



Metabolite Profiling of Balinese Grape (*Vitis vinifera* L. Var. Alphonso Lavallee) Kombucha: GC-MS Analysis

Ida Ayu Manik Damayanti , Nadya Treesna Wulansari ,
Ni Wayan Kesari Dharmapatni , Ni Wayan Sukma Antari 

[The author informations are in the declarations section. This article is published by ETFLIN in Sciences of Phytochemistry, Volume 5, Issue 2, 2026, Page 262-266. DOI: 10.58920/sciphy0502689]

Received: 17 May 2026

Revised: 21 June 2026

Accepted: 16 July 2026

Published: 17 July 2026

Editor: Khafit Wiradimafan



This article is licensed under a Creative Commons Attribution 4.0 International License. © The author(s) (2026).

Keywords: Balinese grape kombucha, GC-MS, Metabolite profiling.

Abstract: Balinese grape (*Vitis vinifera* L. var. *Alphonso Lavallee*) is a local grape variety containing various phytochemical constituents. Fermentation into kombucha using a symbiotic culture of bacteria and yeast (SCOBY) may alter the chemical composition of the substrate through microbial metabolic activity. This study aimed to characterize the metabolite profile of Balinese grape kombucha using Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS analysis detected several chemical constituents, including fatty acid esters, alkaloids, and terpenoid hydrocarbons. The major compounds identified based on mass spectral library matching were 9-Octadecenoic acid methyl ester (E)-, acetic acid n-octadecyl ester, caffeine, and 2, 6, 10, 14, 18, 22-tetracosahexaene, 2, 6, 10, 15, 19, 23-hexamethyl-, (all-E)-. These results describe the metabolite profile detected in the fermented sample and indicate changes in chemical composition following kombucha fermentation. The findings provide preliminary information on the chemical constituents of Balinese grape kombucha and may serve as a basis for further studies involving comparative analyses with unfermented substrates and confirmation of compound identities using complementary analytical approaches.

Introduction

Fermentation using a symbiotic culture of bacteria and yeast (SCOBY), as in kombucha production, is known to modify the chemical composition of plant-based substrates through microbial metabolic activity (1, 2). During fermentation, yeast primarily converts sugars into ethanol and carbon dioxide, while acetic acid bacteria further oxidize ethanol into organic acids such as acetic, gluconic, and glucuronic acids. These sequential biochemical reactions contribute to a dynamic fermentation environment that continuously alters the substrate composition (3, 4). As a result, these biochemical transformations may lead to the formation, degradation, or conversion of diverse metabolites that can be identified using advanced analytical techniques. Such processes result in fermented products with complex chemical profiles that are suitable for metabolite profiling studies.

Balinese grape (*Vitis vinifera* L. var. *Alphonso Lavallee*) is a local grape variety cultivated in Indonesia with distinct agroclimatic conditions that may influence its chemical composition. These environmental factors are known to affect the biosynthesis and accumulation of secondary metabolites in grapes (5, 6). Although grapes are widely recognized for their phytochemical constituents such as

phenolic compounds, organic acids, and other bioactive metabolites, limited information is available regarding the chemical changes and metabolite profile of Balinese grape following fermentation into kombucha. This lack of data is particularly relevant for locally produced fermented beverages, where substrate origin and fermentation conditions may significantly affect the final chemical composition. Therefore, this knowledge gap highlights the need for systematic chemical characterization of Balinese grape kombucha as a locally derived fermented product.

During fermentation, enzymatic activity from bacteria and yeast can alter the original compounds present in fruit substrates through a series of biochemical reactions such as hydrolysis, oxidation, esterification, and microbial metabolism (7, 8). These processes can lead to the breakdown of complex molecules and the formation of new secondary metabolites, thereby contributing to dynamic changes in the chemical composition of the system. As a result, these transformations can significantly affect the overall metabolite profile of the final fermented product. Therefore, a systematic and comprehensive analytical approach is required to identify, detect, and characterize the compounds produced during fermentation in order to better understand the chemical changes occurring in the substrate.

Gas Chromatography-Mass Spectrometry (GC-MS) is analytical technique widely used for the separation, detection, and identification of volatile and semi-volatile compounds in complex mixtures based on their mass-to-charge ratios and retention times. This method enables detailed metabolite profiling by providing both qualitative information on compound identity and insights into the chemical diversity present in complex biological and fermented systems. In the context of fermented products, GC-MS is particularly useful for revealing metabolic transformations that occur during fermentation processes (9). Accordingly, this study aims to characterize the metabolite profile of Balinese grape kombucha using GC-MS analysis as a preliminary investigation of its chemical composition.

