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RESEARCH ARTICLE

# Yield and Physicochemical Characteristics of Kaffir Lime Leaf Essential Oils Subjected to Different Post-Harvest Treatment

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## Abstract

The importance of kaffir lime leaf as a raw material for essential oils (EOs) is gaining attention due to its commercial value, particularly for its citronellal rich EOs used in the perfume and pharmaceutical industries. However, there is currently no established quality reference for kaffir lime leaf EOs, especially considering various post-harvest handling methods. This study aimed to characterize the physicochemical properties and yield of kaffir lime EOs subjected to different post-harvest treatments. Kaffir lime leaves were collected from local farmers in Bogor and underwent several post-harvest treatments: control/ fresh green leaves, milling to produce green leaf flour, drying to produce dry brown leaves, and milling and drying to produce brown leaf flour. The results revealed that post-harvest treatment generally decreased oil yield and increased darkness color, specific gravity, and refractive index of the tested EOs. Furthermore, post-harvest treatment altered the metabolite profile as revealed by GCMS analysis. Both milling and drying, either individually or in combination, resulted in an increase in the relative percentage of caryophyllene and citronellol, while the levels of linalool and citronellal decreased. For the fragrance industry, which requires high citronellal levels, EOs sourced from fresh green leaves showed significantly higher citronellal content (59-65% higher than older leaves), emphasizing the importance of avoiding any drying and milling treatment.

Keywords: citronellal, drying, GCMS, milling, leaf flour.

## Introduction

Kaffir lime leaf is a horticultural commodity valued for its role as a cooking spice and as raw material for essential oils (EOs) (Budiarto et al., 2021a; Budiarto et al., 2021b). Like many horticultural commodities, kaffir lime leaves are perishable due to their high-water content (Poerwanto and Susila, 2014), making them susceptible to damage during the post-harvest period, resulting in yield loss. Yield losses in fresh horticultural commodities typically range from 40-50% (Suwono, 2013), whereas yield loss in citrus fruits and kaffir lime due to inadequate post-harvest handling can reach up to 25% (Asni, 2015). However, specific yield losses in citrus leaf commodities, such as kaffir lime, during the post-harvest period have not been extensively reported.

Post-harvest activities encompass the entire process from harvest until the material is ready for consumption by consumers (Utama and Antara, 2013). Various post-harvest methods can be applied to kaffir lime leaf products, as these products are stored, packaged, and sold in different forms internationally, including fresh green leaves, frozen green leaves, and dried leaves (Wongpornchai, 2012). While the domestic market mainly recognizes fresh green leaves as the primary product of kaffir lime agriculture, there is a growing trend towards diversifying products, such as leaf EOs and leaf flour (Budiarto et al., 2019). Both products undergo drying and milling processes, highlighting the importance of studying these post-harvest treatments as they directly influence quality,

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particularly sensory acceptance (Budiarto et al., 2021a).

Quality assessment of citrus fruit commodities typically involves attributes such as size, weight, color, shape, texture, and cleanliness, which serve as indicators of product excellence (Sutopo, 2011). However, there is a lack of established quality references for kaffir lime leaf and its derivative products. Evaluation of EOs quality can be based on yield and physicochemical characteristics, including specific gravity, refractive index, color, and metabolite profile (Mahulette et al., 2020; Hariyadi et al., 2020). Kaffir lime leaf essential oils contain various metabolites, with citronellal being the predominant and economically significant compound (Efendi et al., 2021; Budiarto et al., 2022) due to its potential as an intermediate compound for fragrance synthesis (Lenardao et al., 2007). Additionally, citronellol, linalool, and caryophyllene, identified as major metabolites (Budiarto and Sholikin, 2022), are essential for assessing the quality of kaffir lime EOs (Riyadi, 2012). Therefore, this study aimed to investigate the yield and physicochemical characteristics of kaffir lime EOs subjected to different post-harvest treatments.

