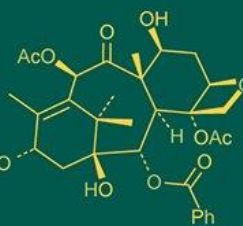
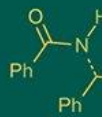


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Kilehira A Marak
Ph.D. Scholar, Department of
Fruit Science, Post Graduate
Institute, Dr. P.D.KV. Akola,
Maharashtra, India

Dr. DM Panchbhai
Professor and Dean, Faculty of
Agriculture, Dr. P. D. K. V.,
Akola, Maharashtra, India

Dr. SR Patil
Department of Fruit Science,
Dr. P. D. K. V., Akola,
Maharashtra, India

Dr. SG Bharad
Professor and Head,
Department of Fruit Science,
Dr. P. D. K. V., Akola,
Maharashtra, India

Dr. Amit G Deshmukh
Associate Professor,
Department of Biotechnology,
Dr. PDKV, Akola, Dr. P. D.
K. V., Akola, Maharashtra,
India

DS Kankal
Assistant Professor
Department of Soil science
Dr. P. D. K. V., Akola,
Maharashtra, India

Sonia Thiyam
Ph.D. Scholar, Department of
Floriculture and Landscaping,
Dr. P. D. K. V., Akola,
Maharashtra, India

Dr. Rohit Y Karde
Assistant Professor,
Department of Agronomy,
BFGCOA, Buldhana,
Maharashtra, India

Corresponding Author:
Kilchira A Marak
Ph.D. Scholar, Department of
Fruit Science, Post Graduate
Institute, Dr. P.D.KV. Akola,
Maharashtra, India

Comparative evaluation of essential oil yield and GC-MS characterization of volatile constituents in different citrus species and plant parts

Kilchira A Marak, DM Panchbhai, SR Patil, SG Bharad, Amit G Deshmukh, DS Kankal, Sonia Thiyam and Rohit Y Karde

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Abstract

The increasing demand for natural bioactive compounds has enhanced interest in the utilization of citrus by-products as alternative sources of essential oils. The present investigation was conducted during 2023-24 and 2024-25 to evaluate essential oil content, oil recovery, oil yield and chemical composition of leaf and peel oils of mandarin (*Citrus reticulata* Blanco), sweet orange (*Citrus sinensis* L. Osbeck) and acid lime (*Citrus aurantifolia* Swingle). Essential oils were extracted through hydrodistillation using a Clevenger-type apparatus and characterized by Gas Chromatography-Mass Spectrometry (GC-MS). Among the citrus species studied, acid lime recorded significantly higher oil recovery (9.16 ml kg⁻¹), oil yield per plant (253.00 ml), and oil yield per hectare (69.50 L ha⁻¹) compared with mandarin and sweet orange. Peel samples exhibited greater oil recovery than leaf samples across all species. GC-MS analysis revealed considerable variation in the chemical profiles of leaf and peel oils among the citrus species. β -Phellandrene was identified as the major constituent in mandarin and sweet orange leaf oils, while D-limonene predominated in acid lime and sweet orange peel oils. The results demonstrate the potential of citrus leaves and peels as valuable raw materials for essential oil extraction, supporting the sustainable utilization and value addition of citrus waste.

Keywords: Citrus species, essential oil, GC-MS, D-limonene, β -phellandrene, citrus peel, citrus leaf

Introduction

Citrus fruits are among the most widely produced crops in the world, and their processing industries generate large amounts of peel as waste. These peels are not just fibrous by-products: they contain essential oils (EOs) rich in volatile compounds that can be recovered and turned into valuable ingredients for food, cosmetic and pharmaceutical uses (Ibrahim *et al.*, 2024) [2]. Growing interest in waste reduction, green ingredients and circular economy models has pushed researchers to examine how citrus peel EOs can replace synthetic additives and improve product safety and quality. Essential oils represent one of the most economically important products obtained from citrus wastes. Citrus oils are widely utilized in food flavouring, pharmaceuticals, cosmetics, perfumery and aromatherapy because of their pleasant aroma and biological properties. The quality and composition of citrus essential oils depend upon species, plant parts, environmental conditions and extraction methods.