Experimental Section

Balinese Grapes Kombucha

Balinese grape kombucha was prepared by infusing 5 g of black tea leaves in 1 L of boiling water. After filtration, 150 g of sucrose was added and stirred until completely dissolved. The sweetened tea was allowed to cool to room temperature before the juice obtained from 500 g of fresh Balinese grapes (*V. vinifera* L. var. *Alphonso Lavallee*) was added. A symbiotic culture of bacteria and yeast (SCOBY), obtained from the laboratory culture collection, together with the corresponding starter liquid, was inoculated into the mixture. Fermentation was carried out in sterile glass bottles covered with a clean cloth to allow air exchange while minimizing contamination. The bottles were incubated under static conditions at 28°C for 14 days. At the end of the fermentation period, the pH of the kombucha was measured and recorded as 3.4.

Compounds Analysis

Gas Chromatography-Mass Spectrometry (GC-MS)

Metabolite profiling was performed using a GCMS-QP2010 Ultra gas chromatography-mass spectrometry system (Shimadzu, Kyoto, Japan) equipped with a DB-17MS capillary column (30 m × 0.25 mm i. d., 0.25 µm film thickness; Agilent J& W, USA). Prior to analysis, 50 mL of kombucha sample was mixed with 10 mL of analytical-grade *n*-hexane and homogenized thoroughly. The mixture was allowed to stand until complete phase separation occurred, after which the upper organic phase

was transferred into a GC-MS vial for analysis. Because *n*-hexane was used as the extraction solvent, the analysis primarily targeted relatively non-polar metabolites. A 1 µL aliquot of the extract was injected into the GC-MS system. The injector temperature was maintained at 300 °C. The oven temperature program was set at 80 °C for 5 min, increased at 10 °C/min to 250 °C and held for 5 min, followed by a further increase at 10 °C/min to 300 °C and held for 10 min. The ion source and interface temperatures were maintained at 200 °C and 250 °C, respectively. Helium (ultra-high purity) was used as the carrier gas under the instrument operating conditions. Compound identification was performed by comparing the obtained mass spectra with those in the WILEY7. LIB and NIST08S. LIB spectral libraries. The identified compounds should be regarded as tentative identifications based on library matching.

Results and Discussion

Balinese grape kombucha is a fermented beverage produced from black tea, sugar, and Balinese grapes (*V. vinifera* L. var. *Alphonso Lavallee*) using a SCOBY starter culture. In this study, kombucha was prepared by steeping 5 g of black tea in 1 L of hot water, followed by the addition of 150 g of sugar and 500 g of Balinese grape juice. The mixture was then inoculated with SCOBY and fermented at 28 °C for 14 days.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was conducted to identify the bioactive compounds present in the fermented extract of Balinese grape kombucha. The analysis revealed various secondary metabolites and volatile compounds that may contribute to the biological activity of kombucha, particularly its antioxidant potential. These compounds consist of phenolic compounds, fatty acids, esters, alcohols, and other bioactive constituents formed during the fermentation process. The diversity of the detected metabolites indicates that microbial fermentation plays a significant role in modifying and enhancing the chemical composition of Balinese grape kombucha. The identified compounds and their characteristics are presented in Table 1.

The compound 9-Octadecenoic acid methyl ester (E)-, commonly known as methyl oleate, is a fatty acid methyl ester that has been reported in a variety of plant-derived

Table 1. Bioactive compounds identified in Balinese grape kombucha using GC-MS analysis.

No	Compounds Name	Percent Area (%)	Molecular Formula	Functional Group	Compound Group	PubChem CID
C1	9-Octadecenoic acid, methyl ester (E)-	0.26	C ₁₉ H ₃₆ O ₂	-COOCH ₃ , C=C	Unsaturated fatty acid ester	637517
C2	Acetic acid n-octadecyl ester	1.17	C ₂₀ H ₄₀ O ₂	-COO-	Fatty acid ester	12366
C3	Caffeine	1.81	C ₈ H ₁₀ N ₄ O ₂	C=O, N-containing heterocycle	Alkaloid	2519
C4	2, 6, 10, 14, 18, 22-Tetracosahexaene, 2, 6, 10, 15, 19, 23-hexamethyl-, (all-E)-	1.45	C ₃₀ H ₅₀	C=C	Terpenoid hydrocarbon	5280483

materials and fermented products (10, 11). In the present study, this compound was detected in the fermented kombucha extract prepared using *n*-hexane, a solvent that preferentially extracts relatively non-polar constituents. Since an unfermented Balinese grape tea control was not analyzed, it cannot be determined whether this compound originated from the grape substrate, the tea substrate, or biochemical changes occurring during fermentation. Therefore, its detection is interpreted only as part of the chemical profile observed in the analyzed sample.

