

GC- MS Profiling of Storage-Related Changes in Phyto Compounds and Lipidome of *Murraya koenigii* Leaves

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Abstract

Curry leaves (*Murraya koenigii*) are highly valued in culinary and traditional medicine due to the rich phytochemical composition. This study investigates the impact of refrigerated storage on the phytochemical integrity of curry leaves using gas chromatography-mass spectrometry (GC-MS). Freshly harvested leaves were compared with samples stored at $4 \pm 1^\circ\text{C}$ for 15 days. GC-MS profiling revealed a significant reduction in sesquiterpenes such as trans- β -caryophyllene (22.19% to 13.36%) and the near disappearance of α - and β -humulene. The samples suggest cold-induced degradation. Additionally, refrigerated samples showed increased level of saturated fatty acids and esterified derivatives such as palmitic acid and ethyl linolenate, indicating lipid remodelling. Functionally important compounds such as quinic acid and cubenol were either reduced or absent after storage. Higher levels of 1,2-benzenedicarboxylic acid, diisooctyl ester- a potential contaminant from polyethylene packaging were detected in refrigerated samples, raising food safety concerns. Chemometric heatmap analysis confirmed distinct metabolic clustering between fresh and stored samples. These findings highlight the adverse effects of cold storage on the aroma, therapeutic potential and nutritional quality of curry leaves emphasizing the need for improved postharvest handling strategies.

INTRODUCTION

Curry leaves (*Murraya koenigii*), are a versatile herb extensively used in culinary and medicinal applications, particularly in South Asia, Native to India, Sri Lanka and Bangladesh, curry leaves are integral to Indian cuisine, enhancing flavour and nutritional value while also serving as a natural flavouring agent [1]. They are rich in phytochemicals such as carbazole alkaloids, saponins and flavonoids which contribute to a wide range of pharmacological activities, including antioxidant and anti-inflammatory properties [2]. Curry leaves are integral to South Asian cuisine and traditional medicine, particularly in India. They possess not only the culinary benefits but also possess a rich array of medicinal benefits [3]. Curry leaves are noted for their high levels of flavonoids and phenolics, which enhance their antioxidant capacity, as evidenced by studies showing substantial radical scavenging activity and ferric-reduction potential [4]. Preservation of phytochemical integrity in curry leaves during postharvest handling and storage is critical due to their perishable nature. Employing light/dark cycles during storage can help maintain the tissue integrity chlorophyll and phytochemical levels [5]. Additionally, preharvest treatments with salicylic acid have been shown to significantly extend shelf life and retain quality by reducing physiological weight loss and preserving essential nutrients. The choice of packaging material such as polypropylene bags, has been found to reduce weight loss and senescence while maintaining high levels of chlorophyll and ascorbic acid [6]. Cold storage of curry leaves can induce significant biochemical and physiological changes, primarily due to chilling injury when stored at typical refrigeration temperatures between ($4-8^\circ\text{C}$). Prolonged refrigeration increases enzymatic activities such as polyphenol oxidase and lipoxygenase, contributing to browning, chlorophyll, degradation, colour loss and antioxidant properties [7]. Only limited studies are available on how the refrigerated storage affects the phytochemical profile of curry leaves. Furthermore, most existing studies focus on microbial spoilage or visual quality rather than chemical composition. GC-MS is a robust tool

for identifying and quantifying volatile and semi-volatile phytochemicals. It provides high-resolution profiling of complex plant matrices and offers detailed insight into how storage influences the abundance of key phytochemicals. This research investigates the phytochemical differences between fresh and refrigerated curry leaves using GC–MS analysis. The findings provide insights into nutraceutical quality and established baseline data for future research on postharvest biochemistry of aromatic herb

MATERIALS AND METHODS

Plant Material Collection and Storage Condition

Fresh curry leaves (*Murraya koenigii* L.) were harvested from healthy, mature plants located in a garden at (Agriculture Research Station, Kovilpatti) (Fig. 5) during early morning hours to minimize volatile compound loss. The leaves were thoroughly washed under running tap water to remove surface contaminants, followed by blot drying with sterile paper towel. The freshly harvested leaves were divided into two equal portions. The fresh sample served as control and was analysed immediately. The remaining portion was placed in perforated food-grade polyethylene bags (80 GSM) and stored at $4 \pm 1^{\circ}\text{C}$ in a domestic refrigerator for 15 days (Fig. 6). Both sets of samples were analyzed under identical conditions after storage for phytochemical profiling.