Across many Citrus species and cultivars, peel EOs are dominated by the monoterpene D-limonene, often accompanied by α -pinene, β -pinene, γ -terpinene, linalool, and related terpenoids that together define the characteristic citrus aroma (Ibrahim *et al.*, 2024; Vasquez-Gomez *et al.*, 2024; Kady *et al.*, 2024) [2, 3, 4]. Despite this common chemical backbone, the exact composition varies markedly with species, hybrid background, growing region, and extraction method, which leads to differences in color, volatility, stability, and biological activity (Lin *et al.*, 2021; Zhang *et al.*, 2022; Yang & Park, 2025) [1, 6, 7]. This diversity allows producers to tailor specific citrus EOs to particular applications, such as strong antibacterial action, enhanced antioxidant protection, or desirable sensory profiles.

From a technological and environmental perspective, using peel EOs adds value to a low-cost residue stream and can help reduce landfill burdens and environmental

contamination from citrus processing (Ibrahim *et al.*, 2024; Grover *et al.*, 2024; Gómez-Mejía *et al.*, 2025) [2, 8, 9]. At the same time, the demonstrated antioxidant and antimicrobial activities of these oils have made them promising candidates as natural preservatives in foods, active components in edible coatings and packaging, and functional ingredients in personal-care formulations (Gupta, 2022; Anastasopoulou *et al.*, 2024) [10, 11]. Recent work also explores advanced processing (molecular distillation, nanoencapsulation, biorefinery schemes) to better stabilize these oils, concentrate key actives like D-limonene, and integrate them into modern product matrices (Vasquez-Gomez *et al.*, 2024; Mori-Mestanza *et al.*, 2025) [3, 12]. Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful analytical technique used for identification of volatile compounds present in essential oils. Therefore, the present investigation was undertaken to evaluate essential oil recovery, oil yield and chemical composition of leaf and peel oils of mandarin, sweet orange and acid lime.

Materials and Methods

The study was conducted during 2023-24 and 2024-25 at the Analytical and Post-Harvest Technology Laboratory, Department of Fruit Science, Faculty of Horticulture and AICRP on Medicinal and Aromatic Plants, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India. Experiment consists of two factors the factor A consists of different citrus species, *i.e.* S1-Mandarin (*Citrus reticulata* Blanco), S2-Sweet orange (*Citrus sinensis* L. Osbeck) and S3-Acid lime (*Citrus aurantifolia* Swingle), whereas, factor B consists of different plant part of citrus, P1-Fresh leaves and P2-Fresh peel.

Leaves oil content (ml)

Fresh citrus leaves were collected from healthy plants and weighed immediately to record fresh leaf weight. A sample of 150 g fresh leaves (without drying) was taken and mixed with distilled water in a leaf: water ratio of 1:3 (w/v) in a round bottom flask of the Clevenger apparatus. The mixture was heated using a heating mantle for about 3 hours until oil collection stopped. The condensed oil and water were collected, and the oil was separated using a separating funnel. The volume of pure essential oil obtained was measured in millilitres (ml) and recorded as leaf oil content. Oil content was calculated by dividing the quantity of essential oil obtained by the weight of fresh citrus peel or leaves used for extraction and multiplying by 100.

Peel oil content (%)

Fresh citrus peel was separated from the fruits and weighed immediately to record fresh peel weight. A sample of 150 g fresh peel (without drying) was taken and mixed with distilled water in a peel: water ratio of 1:3 (w/v) in a round bottom flask of the Clevenger apparatus. The mixture was heated using a heating mantle for about 3 hours until oil collection stopped. The condensed oil and water were collected, and the oil was separated using a separating funnel. Peel oil content percentage was calculated by dividing the quantity of essential oil obtained from fresh citrus peel by the weight of fresh peel used for extraction and multiplying by 100.

Leaf and peel oil yield per plant

Leaf and peel oil yield per plant (ml) was calculated by

multiplying the oil recovery percentage by the total leaves weight per plant. Subsequently, the total leaf oil yield per hectare was determined by multiplying the per-plant yield by the established plant population density.