## Material and Methods

This study used kaffir lime leaves collected from local farmers in Bogor (6.609042, 106.783605, 263 m above sea level), Indonesia. Kaffir lime plants aged at least 1 year, with healthy leaves, were used. Mature, fully developed, green, and bifoliate leaves were utilized for all initial treatments. Up to 1 kg (wet weight) of leaf samples were prepared for each treatment. Kaffir lime leaves were exposed to several post-harvest treatments, i.e., fresh green leaves; green leaf flour; dry brown leaf, and brown leaf flour, as displayed in Figure 1. Fresh green leaves were prepared by conducting sortation and grading, only allowing fresh, mature, green to dark green color, and normal bifoliate leaf shapes, as the unique character of kaffir lime leaf (Budiarto et al., 2021c). Green leaf flour was subjected to oven-drying ($40^{0}$C) for 48 hours so that it experienced a 66% weight loss; then, it was ground using a grinder equipped with a 1 mm sieve. The dry brown leaves had no sortation and grading, were dried at room temperature (25-$31^{0}$C) for 10 days and experienced natural senescence as indicated by dry and brown leaf. Brown leaf flour is dry brown leaves that were ground and filtered with a 1 mm sieve. Treatment selection was guided by prior research findings indicating variations in sensory preference among kaffir lime leaves exposed to fresh, drying, and milling processes (Budiarto et al., 2021a). The morphological appearance of kaffir lime leaf after being subjected to various post-harvest treatments was depicted in Figure 1.


Figure 1. Kaffir lime leaf exposed to various post-harvest treatments: fresh green leaves, green leaf flour, dry brown leaf, and brown leaf flour (from left to right).

Four types of post-harvest treatment were subjected to several analyses: EOs yield, EOs physical and chemical characteristics. Both yield and physical character tests of EOs were conducted at the Indonesian Medicinal and Aromatic Crop Research Institute, Bogor, Indonesia. All samples were subjected to water-steam distillation using a stainless-steel distillery kettle for about 3 hours. Anhydrous sodium sulfate was used to dry obtained EOs, then its volume was measured. The yield (%) was the ratio of obtained EOs volume against the weight of raw material used to distillate, following previous study by Efendi et al. (2021).

EOs physical variables, i.e., color, specific gravity, and refractive index were analyzed. Color EOs are observed directly with an observation distance between the eye and the sample of about 30 cm. Specific gravity was determined based on the ratio of oil to water's weight at the same volume and temperature, using a pycnometer (Iwaki, Picno-5M) with a capacity of 5 ml. The refractive index is measured with a refractometer based on the refractive angle of essential oils, which is maintained at constant temperature conditions. The measurement temperature is stable at $29^{0}$C, while the reference temperature for working the refractive index test is $20^{0}$C.

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EOs chemical characteristics included citronellal absolute quantification and qualitative metabolite profile using targeted- and untargeted GC-MS analysis, respectively. Present study used (i) Agilent 7890 Gas Chromatography interfaced to 5975 Mass Spectrometry (Agilent Technologies Inc, USA) with 1 μL injection volume and a split ratio of 10:1, (ii) Agilent 19091N-133HP-INNOWax Polyethylene Glyco column with a length, thickness, and diameter of 30 m, 0.25 μm, and 250 μm, respectively; and (iii) helium as carrier gas. Present study employed several conditions: a constant helium flow with a rate of 0.9 μl per minute column pressure of 7.06 psi; designed column regime from $60^0$C to $210^0$C ($2^0$C incremental per minute up to $150^0$C, kept at $150^0$C for 1 minute, $20^0$C incremental per minute up to $210^0$C, and then kept at $210^0$C for 10 minutes) and designed injector regime of about $230^0$C. For targeted GC-MS analysis, the present study involved a standard compound of citronellal, divided into 10 different concentrations (1%-10%) to make a proper regression curve for quantifying citronellal content in kaffir lime EOs, following the previous method (Efendi et al., 2021). Untargeted GC-MS analysis, which employed similar conditions to targeted ones, was applied to EOs of 4 treatments and resulted in the EOs qualitative metabolite profile. An initial selection of metabolites to compose leaf EOs profile was carried out by eliminating certain annotated metabolites with low quality, i.e., lower than 80%.