Sample preparation for GC-MS Analysis

5g of fresh and refrigerated curry leaves were separately ground using a chilled mortar and pestle under liquid nitrogen to prevent degradation of volatile components. The powdered samples were then subjected to methanolic extraction. Each sample was mixed with 25 mL of HPLC- grade methanol. Extracts were sonicated for 30 mins in an ultrasonic bath at room temperature. The mixtures were incubated on a shaker at 150 rpm for 24 hours. The extracts were then filtered using Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C . Final concentrates were reconstituted in 1mL methanol, filtered through a syringe filter and stored in amber vials at 4°C to avoid oxidation until GC-MS analysis

GC-MS Instrumentation and Operating Conditions

Qualitative profiling of phytochemical constituents was performed using a Shimadzu GCMS-QP2010 Plus System equipped with an AOC-20i autosampler. The instrument was fitted with RTX-5MS capillary column (30 m $\times$ 0.32 mm internal diameter 0.50 μm film thickness). The system operated in electron impact (EI) ionization mode at 70eV, with ultra-high-purity helium (99.9%) as the carrier gas at a constant flow rate of 1.73 mL min^{-1} . A 0.5 μL aliquot of the sample was injected in split mode (split ratio 10:1). The injector temperature was maintained at 250°C , and the ion source temperature at 200°C . The oven program started at 50°C (isothermal for 2 min), then increased at $8^{\circ}\text{C min}^{-1}$ to 280°C and was held for 20

minutes. The total runtime was approximately 24 minutes, as indicated by the total ion chromatogram (TIC) profile. Mass Spectra were recorded across a mass to charge (m/z) range of 40–450 with a scan interval of 0.30 seconds. Prominent compound peaks were observed between approximately 6.2 min and 21.6 min with a major peak at 20.056 min indicating a major constituent. Chromatographic data were acquired and processed using TurboMass software version 5.2.0.

Compound Identification and Data Analysis

Chromatograms were interpreted using MSD Chem Station software, and the mass spectra of the separated compounds were matched with those in the NIST 14 mass spectral library. Only peaks with a matching score > 85% were considered reliable for compound identification. The relative percentage of each compound was calculated based on its peak area relative to the total peak area. Comparative analysis was conducted to examine the loss or appearance of specific compounds variation in the relative abundance of key bioactive constituents and shifts in compound classes (terpenes, phenolics, aldehydes etc.)

RESULTS

Depression of bioactive volatiles following refrigeration

The phytoactive profiling revealed a marked reduction in the key volatile compounds associated with the characteristic aroma and freshness of curry leaves upon refrigeration. In particular, caryophyllene - a prominent sesquiterpene known for its spicy, woody scent and antimicrobial properties decreased from 22.19% in fresh samples to 13.36% in refrigerated samples, indicating a 39.8% reduction (Fig. 3). Similarly, phytol - a diterpene alcohol contributing to green freshness of the leaves, declined from 7.38% in fresh leaf samples to 2.01% in refrigerated samples, representing a 72.8% reduction. Both compounds are integral to the sensory freshness and biofunctional quality of curry leaves. Their significant depletion suggests that cold conditions adversely affect the aromatic profile diminishing the organoleptic appeal and perceived freshness of the leaves. GC-MS profiling revealed a significant reduction in major sesquiterpene constituents following refrigerated storage. Trans- β -caryophyllene, a sesquiterpene hydrocarbon known for its anti-inflammatory, antimicrobial and analgesic properties was the predominant compound in fresh curry leaves, accounting for 22.19% of the total ion chromatogram (TIC). However, in refrigerated leaves, its relative abundance declined to 13.36%, indicating a 39.8% reduction. Similarly, α -humulene and β -humulene collectively contributing over 11% in fresh samples were markedly reduced or absent in refrigerated curry leaves samples. These reductions suggest that cold storage destabilizes volatile aromatic compounds through oxidation evaporation or enzymatic degradation. Since these terpenoids are largely responsible for the distinctive aroma and therapeutic potential of curry leaves, their degradation directly compromises to both sensory and pharmacological quality.