Leaf and peel oil yield per hectare

Leaf and peel oil per hectare was calculated by multiplying the per-plant oil yield by the plant population of 277. The calculated leaf oil yields per hectare for the respective citrus varieties were then analyzed to assess the potential economic productivity of the plantation. Estimated leaf and peel oil yield per hectare (l/ha) was calculated by multiplying the peel oil yield per plant by the total number of plants per hectare. The value obtained in millilitres was then converted into litres.

GCMS profiling of Chemical components in different essential oil

All required glassware were cleaned and dried properly, and the GC-MS instrument was calibrated before analysis. The materials used included GC-MS instrument, analytical balance, micropipettes (20-200 μ L and 100-1000 μ L), 2 mL GC vials, ethanol, and Citrus oil sample. Twenty microlitres of Citrus Essential oil sample were taken in a 2 mL GC vial, and 980 μ L of ethanol was added. The vial was closed properly, shaken well, and vortexed for 1 minute to ensure uniform mixing. After mixing, 100 μ L of the upper layer was taken and transferred into another 2 mL GC vial. Then, 900 μ L of ethanol was added for further dilution, and the solution was mixed properly. The GC-MS instrument was set according to the required chromatographic conditions. The initial oven temperature was maintained at 40 °C and increased at a ramp rate of 10 °C per minute up to 200 °C with a hold time of 13 minutes. The total run time was 32 minutes. The injector temperature was set at 200 °C with split mode (25:1). HP-5 column (30 m $\times$ 250 μ m $\times$ 0.25 μ m) was used with constant column flow of 2 mL. The MSD transfer line temperature was maintained at 200 °C, and the acquisition type was set as scan. One microlitre of the prepared diluted sample was injected into the GC-MS instrument, and the run was started. After completion of the analysis, the chromatogram was recorded, and the volatile oil compounds present in the sample were identified.

Results

Essential oil content in different citrus species

It is evident from the data depicted in Fig.1 that, different citrus species recorded varied values accounting for different plant parts. Among the species, acid lime (S₃) recorded the highest mean essential oil content, followed by mandarin (S₁), whereas the lowest mean essential oil content was observed in sweet orange (S₂). With respect to plant parts, both leaves (P₁) and peel (P₂) recorded an almost identical mean essential oil content of 0.63%. The treatment combinations mandarin leaves (S₁P₁) and mandarin peel (S₁P₂) recorded essential oil contents of 0.67% and 0.66%, respectively. The lowest essential oil content was observed in sweet orange leaves (S₂P₁) (0.36%), followed by sweet orange peel (S₂P₂) (0.41%). However, the interaction effect between citrus species and plant parts was found to be non-significant.

Essential oil yield varied significantly among citrus species and plant part used. Similarly to our findings in citrus *reticulata* the yield of *petitgrain* of several citrus genotypes,

extracted through hydrodistillation of fresh leaves varied from 0.05 to 0.60%. Mandarin showed moderate oil content, while sweet orange exhibited comparatively lower oil concentration. (M. Sulzbach *et al.*, 2021) ^[13]. Sweet orange leaves 0.4% yielded from fresh leaves through steam distillation (Tan Phat Dao *et al.*, 2019) ^[14]. The higher oil content in acid lime may be attributed to greater accumulation of volatile terpenoids and essential oil glands in the peel and leaves similar to our findings (Budiarto *et al.*, 2024) ^[15] in kaffir lime leaves lime oil extracted from fresh green leaves which yield highest 1.5%.

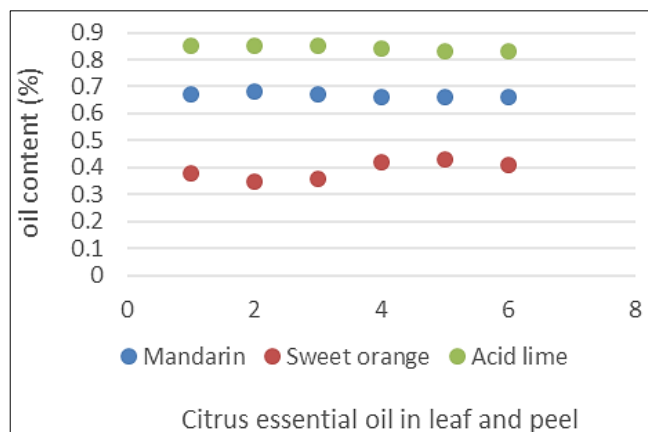


Fig 1: Leaf and Peel essential oil content of Mandarin, Sweet orange and acid lime