*Data Analysis*

Color data was visually compared by displaying a figure of four treatments. Data of the variable of oil yield, specific gravity, refractive index, and leaf metabolite profile was described comparatively among four post-harvest treatments. The data of citronellal content was subjected to analysis of variance and then continued by Duncan multiple range test (DMRT) at α 5% level in Statistical Tool for Agricultural Research (STAR) version 2.0.1.

## Results and Discussion

*Post-harvest Treatment Decreased Yield and Physical Quality of Kaffir Lime Leaf Essential Oils*

The yield of kaffir lime EOs is significantly affected by post-harvest treatment. Yield is calculated as the ratio of the weight of obtained EOs to the weight of refined raw materials and is expressed as a percentage. Typically, kaffir lime leaf EOs are extracted from relatively fresh green leaves, which yield the highest oil yield at 1.5% (Figure 2a). However, various post-harvest treatments, including drying, grinding, and their combinations, lead to a reduction in oil yield.

The oil yield decreased by 48% when green leaves were ground into powder compared to EOs obtained

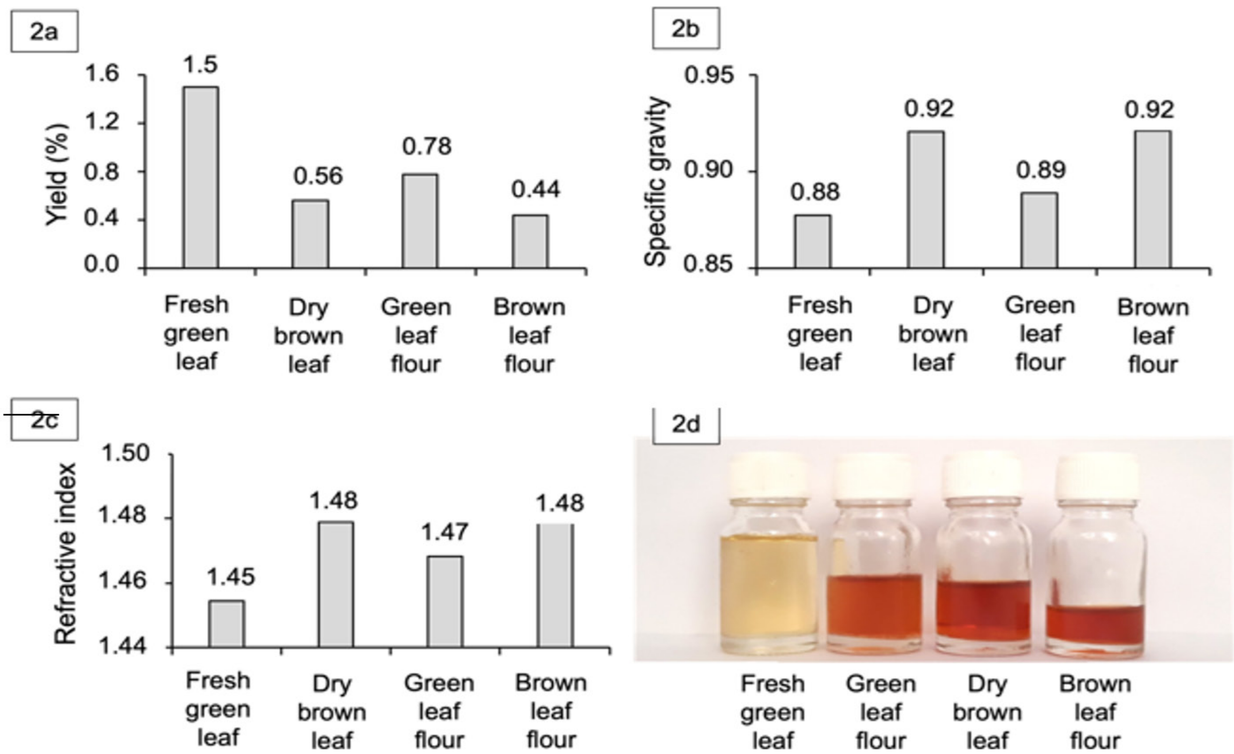


Figure 2. Essential oils yield (a) and specific gravity (b), refractive index (c), and color (d) of kaffir lime leaf after various post-harvest treatments.