Acetic acid *n*-octadecyl ester is a long-chain fatty acid ester that is primarily associated with lipid-related structural components. Compounds belonging to this class are commonly associated with lipid-derived constituents and may originate from biochemical transformations occurring during fermentation (12). Long-chain fatty acid esters are generally related to the lipid fraction of biological materials and may contribute to the overall chemical characteristics of fermented products (13, 14). Although fatty acid esters have been reported in various plant-based and fermented matrices, there is currently limited evidence demonstrating direct antioxidant activity for Acetic acid *n*-octadecyl ester specifically. Similar to methyl oleate, the presence of this compound in the present study cannot be specifically attributed to the fermentation process because no unfermented control was included for comparison. Furthermore, the use of *n*-hexane as the extraction solvent favors the recovery of hydrophobic compounds, which may influence the composition of metabolites detected by GC-MS. The detection of this compound highlights the chemical complexity of the fermented beverage and indicates that fermentation may influence the formation and modification of lipid-related metabolites.

Caffeine is an alkaloid compound naturally present in grapes or possibly derived from fermentation related sources. In kombucha beverages, caffeine is generally derived from the tea substrate used during fermentation and may remain present throughout the fermentation process, although its concentration can vary depending on fermentation conditions and microbial activity (15). Structurally, caffeine contains a purine like heterocyclic ring with nitrogen-containing functional groups, which contribute to its chemical stability and characteristic properties (16). The presence of caffeine in fermented beverages has been frequently reported in previous studies investigating kombucha composition. As one of the major tea-derived metabolites, caffeine serves as an indicator of the contribution of the tea substrate to the overall chemical profile of kombucha. Caffeine was identified through GC-MS analysis and represents one of the compounds contributing to the metabolite composition of Balinese grape kombucha.

2, 6, 10, 14, 18, 22-Tetracosahexaene, 2, 6, 10, 15, 19, 23-hexamethyl-, (all-*E*)- was identified in the GC-MS analysis as a terpenoid hydrocarbon compound corresponding to squalene (C₃₀H₅₀) (17, 18). Squalene is a naturally occurring triterpenoid widely distributed in plants, fruits, vegetable oils, and various biological materials. As an intermediate in terpenoid and sterol biosynthesis, squalene is commonly detected in metabolomic studies of plant-derived products and fermented materials (19). Squalene has been reported in various plant-derived materials and represents a naturally

occurring triterpenoid hydrocarbon. However, because the present study did not analyze the unfermented grape substrate or tea base, it cannot be concluded whether the detected squalene originated from the raw materials or was associated with biochemical transformations during fermentation. Confirmation of its identity would require additional analytical approaches using authentic reference standards or complementary spectroscopic techniques.

Overall, the GC-MS analysis demonstrated the presence of several non-polar metabolites in the *n*-hexane extract of fermented Balinese grape kombucha. The detected compounds indicate the chemical complexity of the analyzed sample; however, the present data do not permit conclusions regarding the origin of individual metabolites, changes induced by fermentation, or their biological significance. Future studies incorporating unfermented control samples, quantitative analyses, chromatographic peak evaluation, and compound confirmation using authentic standards are required to better characterize the chemical changes associated with kombucha fermentation.

Conclusion

GC-MS analysis of Balinese grape kombucha (*V. vinifera* L. var. *Alphonso Lavalley*) tentatively identified several chemical constituents, including 9-Octadecenoic acid methyl ester (*E*)-, acetic acid *n*-octadecyl ester, caffeine, and 2, 6, 10, 14, 18, 22-tetracosahexaene, 2, 6, 10, 15, 19, 23-hexamethyl-, (all-*E*)-, based on mass spectral library matching. These compounds were classified as fatty acid esters, alkaloids, and terpenoid hydrocarbons, reflecting the diversity of relatively non-polar metabolites detected in the *n*-hexane extract of the fermented sample. Therefore, the present findings provide a preliminary characterization of the metabolite composition of fermented Balinese grape kombucha under the analytical conditions employed.

