11H14	17.832
20	25.668	Bicyclic compound	C14H20	3.380
21	25.940	Benzene (3-methyl-2-butenyl)	C11H14	2.618
22	26.044	Dispiro compound	C12H18	0.467
23	26.172	Docosahexaenoic acid, methyl ester	C23H34O2	15.461
24	26.329	Docosahexaenoic acid, methyl ester	C23H34O2	3.189
25	26.652	Docosahexaenoic acid, methyl ester	C23H34O2	2.477
26	29.032	Heptasiloxane, hexadecamethyl	C16H48O6Si7	1.422
27	29.376	Terephthalic acid ester	C27H44O4	0.617
28	30.068	Heptasiloxane, hexadecamethyl	C16H48O6Si7	1.266
29	32.032	Cyclodecasiloxane	C20H60O10Si10	0.987
30	33.349	Heptasiloxane	C16H48O6Si7	0.882

Note: *Based on comparison of MS spectrum with database of WILEY7.LIB

kombucha substrates such as apple, grape, or berry fruits, buni fruit remains underutilized despite its comparable phytochemical richness and unique indigenous character. This underexplored potential highlight the significance of utilizing buni fruit as an innovative fermentation substrate that support local biodiversity censrvation, diversification of functional beverage, and the development of value added product from indigenous tropical resources. This provides an opportunity to develop novel kombucha products derived from local biodiversity while simultaneously increasing the economic value and utilization of native tropical fruits

However, the use of buni fruit in fermented products, particularly kombucha, remains very limited and has not been extensively studied, especially regarding the characterization of its volatile compounds using analytical techniques such as Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS is widely used in kombucha studies to identify volatile metabolites and understand the metabolic pathways that occur during fermentation (8). In addition, a number of volatile compounds can be detected in kombucha, indicating that biochemical processes occur during fermentation (8). GC-MS analysis also plays a role in linking the composition of the microbiota to the formation of aroma compounds in kombucha (1). Therefore, exploring buni fruit as a substrate in kombucha fermentation not only has the potential to enhance the product's functional value but can also serve as a foundation for the development of functional foods based on local resources.

Methodology

Experiment Design

This study used a descriptive experimental design to analyze volatile compounds in buni fruit kombucha after 8 days of fermentation using GC-MS. The fermentation period was selected for that day because previous research has shown that the intermediate stages of fermentation significantly influence the biochemical characteristics of kombucha, including total acidity, phenolic compounds, alcohol formation, antioxidant activity, and sensory quality (9). In addition, kombucha fermented during that period was reported to have good physicochemical and sensory characteristics without excessive acidification (10). This study was replicated during the kombucha fermentation stage, while GC–MS analysis was performed only once on samples collected on the eighth day of fermentation, as the analysis was intended to identify volatile compounds. The results of the GC-MS analysis were used for the qualitative identification of the compound profiles. Compound identification was performed using WILEY and NIST spectral libraries.

The Process of Making Kombucha Tea

Water (1 L) was heated in a water bath for 15 minutes after reaching 90 °C. Subsequently, 10% (w/v) granulated sugar was added and dissolved, followed by the addition of 2.5% (w/v) buni fruit The mixture was stirred and allowed to stand for 5 minutes, then filtered into a sterile jar and adjusted to a final volume of 1 L with boiled water. After cooling to 30 °C, a 10% (w/v) SCOBY sheet was introduced. The jar was covered with a sterile cloth and secured (11). Fermentation proceeded throughout the treatment period, and the kombucha was harvested by separating the cellulose layer formed from the fermentation medium for subsequent analysis.

GC-MS Analysis

Analysis of volatile compounds in buni fruit kombucha samples was performed using a Shimadzu GCMS-QP2010 instrument. A total of 50 mL of kombucha sample was pipetted into a container, then 10 mL of n-hexane was added to extract the volatile compounds and separate the aqueous phase from the non-polar components. The mixture was then homogenized until thoroughly mixed and allowed to stand until two completely separate phases formed. The upper layer was collected and transferred into a sample vial. Subsequently, 1 µL of the sample was injected into the GC-MS system for further analysis. Compound identification was performed by comparing the obtained mass spectra with the WILEY7. LIB and NIST08S. LIB spectral libraries. The detected compounds were then analyzed based on retention time and percentage of peak area to determine the relative composition of volatile compounds in the sample (12).