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from fresh green leaves, resulting in a difference in oil yield of 0.72% between the two treatments. Similarly, the oil yield decreased by 63% when dried brown leaves were used, with a difference in oil yield of 0.94% compared to EOs from fresh green leaves. Furthermore, the oil yield decreased by 71% when brown leaf powder underwent both grinding and drying, resulting in a difference in oil yield of 1.11% compared to EOs from fresh green leaves (Figure 2a).

In addition to yield, post-harvest treatment of leaf raw materials also impacts the physical quality of essential oils (EOs), particularly specific gravity and refractive index (Figure 2b-c). Specific gravity, a key attribute of EOs, is determined by comparing the weight of oil to that of water at the same volume and temperature. The refractive index, another essential quality indicator, is measured based on the angle of refraction of EOs under fixed temperature conditions. The highest values of specific gravity and refractive index were observed in EOs derived from brown dry leaf powder, followed by those from dried brown leaves and green leaf powder. Conversely, EOs from fresh green leaves exhibited the lowest specific gravity and refractive index values (Figure 2b-c). The standard range for specific gravity of kaffir lime leaf EOs is typically 0.830 – 0.910 (Riyadi, 2012). While the specific gravity of EOs obtained from both fresh green leaves and green leaf powder fell within this standard range, those from the other two treatments exceeded it. Similarly, the standard range for refractive index of lime leaf EOs is 1.445 – 1.465 (Riyadi, 2012), with EOs from fresh green leaves and green leaf powder falling within this range, whereas those from brown leaf powder and brown dry leaf powder exceeded it. It is evident that post-harvest treatment leads to an increase in specific gravity and refractive index values of kaffir lime leaf essential oil, likely due to associated changes in EO color.

The color of kaffir lime leaf essential oils is also affected by the post-harvest treatment of raw materials (Figure 2d). EOs extracted from fresh, green kaffir lime leaves typically exhibit a bright, clear yellow color, while those from green leaf powder tend to display a more orange hue. Additionally, EOs derived from brown leaf powder and brown dry leaves appear even darker orange (Figure 2d). This variation in color could influence consumer preferences for kaffir lime leaf essential oil. For instance, in the case of patchouli essential oil, consumers generally prefer lighter colors over darker ones. The changes observed in physical quality, including color, specific gravity, and refractive index, may be attributed to alterations in the composition and proportion of metabolites within the essential oil.

*Post-harvest Treatment Decreased Citronellal Content and Changed Metabolite Profile*

Post-harvest treatments applied to kaffir lime leaf raw materials induce changes in the composition of the produced essential oils. Typically, a standard kaffir lime leaf essential oil contains at least four compound components: citronellal, citronellol, linalool, and caryophyllene (Riyadi 2012). All these compounds belong to the terpene group, with three being monoterpenes and only caryophyllene belonging to the sesquiterpene subgroup. The present study demonstrates changes in the proportions of these four components (Table 1). The relative amount of citronellol and caryophyllene tends to increase, while citronellal and linalool tend to decrease. Specifically, the increase in citronellol content in essential oils derived from dried brown leaves, dry brown leaf powder, and green leaves is 20%, 13%, and 9%, respectively. Similarly, the increase in caryophyllene content in essential oils derived from dried brown leaves, dried brown leaf powder, and green leaf powder is 9%, 14%, and 9%, respectively. The levels of linalool in oil from green leaf powder decreased by 0.5%, and this compound was not detected in essential oils derived from dried brown leaves and brown leaf powder (Table 1). These changes are undesirable as they result in essential oils that do not meet the standard commercial quality criteria for kaffir lime essential oils, which typically contain 65-75% citronellal, 3.5-5.5% linalool, 1.9-6% citronellol, and 0-2.5% caryophyllene (Riyadi, 2012). Table 1 also illustrates the increase in phenolic compounds (eugenol and myristicin) in essential oils derived from brown leaves, reflecting the effects of leaf aging. Previous studies have shown that older leaves tend to have higher phenol levels due to cell wall degradation with aging (Pristiana et al., 2017). Lignin, a key component of cell walls, is derived from phenylpropanoid alcohols such as coniferyl, coumaryl, and sinapyl alcohol, which are synthesized from phenylalanine through various cinnamic acid derivatives (Mastuti, 2016). This degradation during leaf aging likely contributes to the increased phenolic compounds observed in dried brown kaffir lime leaves. Similar findings have been reported for dried gambier leaves, where higher drying temperatures led to greater release of phenolic compounds from cell walls (Wazir et al., 2011).