Reduction of functionally significant phytochemicals

Several low-abundance constituents with high biological significance exhibited storage-induced degradation. Quinic acid (1.43%), a precursor in chlorogenic acid biosynthesis and known for its antioxidant and antimicrobial properties was detected only in the fresh curry leaf samples. Similarly, epiglobulol and cubenol, sesquiterpene alcohols with reported anti-inflammatory and cytotoxic effects were either significantly reduced or absent in refrigerated samples. The selective degradation of these compounds under cold storage highlights the vulnerability of structurally complex oxygenated phytochemicals to hydrolysis and oxidative stress. Their loss likely contributes to a reduced health-promoting potential and decreased shelf stability of refrigerated curry leaves.

Contaminant Accumulation Associated with Polyethylene Packaging

Both samples contained traces of 1,2-benzenedicarboxylic acid, diisooctyl ester, a widely used plasticizer known to leach from polymeric food packaging. Its concentration increased in refrigerated curry leaf samples (3.11%) compared to fresh curry leaf samples (1.21%), suggesting progressive migration into the plant matrix during extended refrigerated contact. This finding underscores a potential food-safety concern, particularly in the long-term storage in polyethylene bags and warrants further evaluation regarding permissible exposure levels and material alternatives.

Chemometric Heatmap Analysis Confirms Distinct Metabolic Profiles

Heatmap visualization based on GC-MS parameters (Area%, Height%, and A/H ratio) revealed distinct clustering patterns between fresh (Fig. 1) and refrigerated samples (Fig. 2). Fresh curry leaves showed dominant clusters associated with sesquiterpenes and polyunsaturated lipid derivatives reflecting a metabolically active and biochemically rich profile. In contrast, refrigerated samples were characterized by clusters of saturated fatty acids, long-chain alcohols and esterified compounds, indicative of cold-induced compositional shifts and phytochemical simplification. These observations confirm that refrigeration strongly influences the volatile and non-volatile phytochemical matrix of curry leaves affecting, not only aroma and shelf life but also nutraceutical and functional attributes. The hierarchical dendrogram (Fig. 4.) groups compounds based on the similarity of their normalized profiles across all measured parameters. Shorter branch lengths indicate closely linked compounds ethyl 9,12,15-octadecatrienoate and 9,12,15-octadecatrienoic acid suggesting structural and biosynthetic similarity reflected in their GC-MS behaviour. Longer branches and isolated clusters such as 1-chloroicosane or n-hexadecanoic acid (Z) indicate the outliers with unique compound behaviours in volatility and chemical stability. Dynamics in the abundance of biophytoactive compounds in fresh and refrigerated curry leaf samples is tabulated below (Table. 1)