Results and Discussion

GC-MS analysis of buni fruit kombucha after 8 days of fermentation identified 30 volatile compounds in the

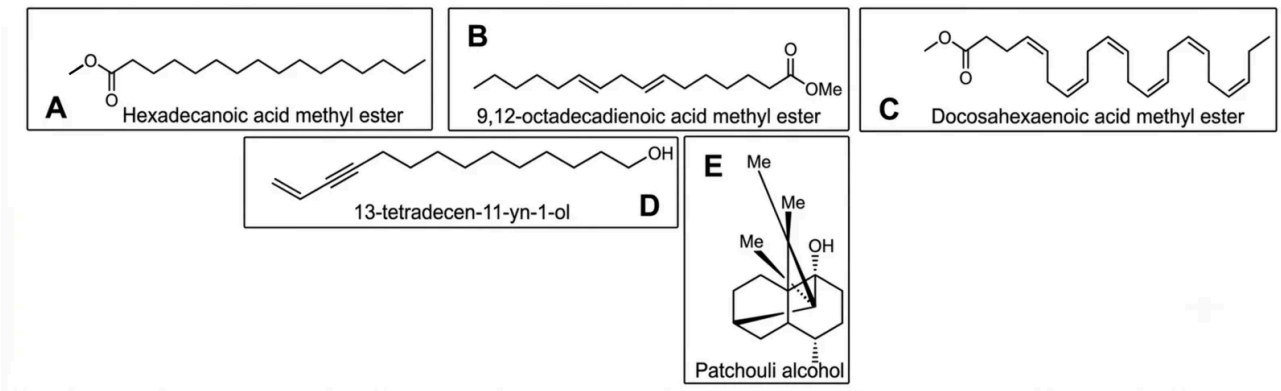


Figure 1. Chemical structure of volatile compounds in buni fruit kombucha: (A) Hexadecanoic acid methyl ester, (B) 9,12-octadecadienoic acid methyl ester, (C) docosahexaenoic acid methyl ester, (D) 13-tetradecen-11-yn-1-ol, (E) Patchouli alcohol.

fermented product. The detected compounds belong to several major groups, namely fatty acid esters, fatty acids and their derivatives, alcohols, aromatic compounds, amides, and siloxanes. The volatile compounds identified in the buni fruit after 8 days of fermentation are shown in **Table 1** below.

Kombucha made from buni fruit, fermented for 8 days, produces a dark purple color. Based on the analysis of volatile compounds in buni fruit kombucha, approximately 30 volatile compounds were identified (**Table 1**). Hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, 9-octadecenoic acid methyl ester, (e)-9-octadecenoic acid ethyl ester, octadecanoic acid ethyl ester, eicosanoic acid ester, and docosahexaenoic acid methyl ester are classified as esters and lipids. Meanwhile, the alcohol group included patchouli alcohol and 13-tetradecen-11-yn-1-ol. Some compounds, such as siloxanes and certain hydrocarbon derivatives detected in the analysis, are likely instrumental artifacts or contamination during the analytical process, therefore, they were not included in the interpretation of the sample's chemical composition. The structure of several main volatile compounds found in buni fruit kombucha can be seen in **Figure 1**.

Hexadecanoic acid methyl ester, 9, 12-octadecadienoic acid methyl ester, and docosahexaenoic acid methyl ester are compounds classified as fatty acid esters in fermented kombucha. Hexadecanoic acid methyl ester is found in Hordeum vulgare seeds as a product of lipid metabolism and has potential antimicrobial and anti-inflammatory properties (13). In addition, this compound is derived from palmitic acid in soybeans through the fermentation of food products (douchi) by microorganisms during the fermentation process (14). 9-Octadecenoic acid methyl ester is a form of oleic acid derived from the lipid metabolism of plant seeds, and is used to describe the profile and quality of oils from Satureja species (15). This compound also shows potential as a bioactive anticancer candidate (16). Docosahexaenoic acid methyl ester is a fatty acid methyl ester with antifungal potential (17). The predominance of fatty acid esters is likely due to the metabolic activity of microorganisms, which plays a key role in shaping the volatile profile. The results of this study are consistent that the major volatile compounds in kombucha are dominated by esters, acids, alcohols, and terpenes formed during fermentation (1). In that study, the formation of volatile compounds was reported to be closely correlated with the composition of the microbiota. In fresh buni fruit, fatty acids and their derivatives namely hexadecanoic acid, 9, 12-octadecadienoic acid, and ester derivatives were identified (18). This indicates the presence of lipid pathways in the fruit. Esters play a key role in influencing the final characteristics of kombucha after fermentation, imparting fruity, sweet, and floral aroma profiles. These GC-MS results provide further evidence of the biochemical transformations occurring during fermentation, which are facilitated by microorganisms within the Symbiotic Culture of Bacteria and Yeast (SCOBY). Kombucha based fermentation significantly increases the number and complexity of volatile compounds in grape pulp, particularly esters that contribute to the formation of fruity and sweet aromas (19). Furthermore, the formation of new compounds not present in the starting material indicates active biotransformation by microorganisms. Patchouli alcohol is a type of alcohol identified in this study. Previous studies have reported that this compound possesses anti-inflammatory, antioxidant, antimicrobial, and immunomodulatory activities (20). Restoring the integrity of the intestinal barrier and reducing inflammation in cases of digestive disorders are also benefits of this compound (21). The presence of alcohol in this study particularly ethanol, is the primary fermentation product generated by yeast activity during the early stages of kombucha fermentation Sales, Cunha (22). This compound acts as an intermediate metabolite that not only contributes to the aroma profile but also serves as an important precursor in the formation of esters through reactions with organic acids.