Citronellal is the primary component found in lime leaf essential oils, comprising the largest proportion in the GCMS profile (Agusta 2000; Tinjan and Jirapakkul 2007; Ratseewo et al. 2016; Budiarto and Sholikin 2022). This compound holds commercial value due to its non-toxic nature, making it useful in the perfume and pharmaceutical industries as an

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Table 1. Untargeted GCMS analysis result relative metabolite amount (%) of kaffir lime leaf EOs in response to various post-harvest treatment.

| Metabolites (%) | Fresh green leaves | Green leaf flour | Dry brown leaf | Brown leaf flour |
|---|---|---|---|---|
| 1. Citronellal | 74.56 | 39.06 | 21 | 9.8 |
| 2. Citronellol | 2.91 | 12.19 | 23.15 | 16.15 |
| 3. Caryophyllene | 0.89 | 10.22 | 10.38 | 15.13 |
| 4. Myristicin | 0.49 | 4.08 | 10.61 | 12.73 |
| 5. Linalool | 3.88 | 3.43 | nd | nd |
| 6. Caryophyllene oxide | 0.7 | nd | 4.91 | 2.81 |
| 7. Citronellic acid | 1.12 | 2.5 | nd | nd |
| 8. Isopulegol | 1.01 | 0.87 | nd | nd |
| 9. Beta Myrcene | nd | 0.33 | nd | nd |
| 10. Patchouli alcohol | nd | nd | 1.25 | 0.8 |
| 11. Nerolidol | nd | 2.78 | 4.1 | 1.36 |
| 12. Eugenol | nd | 2.93 | 0.45 | 14.06 |
| 13. D-Limonene | 0.46 | nd | nd | nd |

Note: nd – not detected.

intermediate ingredient in the synthesis of isopulegol, menthol, and citronellol (Lenardao et al. 2007; Api et al. 2021). Citronellal is also present in the essential oil of citronella leaves (*Cymbopogan winterianus* Jowitt), albeit in a lower relative percentage of about 40% (Lestari et al. 2012). Experimental results have shown that kaffir lime leaves contain citronellal in relative levels ranging from 66.8-85.5%, higher than that found in citronella, indicating the potential of kaffir lime leaves to substitute citronella in terms of citronellal production.

The relative metabolite amount (%) in Table 1 can serve as input for calculating citronellal levels in various kaffir lime leaf essential oils using the regression equation on the optimization curve of citronellal (Figure 3a). Post-harvest treatment significantly impacts the citronellal levels in essential oils (Figure 3b). Drying green kaffir lime leaves for 10 days to produce dried brown leaves results in significantly lower citronellal levels compared to essential oils from green leaves, with a gap of 59%. The most significant reduction in citronellal content is observed in essential oils derived from brown leaf powder, with a gap of 65% compared to essential oils from green leaves. This reduction is attributed to the air-drying and powdering treatment of brown leaf powder. Citronellal levels in essential oils from green leaf powder also decrease by 34% compared to those from fresh green leaves. Besides the milling process, this decrease is also attributed to oven treatment. Both milling and drying are believed to expedite the conversion of citronellal to citronellol. Previous research has suggested that this conversion occurs through the reduction of the aldehyde group to the alcohol group in citronella oil (Wijayanti, 2015). The reduction in citronellal content is 31% lower in the oven-drying method compared to air-drying. However, this study is still limited to providing basic chemical information for the quality assessment of kaffir lime leaf essential oils and requires further clarification through other chemical analysis approaches.