Essential oil yield per plant in different citrus species

Among the different citrus plant parts illustrated in fig no.2 where mandarin peel (S_1P_2) recorded the highest essential oil yield per plant, followed by acid lime peel (S_3P_2) and acid lime leaves (S_3P_1). The lowest essential oil yield per plant was observed in sweet orange leaves (S_2P_1), while sweet orange peel (S_2P_2) recorded.

Mandarin showing the highest oil yield per plant, followed by acid lime and then sweet orange, aligns with reports that oil content varies greatly among citrus species and cultivars, sometimes by several-fold (Catucuamba *et al.*, 2026; Luro *et al.*, 2020; Kamal *et al.*, 2011) ^[16, 18, 17]. For example, peel oil yields differed markedly among oranges, mandarins, grapefruits, and limes, with some varieties producing up to ~3-4 times more oil than others under the same extraction conditions (Catucuamba *et al.*, 2026; Luro *et al.*, 2020; Kamal *et al.*, 2011) ^[16, 18, 17]. These differences are attributed to genetic factors and intrinsic anatomical traits such as oil gland density (Santos *et al.*, 2023; Luro *et al.*, 2020) ^[20, 18].

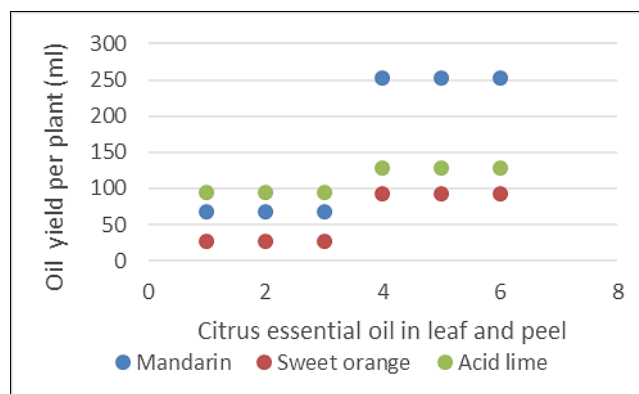


Fig 2: Leaf and Peel oil yield of mandarin, sweet orange and acid lime per plant

Estimated essential oil yield per hectare

Mandarin peel (S_1P_2) recorded the highest essential oil yield per hectare, followed by acid lime peel (S_3P_2) and acid lime leaves (S_3P_1). The lowest essential oil yield per hectare was observed in sweet orange leaves (S_2P_1) (7.40 L ha⁻¹), while sweet orange peel (S_2P_2) recorded 25.67 L ha⁻¹. shown in fig. No. 3

Studies consistently report marked variability among citrus species and cultivars in peel oil yield, even under similar extraction conditions (Catucuamba *et al.*, 2026; Luro *et al.*, 2020; Santos *et al.*, 2023; Kamal *et al.*, 2011; Azevedo *et al.*, 2019) ^[16, 18, 20, 17, 21]. Mandarin-or mandarin-derived genotypes often show relatively high peel oil content, while sweet orange yields are moderate but highly cultivar-dependent (Luro *et al.*, 2020; Santos *et al.*, 2023; Kamal *et al.*, 2011; Azevedo *et al.*, 2019) ^[18, 20, 17, 21]. This aligns with your higher per-hectare yield in mandarin compared with sweet orange and acid lime. Furthermore, these findings suggest that refining extraction protocols specifically for Mandarin peel could substantially enhance industrial efficiency.