Abbreviations

GC-MS, Gas Chromatography-Mass Spectrometry; RT, Retention Time; CID, Compound Identification Number; SCOBY, Symbiotic Culture of Bacteria and Yeast.

Declaration

Author Information

Ida Ayu Manik Damayanti

*Corresponding author

Bachelor of Clinic and Community Pharmacy Program, Faculty of Health, Institute of Technology and Health Bali.

Contribution: Conceptualization, Formal Analysis, Methodology, Resources, Validation, Writing – Original Draft.

Nadya Treesna Wulansari

Bachelor of Food Technology Program, Faculty of Technology, Institute of Technology and Health Bali.

Contribution: Formal Analysis, Methodology, Validation.

Ni Wayan Kesari Dharmapatni

Bachelor of Nursing, Faculty of Health, Institute of Technology and Health Bali.

Contribution: Resources, Validation.

Ni Wayan Sukma Antari

Bachelor of Clinic and Community Pharmacy Program,
Faculty of Health, Institute of Technology and Health Bali.

Contribution: Formal Analysis, Validation.

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.

References

- Antolak H, Piechota D, Kucharska A. Kombucha Tea—A Double Power of Bioactive Compounds from Tea and Symbiotic Culture of Bacteria and Yeasts (SCOBY). *Antioxidants*. 2021;10(10):1541. doi: <https://doi.org/10.3390/antiox10101541>
- Tandhanskul A, Krungkaew S, Li L, Kham SA, Yonekura L. Kombucha as a Sustainable Source of Metabiotics: Potential, Applications, and Future Perspectives. *Beverages*. 2025;11(6):173. doi: <https://doi.org/10.3390/beverages11060173>
- Nguyen NN, Nguyen QD. Evolution of Kombucha Tea from Isolated Acetic Acid Bacteria, Lactic Acid Bacteria and Yeast in Single- and Mixed-Cultures: Characteristics, Bioactivities, Fermentation Performance and Kinetics. *Food Biotechnology*. 2024;38(1):86–117. doi: <https://doi.org/10.1080/08905436.2024.2306505>
- Chen FI, Chang YT, Khoirunnida FL, Pan HY, Chuang PY, Lu YC. The effect of different yeast and bacterial strain combinations on the physicochemical and metabolic profile of kombucha fermentation. *International Journal of Gastronomy and Food Science*. 2025;42(1):101296. doi: <https://doi.org/10.1016/j.ijgfs.2025.101296>
- Doğan O, Yazar K. Effect of Different Seaweed Extracts on Yield, Quality and Physiological Characteristics of the Alphonse Lavallée (*Vitis vinifera* L.) Grape Variety. *Horticulturae*. 2025;11(9):1118. doi: <https://doi.org/10.3390/horticulturae11091118>
- Zabrina PT, Puspitasari L, Damayanti IAM. EFEKTIVITAS EKSTRAK BUAH ANGGUR BALI (*Vitis vinifera* L. Var. Alphonso Lavallee) TERHADAP HISTOLOGI SEL BETA PANKREAS *Mus musculus* L. DENGAN HIPERGLIKEMIA. *Berita Biologi*. 2024;23(3):335–343. doi: https://doi.org/10.55981/berita_biologi.2024.5508
- Karpova A, Adesina D, Tian F, Naik AS. Seaweed (*Laminaria digitata*) and Honey Kombucha: A Fermented Antioxidant-Rich Beverage. *Fermentation*. 2025;11(7):379. doi: <https://doi.org/10.3390/fermentation11070379>