This is the first study reporting the effects of postharvest treatments on kaffir lime leaf EOs quality. Our findings reveal that leaf drying and milling significantly diminish EOs yield, alter the composition of major metabolites, and notably decrease citronellal content. The reduction in citronellal content is particularly concerning as it correlates with diminished fragrance quality. Our work underscores the importance of considering these factors when developing citronellal-rich kaffir lime products.

Citronellal holds significant economic value owing to its powerful lemony scent (Agouillal et al. 2017; Wany et al. 2013), making it a crucial intermediate in perfume manufacturing, pharmaceutical synthesis, and the production of compounds like isopulegol, menthol, and citronellol (Yahya et al. 2021; Lenardao et al. 2007; Jacob et al. 2003). Moreover, beyond its aromatic properties, citronellal exhibits diverse pharmacological benefits, including antibacterial (Vimol et al. 2012), antifungal (Rammanee and Hongpattarakere 2011; Ouyang et al. 2021), anti-inflammatory (Lota et al. 2002), and mosquito-repellent properties (Sharma et al. 2019; Dicken and Bohbot 2013; Nerio et al. 2010).

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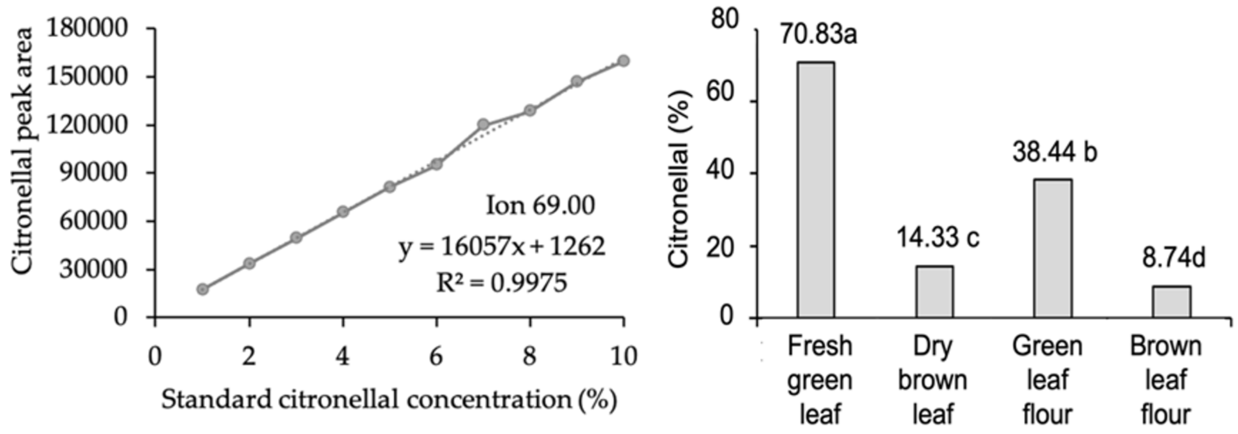


Figure 3. Optimization curve of absolute content of citronellal in ion 69 (a) and citronellal levels in lime leaf EOs subjected to different post-harvest treatments (b). (P1): fresh green leaves, (P2): green leaf flour, (P3): dry brown leaf, and (P4): brown leaf flour.

Enhancing secondary metabolite content, such as citronellal, through agricultural practices compared to wild samples presents an intriguing avenue for future research (Zhang and Guo 2020). Subsequent studies should focus on formulating on-farm cultivation recommendations aimed at maintaining high citronellal content. Additionally, optimizing the drying and milling processes to produce kaffir lime leaf EOs and leaf flour with elevated citronellal levels represents another promising area for future investigation.

## Conclusion

Post-harvest treatments, including drying and milling, implemented to produce green leaf powder, dried brown leaf powder, and dried brown leaves, induce changes in the yield and physical attributes of essential oils (EOs). These modifications include: (i) a reduction in oil yield; (ii) an elevation in specific gravity and refractive index; and (iii) a deepening of EOs' color due to shifts in metabolite proportions. The relative percentage of caryophyllene and citronellol tends to escalate, whereas levels of linalool and citronellal diminish with these post-harvest treatments. These findings underscore the importance of sourcing EOs from fresh green leaves for industries necessitating high citronellal content in fragrances, thereby avoiding drying and milling treatments.

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