DISCUSSION

β -Caryophyllene, a sesquiterpene responsible for the characteristic woody and spicy aroma of curry leaves exhibited a marked decline of 39.8% under refrigerated conditions. This compound is integral to olfactory identity of leaves, possesses notable antimicrobial and anti-inflammatory properties, enhancing the functional value of the curry leaves. The decrease aligns with earlier findings indicating that storage conditions substantially influence terpene stability and volatility [8]. Phytol, a diterpene alcohol derived from chlorophyll metabolism also showed a substantial reduction (72.8%) following refrigeration. As a compound contributing to the green freshness of curry leaves. Phytol plays a dual role- enhancing sensory freshness and participating in key physiological processes such as energy and glucolipid metabolism [9]. Its significant depletion may indicate broader metabolic disturbance under cold stress, potentially impairing membrane stability. The present investigation underscores the profound influence of refrigeration on both volatile and non-volatile phytochemical constituents of curry leaves with the implications for both sensory quality and functional efficacy. The significant loss of major sesquiterpenes- including trans- β -caryophyllene (39.8% reduction), α -humulene, and β -humulene reflects the susceptibility of thermolabile and oxygen-sensitive volatiles to degradation through enzymatic hydrolysis, oxidation or volatilisation. These findings corroborate earlier reports in *Ocimum basilicum*, where low-temperature storage elicited pronounced losses in aroma-related volatiles, with temperature exerting a more dominant effect than modified-atmosphere packaging [10]. Similar patterns were observed in tomatoes where cold storage led to the downregulation of volatile-producing enzymes like lipoxygenase and hydroperoxide lyase, resulting in decreased aldehyde and alcohol concentrations essential for flavour retention [11]. The degradation of volatile sesquiterpenes compromises organoleptic characteristics and diminishes the pharmacological potential of curry leaves, particularly their anti-inflammatory, antimicrobial and analgesic activities, attributed to compounds such as trans- β -caryophyllene and epiglobulol. The significant reduction or absence of low abundance, yet bioactive constituents like quinic acid, cubenol and epiglobulol further suggests that structurally complex oxygenated phytochemicals are particularly vulnerable to cold-induced hydrolytic and oxidative degradation. These losses align with findings in *Brassica oleracea* var. *acephala* [12], where chilling stress induced oxidative damage and reduced phytochemical density, highlighting a broader trend across leafy vegetables.

Cold storage also prompted significant reprogramming of the liquid metabolic landscape marked by a decline in polyunsaturated fatty acids (PUFAs) such as α -linolenic acid (9.88%) and phytol isomers (7.38%) in fresh samples along with a concomitant increase in saturated and monounsaturated compounds such as palmitic acid (20.09%) and 9-octadecen-1-ol (15.70%). This shift suggests of lipid remodelling under cold stress likely mediated by cold-responsive enzymes such as desaturases and lipases. Notably, the accumulation of ethyl esters such as ethyl linolenate and ethyl palmitate in refrigerated samples suggested ongoing esterification reactions, potentially catalyzed by residual enzymatic activity. This is consistent with lipidomic studies in *Arabidopsis thaliana* and *Secale cereale*, which reported alterations in membrane lipid classes and accumulation of cold-adaptive lipid species during low-temperature exposure [13]

Moreover, the increased presence of long chain alcohol and esterified lipids in refrigerated samples mirrors findings in stored cabbage and potato leaves where

cold conditions increased accumulation of C18:1 fatty acids and etherified derivatives, respectively [14]. While some esterified derivatives may retain partial bioactivity, their altered polarity, volatility and absorption profiles may limit their therapeutic and sensory relevance. Additionally, certain ester derivatives like phytol esters have been associated with dietary risk in vulnerable populations, such as individuals with Refsum's disease, necessitating careful evaluation of cold induced lipid transformations [15].

A further concern is the detection and increase of plasticiser contaminants such as 1,2-benzenedicarboxylic acid, diisooctyl ester, which increased in refrigerated samples (3.11%) compared to fresh samples (1.12%). This increase suggests time-dependent leaching from polyethylene packaging under cold and moist conditions. Such findings, parallel, broader concerns regarding the accumulation of phthalate esters (PAEs), including DEHP, in vegetables stored in plastic-film greenhouses are exposed to microplastics via atmospheric deposition or irrigation runoff [4, 16]. Given the documented endocrine-disrupting and carcinogenic potential of these compounds, their presence in refrigerated produce underscores the need for safer storage materials and stricter regulatory oversight.