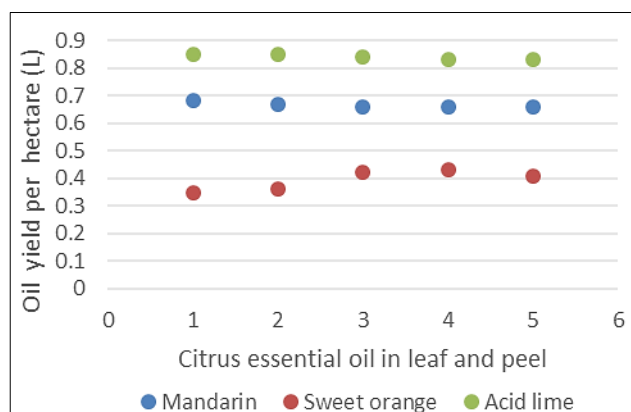


Fig 3: Leaf and Peel oil yield of mandarin, sweet orange and acid lime per hectare.

Citrus oil components

The GC-MS analysis demonstrated considerable variation in the chemical composition of essential oils among citrus species and plant parts. The predominance of β -Phellandrene in mandarin leaf, mandarin peel and sweet orange leaf oils indicates its important role in imparting the characteristic aroma and biological activity of these oils. β -Phellandrene is known for its contribution to plant defence mechanisms and its potential antimicrobial properties. In contrast, the essential oils obtained from sweet orange peel and acid lime tissues were characterized by a high concentration of D-Limonene, a monoterpene widely recognized for its pleasant citrus fragrance, antioxidant activity, antimicrobial properties and extensive industrial applications in food, pharmaceutical, cosmetic and fragrance industries.

The higher proportion of oxygenated compounds such as Citral, Neral, Linalool and Geranyl derivatives in acid lime oils further contributed to their distinctive aroma profile and potential therapeutic value. Variations in the number and concentration of volatile compounds among species and plant parts may be attributed to genetic differences, biosynthetic pathways, physiological characteristics and the distribution of oil glands within citrus tissues. Overall, the results indicate that citrus wastes, particularly leaves and peels, are rich sources of valuable volatile compounds and

can be effectively utilized for the extraction of essential oils with applications in pharmaceutical, nutraceutical, cosmetic, food flavouring and aromatherapy industries.

Many studies report D-limonene as the main constituent in citrus peel oils, often forming 70-97% of the oil in orange, lemon, mandarin and other Citrus peels (Vasquez-Gomez *et al.*, 2024; Anastasopoulou *et al.*, 2024; Luro *et al.*, 2023; Boughendjioua *et al.*, 2020; Li *et al.*, 2022; Mohammed *et al.*, 2024; Shalaby *et al.*, 2023; Ferrer *et al.*, 2022; Yang & Park, 2025) [3, 11, 18, 20, 23, 24, 25, 28, 7]. However, some mandarin and sweet orange peels can show higher levels of β -phellandrene or other terpenes, with β -phellandrene and related monoterpenes identified as major or discriminant compounds in certain cultivars and peel extracts (Luro *et al.*, 2023; Zhang *et al.*, 2022; Ferrer *et al.*, 2022) [18, 6, 28]. Leaf oils usually have more diverse profiles than peels, with higher proportions of oxygenated monoterpenes and sesquiterpenes and multiple chemotypes (e.g., sabinene/linalool, γ -terpinene/linalool, methyl N-methylantranilate) (Lota *et al.*, 2001; Fanciullino *et al.*, 2006; Dev & Mol, 2024) [30, 29, 27].

Conclusion

The results revealed significant differences among citrus species and plant parts with respect to essential oil content, oil yield and chemical composition. Acid lime emerged as the most promising species for essential oil extraction owing to its superior oil content, while mandarin exhibited higher oil recovery on a per plant and per hectare basis. Peel tissues consistently recorded greater oil yield than leaves due to the abundance of oil glands present in the flavedo region. The GC-MS analysis further confirmed the richness of citrus wastes in volatile compounds of industrial importance. The dominance of β -Phellandrene in mandarin leaf, mandarin peel and sweet orange leaf oils, and D-Limonene in sweet orange peel and acid lime oils, highlights their potential utilization in flavour, fragrance, cosmetic, pharmaceutical and food industries. The presence of other important compounds such as β -Ocimene, Citral, Neral, Linalool, Geranyl oleate and Alloaromadendrene further enhances the commercial value of these essential oils.

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