- Chen F, Wang H, Lin Z, Hu J, Wu Y, Shi L, *et al.* Enzymatic and non-enzymatic bioactive compounds, and antioxidant and antimicrobial activities of the extract from one selected wild berry (*Rubus coreanus*) as novel natural agent for food preservation. *LWT*. 2022;171(1):114133. doi: <https://doi.org/10.1016/j.lwt.2022.114133>
- Foster SC, Tipton L, Perrault Uptmor KA. Evaluating chromatographic techniques for the aroma profiling of kombucha. *Anal Bioanal Chem*. 2025;418(10):3059–3073. doi: <https://doi.org/10.1007/s00216-025-06257-5>
- A'yuni Q, Mok K, Nakphaichit M, Jangchud K, Pirak T. Thai Cannabis sativa Leaves as a Functional Ingredient for Quality Improvement and Lactic Acid Bacterial Growth Enhancement in Kombucha. *Foods*. 2025;14(6):942. doi: <https://doi.org/10.3390/foods14060942>
- Xu WY, Zhou WT, Luo JZ, Jiang YY, Zhang K, Zhang SY, *et al.* Lipid metabolism of *Acetobacter pasteurianus* and its main components with hypoglycemic effects. *World J Diabetes*. 2025;16(6):1–18. doi: <https://doi.org/10.4239/wjd.v16.i6.103370>
- Wang B, Rutherford-Markwick K, Zhang XX, Mutukumira AN. Isolation and characterisation of dominant acetic acid bacteria and yeast isolated from Kombucha samples at point of sale in New Zealand. *Current Research in Food Science*. 2022;5(1):835–844. doi: <https://doi.org/10.1016/j.crfs.2022.04.013>
- Yang Y, Li H, Xia Y, Li S, Wang G, Ni L, *et al.* Unraveling physicochemical characteristics, microbial community structure and functional enzymes governing fatty acid ethyl esters formation during Chinese rice wine brewing. *Lwt*. 2024;198(1):116035. doi: <https://doi.org/10.1016/j.lwt.2024.116035>
- Peng X, Yue Q, Chi Q, Liu Y, Tian T, Dai S, *et al.* Microbial Diversity and Flavor Regularity of Soy Milk Fermented Using Kombucha. *Foods*. 2023;12(4):884. doi: <https://doi.org/10.3390/foods12040884>
- Huang G, Huang Y, Sun Y, Lu T, Cao Q, Chen X. Characterization of kombucha prepared from black tea and coffee leaves: A comparative analysis of physiochemical properties, bioactive components, and bioactivities. *Journal of Food Science*. 2024;89(6):3430–3444. doi: <https://doi.org/10.1111/1750-3841.17027>
- Zhang S, Jin J, Chen J, Ercisli S, Chen L. Purine alkaloids in tea plants: component, biosynthetic mechanism and genetic variation. *Bpr*. 2022;2(1):1–9. doi: <https://doi.org/10.48130/bpr-2022-0013>
- Wulandari AP, Rossiana N, Wandira A. Analysis of the Bioactive Compounds N-Hexane Extract of Ramie Leaves and Flowers (*Boehmeria nivea*) and Antibacterial Test Against *Escherichia coli* and *Klebsiella pneumoniae*. *J Bio Bio Edu*. 2022;14(3):356–363. doi: <https://doi.org/10.15294/biosaintifika.v14i3.38379>
- Murugan S, Duraisamy S, Balakrishnan S, Kumarasamy A, Raju A. Bioprocess optimization of PHB production

utilizing agro (sago) waste as a sustainable substrate by the native isolate, *Bacillus cereus*. Research Square. 2021. doi: <https://doi.org/10.21203/rs.3.rs-334519/v1>

19. Micera M, Botto A, Geddo F, Antoniotti S, Berteà CM, Levi R, *et al.* Squalene: More than a Step toward Sterols. *Antioxidants*. 2020;9(8):688. doi: <https://doi.org/10.3390/antiox9080688>

Additional Information

How to Cite

APA 7th Edition: Damayanti, I., Wulansari, N., Dharmapatni, N. & Antari, N. (2026). Metabolite Profiling of Balinese Grape (*Vitis vinifera* L. Var. Alphonso Lavalée) Kombucha: GC-MS Analysis. *Sciences of Phytochemistry*, 5(2), 262-266. <https://doi.org/10.58920/sciphy0502689>

Vancouver: Damayanti I, Wulansari N, Dharmapatni N, Antari N. Metabolite Profiling of Balinese Grape (*Vitis vinifera* L. Var. Alphonso Lavalée) Kombucha: GC-MS Analysis. *Sciences of Phytochemistry*. 2026;5(2):262-266. <https://doi.org/10.58920/sciphy0502689>

Harvard: Damayanti, I., Wulansari, N., Dharmapatni, N. & Antari, N. (2026) 'Metabolite Profiling of Balinese Grape (*Vitis vinifera* L. Var. Alphonso Lavalée) Kombucha: GC-MS Analysis', *Sciences of Phytochemistry*, 5(2), pp. 262-266. doi: 10.58920/sciphy0502689

Publisher Note

All claims expressed in this article are solely those of the authors and do not necessarily reflect the views of the publisher, the editors, or the reviewers. Any product that may be evaluated in this article, or claim made by its manufacturer, is not guaranteed or endorsed by the publisher. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access

This article is licensed under a Creative Commons Attribution 4.0 International License. You may share and adapt the material with proper credit to the original author(s) and source, include a link to the license, and indicate if changes were made.