Chemometric heatmap analysis further validated these compositional shifts, revealing distinct clustering patterns between fresh and refrigerated samples. Fresh leaves were characterized by clusters rich in sesquiterpenes and polyunsaturated lipid derivatives, indicative of a metabolically active phytochemical profile. In contrast, refrigerated samples clustered with saturated fatty acids, long-chain alcohols and esterified compounds, reflecting phytochemical simplification. These findings align with previous work on *Eugenia uniflora* [18], where fresh samples displayed a sesquiterpene-dominant profile that diminished under cold storage. This transition from a diverse to a simplified phytochemical matrix under refrigeration may reduce nutraceutical efficacy, flavour fidelity and shelf appeal of the plant product. Collectively, these findings emphasize that refrigeration, while widely adopted for shelf-life extension imposes significant biochemical and functional trade-offs in perishable herbs like curry leaves. The decline in key aroma volatiles, loss of bioactive phytochemicals and accumulation of undesirable lipids and contaminants presents a compelling case for re-evaluating cold storage protocols. Future research should focus on optimizing storage parameters such as packaging type, humidity control, intermittent warming and exploring bio-based preservation alternatives to retain both the sensory and the therapeutic quality. GC-MS analysis also revealed that refrigerated curry leaf samples contained unique compounds absent in fresh samples, including benzoic acid, phenylmethyl ester, ethyl esters of fatty acids (ethyl 9,12,15-octadecatrienoate, hexadecenoic acid, ethyl ester and 1,2-benzenedicarboxylic acid, diisooctyl ester). These are products of lipid peroxidation and esterification driven by oxidative or enzymatic degradation during storage; their presence reflects biochemical ageing and reduced shelf stability. Notably, benzoic esters and phthalates are associated with cytotoxicity and endocrine disruption, acting through membrane destabilization and interference with signalling pathways.

Phthalates, in particular, can leach from packaging and alter membrane dynamics, with evidence of hormonal disturbances *in vitro* and across species [19–22].

CONCLUSION

These changes suggest that storage of curry leaves in refrigeration even with the food grade packaging materials, may facilitate chemical degradation that compromises both quality and safety. So it is need of an hour to come up with the Improved strategies of food materials especially curry leaves are essential to retain the bioactive integrity of curry leaves.

Declarations

Ethics Approval and Consent to Participate:

This article does not contain any studies with human participants or animals performed by any of the authors. The plant material used in this study was cultivated at the Agricultural Research Station, Kovilpatti and collection was conducted in accordance with institutional guidelines. License and permissions were not applicable.

Consent to Publication:

All authors have read and approved the final manuscript and consent to its publication.

Clinical Trial Registration

Not applicable – this study does not involve clinical trials.

Competing Interests:

The authors declare that they have no competing interests.

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Author Contribution

Conceptualization & Methodology- V. S. A, Investigation & Data Curation- P.K , M. S. S, M.K , Formal Analysis & Visualization-G. B, T.P, K. S, M. I, Writing – Original Draft- V. S. A, Writing – Review & Editing- R. K, Supervision & Project Administration-R. K. All authors reviewed and approved the final manuscript.