The compound 13-tetradecen-11-yn-1-ol identified in this study is a long chain alcohol derived from lipids. The presence of this compound indicates lipid metabolism activity by microorganisms in the kombucha fermentation system. Previous studies have reported the antibacterial potential of this compound, which has also been identified as a major component in several plant essential oils (23). The compounds detected may be associated with the bioactivity previously reported in the literature, thereby supporting the role of fermentation in producing functional metabolites in kombucha made from buni fruit. Volatile compounds such as esters and alcohols were also identified with black tea based kombucha (24). Kombucha fermentation results in dynamic changes in volatile compounds. During the kombucha fermentation process, volatile compounds consisting of carboxylic acids, alcohols, ketones, esters, aldehydes, phenols, and terpenes were detected (25). In addition, volatile compounds such as alcohols, esters, acids, aldehydes, ketones, and terpenoids were also detected in kombucha made from raw Pu-erh tea (26).

This indicates that volatile compounds are indeed produced during fermentation. During fermentation, the microorganisms in the SCOBY actively convert the initial substrate into various new metabolites through metabolic pathways, including the conversion of sugars into alcohols, oxidation into organic acids, and the formation of volatile compounds such as esters. These biotransformation processes not only increase the complexity of the chemical composition but also contribute to the sensory characteristics and potential bioactivity of the final product.

In previous studies, alcohols, esters, and aldehydes were identified as the main contributors to the characteristic flavor profile of blackberry juice beverage fermented with a kombucha consortium (27). Kombucha fermentation produces various new metabolites with biological activity and is significantly influenced by the type of substrate and the dynamics of the microbiota (28). The occurrence of biotransformation during fermentation can serve as a basis for improving the characteristics of kombucha, both in terms of sensory and functional aspects. Nevertheless, further studies involving quantitative analysis and direct bioactivity assays are required to confirm the functional effects of these compounds in buni kombucha. Several compounds identified in this study have previously been reported to possess antimicrobial, antioxidant, anti-inflammatory, anticancer, and immunomodulatory activities in other

studies. However, the present study was limited to the identification of volatile compounds using GC-MS analysis and did not directly evaluate the biological activities of the kombucha product.

Conclusion

Kombucha made from buni fruit was found to contain approximately 30 volatile compounds. The dominant compounds include hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, 9-octadecenoic acid methyl ester, (E)-9-octadecenoic acid ethyl ester, octadecanoic acid ethyl ester, eicosanoic acid ester, and docosahexaenoic acid methyl ester, which are classified as fatty acid esters and lipids. Additionally, the identified alcohol compounds are patchouli alcohol and 13-tetradecen-11-yn-1-ol. The resulting compound profile indicates the important role of biotransformation during fermentation in shaping the sensory characteristics and bioactivity potential of kombucha tea.

Abbreviations

GC-MS = Gas Chromatography-Mass Spectrometry; SCOBY = Symbiotic Culture of Bacteria and Yeast.

Declaration

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability

All data generated or analyzed during this study are included in this published article.

Ethics Statement

Not applicable.

Funding Information

This work received no external funding.

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Additional Information

How to Cite

APA 7th Edition: Wulansari, N., Dharmapatni, N., Damayanti, I. & Raningsih, N. (2026). Identification and Characterization of Volatile Compounds in Antidesma bunius (Buni) Fruit Kombucha During Fermentation. Sciences of Phytochemistry, 5(2), 214-220. https://doi.org/10.58920/sciphy0501676

Vancouver: Wulansari N, Dharmapatni N, Damayanti I, Raningsih N. Identification and Characterization of Volatile Compounds in Antidesma bunius (Buni) Fruit Kombucha During Fermentation. Sciences of Phytochemistry. 2026;5(2):214-220. https://doi.org/10.58920/sciphy0501676

Harvard : Wulansari, N., Dharmapatni, N., Damayanti,

I. & Raningsih, N. (2026)'Identification and Characterization of Volatile Compounds in Antidesma bunius (Buni) Fruit Kombucha During Fermentation', Sciences of Phytochemistry, 5(2), pp. 214-220. doi: 10.58920/sciphy0501676

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