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Data Availability

All data supporting the findings of this study are available within the paper

References

1. Kumari A, Kasaudhan P, Fernandes AH. *Int. J. Res. Anal. Rev.*, 2022, vol. 9, no. 6, pp. 1269–1274. Available from: IJRAR Official Website.
2. Kangane A, Patel B, Rao C. J. *Ethnopharmacol.*, 2025, vol. 250, pp. 45–59. <https://doi.org/10.1016/j.jep.2025.01.002>
3. Abeysinghe DT, Alwis DDD, Kumara KAH, Chandrika UG. *Evid -Based Complement Altern Med.* 2021;2021:5523252. <https://doi.org/10.1155/2021/5523252>.
4. Mandal S. *Int J Clin Exp Physiol.* 2016;3(2):59–65. <https://doi.org/10.4103/2348-8093.185203>.
5. Liu JD, Goodspeed D, Sheng Z, Li B, Yang Y, Kliebenstein DJ, Braam J, *Plant Biol BMC.* 2015, vol. 15, p. 92. <https://doi.org/10.1186/s12870-015-0474-9>
6. Venkatesan K. *Pharma Innov. J.*, 2022, vol. 11, no. 12, pp. 975–7. <https://doi.org/10.22271/tpi.2022.v11.i12m.17358>
7. Venkatachalam K, *Acta Hortic.* 2019, vol. 1245, pp. 145–52. <https://doi.org/10.17660/ActaHortic.2019.1245.21>
8. Sánchez-Muñoz BA, Aguilar MI, King-Díaz B, Rivero JF, Lotina-Hennsen B. *Molecules*, 2012, vol. 17, no. 2, pp. 1437–47. <https://doi.org/10.3390/molecules17021437>
9. Lin X, Zhu X, Jiang Q, Shu G, *Chin. J Anim Nutr.* 2012;24(12):1866–70. <https://doi.org/10.5555/20193152095>.
10. Mitcham EJ. *Front Plant Sci.* 2023;14:1218734. <https://doi.org/10.3389/fpls.2023.1218734>.
11. Baenas N, Bravo S, García-Alonso FJ, Gil JV, Periago MJ. *Eur Food Res Technol.* 2021;247(4):975–84. <https://doi.org/10.1007/s00217-020-03678-7>.
12. Du Y, Wang M, Tong M, Wu D, Chu J, Yao X. *Postharvest Biol Technol.* 2024;199:112634. <https://doi.org/10.1016/j.postharvbio.2023.112634>.
13. Vu HS, Shiva S, Hall AS, Welte R. in *Lipidomics* P, ed. Welte R, editors, New York: Humana, 2014, pp. 199–215. https://doi.org/10.1007/978-1-4939-0844-8_15
14. Syukri D, Nakano K, Agric UJ. *Res.* 2023;11(4):207–14. <https://doi.org/10.13189/ujar.2023.110401>.
15. Krauß S, Michaelis L, Vetter W. *PLoS One*, 2017, vol. 12, no. 11, p. e0188035. <https://doi.org/10.1371/journal.pone.0188035>

16. Ma TT, Wu LH, Chen L, Zhang H, Teng Y, Luo Y. *Environ Sci Pollut Res*. 2015;22:12018–28. <https://doi.org/10.1007/s11356-015-4401-2>.
17. Mandal RSK, *Multidiscip R*. 2024, vol. 8, p. 2025088. <https://doi.org/10.31893/multirev.2025088>
18. Onayade O, Adebajo A, Looman A. *Niger J Nat Prod Med*. 1999;3(1):79–82. <https://doi.org/10.4314/njnpm.v3i1.11769>.
19. Harris CA, Sumpter JP. In: Metzler M, editor. *Endocrine Disruptors*. Berlin: Springer; 2001. pp. 169–201. https://doi.org/10.1007/10690734_9.
20. Dutta S, Haggerty DK, Rappolee DA, Ruden DM. *Front Genet*. 2020;11:405. <https://doi.org/10.3389/fgene.2020.00405>.
21. Gao P, Luo Y, Gou X, Xia P, Wang P, Yan L, Zhang S, Guo J, Zhang X, Yu H, Shi W. *Environ Sci Technol*. 2023;57(36):13664–77. <https://doi.org/10.1021/acs.est.3c03282>.
22. Naz Z, Shrestha RM, Moin ST, Monticelli L. Interaction of phthalates with lipid bilayer membranes. *J Phys Chem B*. 2022;126(20):4679–88. <https://doi.org/10.1021/acs.jpcc.2c02007>.

Tables

Table 1. Dynamics in the abundance of biophytoactive compounds in fresh and refrigerated curry leaf samples

Compound	Fresh curry leaf sample (%)	Refrigerated curry leaf sample (%)
Caryophyllene	22.0	13.8
n-Hexadecanoic acid	17.0	20.0
Phytol	7.5	2.0
Ethyl 9,12,16-octadecatrienoate	0.9	3.7
1,2-Benzenedicarboxylic acid, diisooctyl ester	1.0	3.5
Benzoic acid, phenylmethyl ester	0.1	1.2
Hexadecanoic acid, ethyl ester	0.7	0.5
Docosanoic acid, ethyl ester	0.2	0.5

Figures

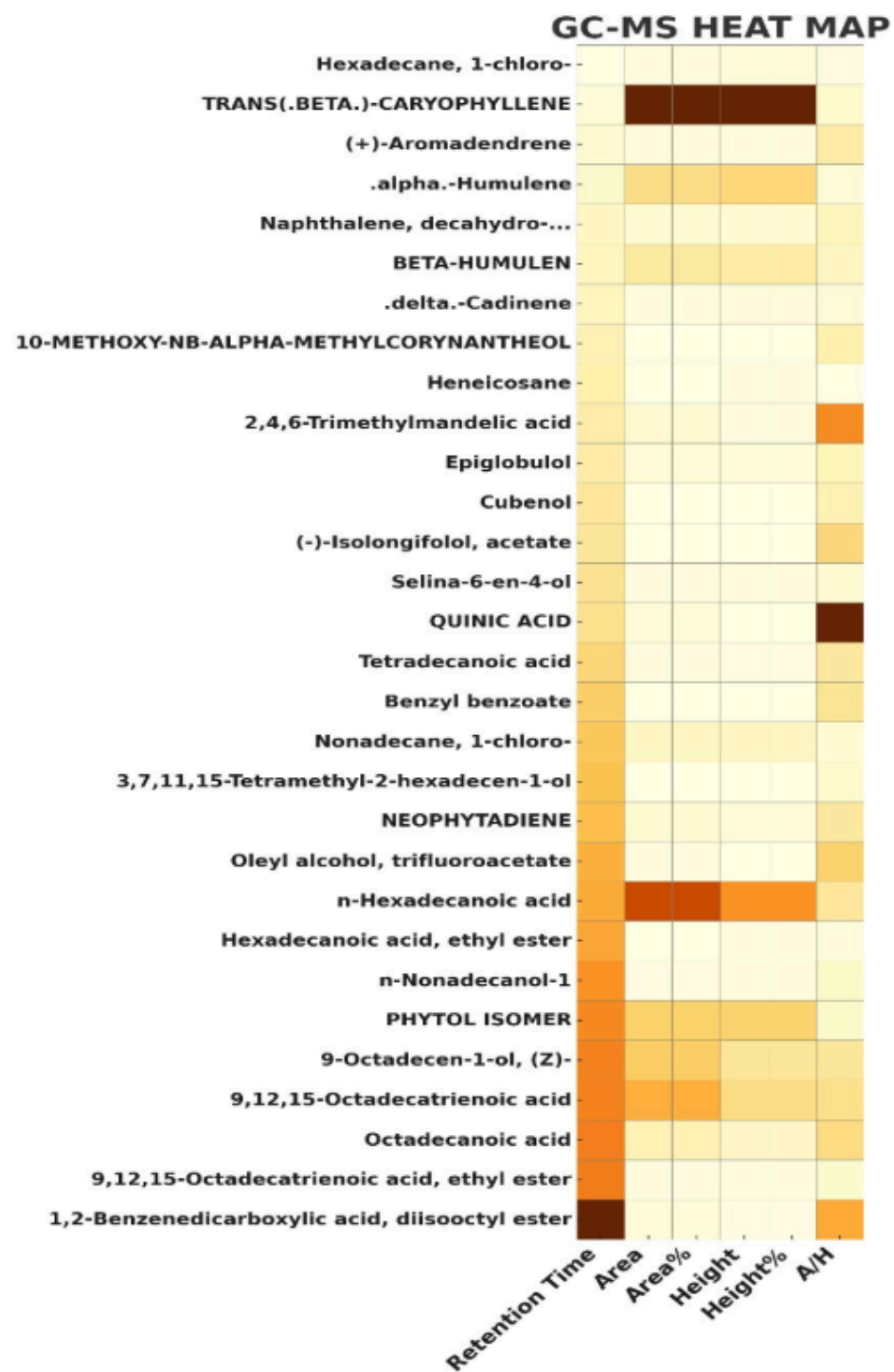


Figure 1

GC-MS profiling and heatmap representation of bio volatiles in fresh curry leaves.

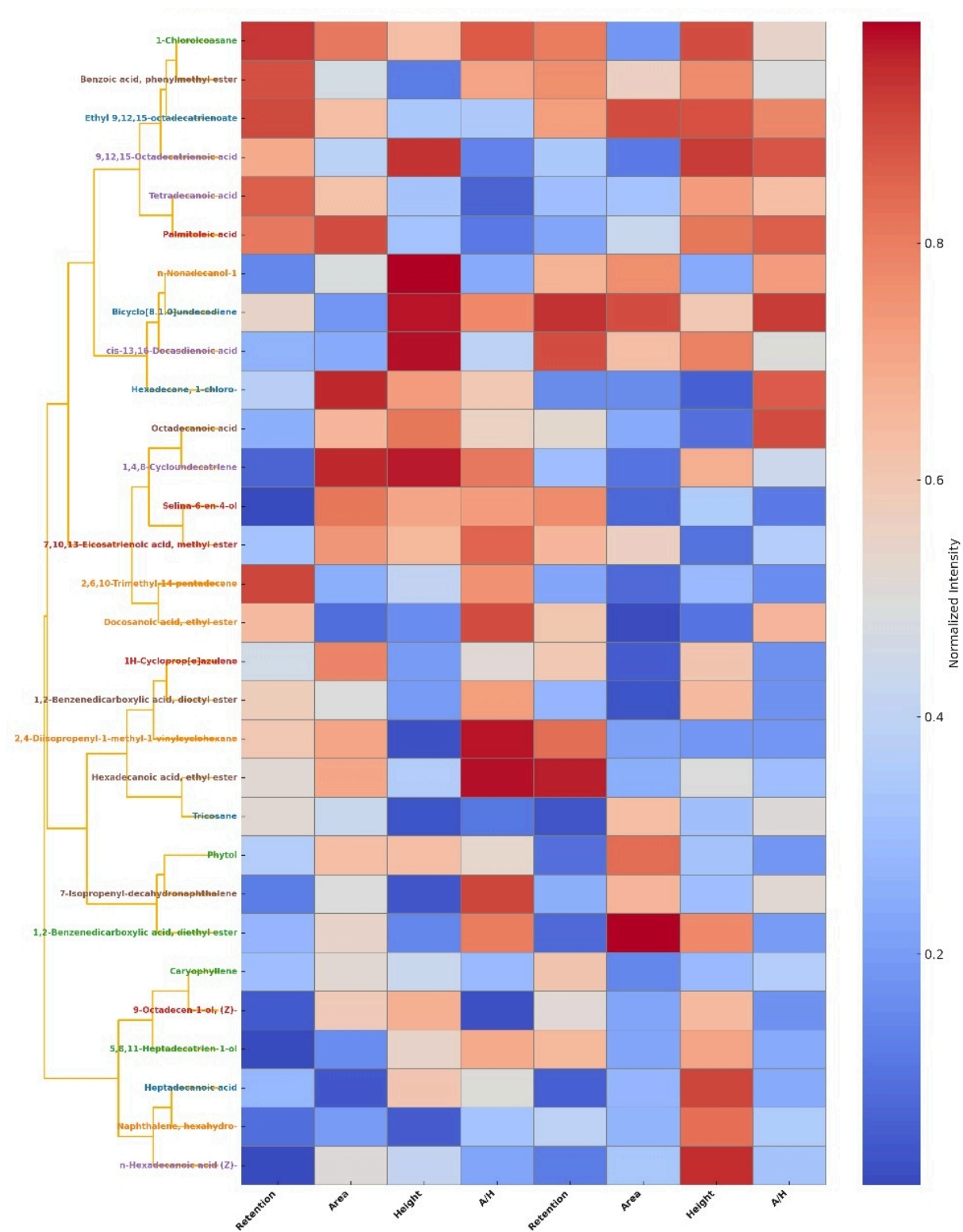


Figure 4

Comparative analysis of Phytobiovolatiles between fresh and refrigerated curry leaf samples.