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The Effects of Preharvest Mild Shading on the Quality and Production of Essential Oil from Kaffir Lime Leaves (*Citrus hystrix*)

Rahmat Budiarto[A], Roedhy Poerwanto[B], Edi Santosa[B], Darda Efendi[B], Andria Agusta[C]

[A]Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran, Sumedang 45363, Indonesia
[B]Department of Agronomy and Horticulture, IPB University, Bogor 16680, Indonesia
[C]Research Center for Chemistry, National Research and Innovation Agency of Republic of Indonesia, Kawasan Puspitek Serpong, Banten 15314, Indonesia

*Corresponding author; email: rahmat.budiarto@unpad.ac.id

Abstract

Kaffir lime (*Citrus hystrix* DC) is a less popular citrus species commonly used as food spice and source of essential oil. Early studies report the success of preharvest mild shading to increase leaf yield, although there is still limited information on the effect of preharvest shading on the quality of essential oil produced. The aim of this current study is to evaluate the effect of preharvest mild shading factor on the yield, physical characteristics and metabolite fingerprinting of kaffir lime leaves essential oil (KLLEO). One-year-old kaffir lime trees were sampled in two preharvest treatments, i.e., open sun and mild shading (24% light reduction) at Pasir Kuda experimental field, Bogor, Indonesia. Statistical analysis showed that there was no significant effect of preharvest treatment on yield and physical characteristics (color, specific gravity and refractive index) of KLLEO. In contrast, there was a metabolite fingerprinting variation of KLLEO as an effect of mild shading. The relative percentage of bergamol, citronellol, caryophyllene oxide, citronellic acid, isopulegol, isopulegyl formate, limonene, linalool and linalool oxide were increased by mild shading. On the other hand, the main metabolite (citronellal) was significantly reduced by about 10% in shading treatment, as compared to the open-sun ones.

Keywords: citronellal, citronellol, *Citrus hystrix*, refractive index, specific gravity

Introduction

Citrus is one of the major horticultural crops grown worldwide, and they are the most traded horticultural commodity with a high economic value (FAO, 2016). Citrus is consumed in various ways, including among others, as fresh fruit, juice, food flavor, aromatherapy. To meet the high demand for citrus and its derivate product in the market, there is a need to improve the citrus production and quality of the fruits. Citrus growth and yield could be manipulated through certain cultural practices, such as root pruning (Budiarto et al., 2019a), canopy manipulation (Budiarto et al., 2018), and shading (Budiarto et al., 2019b).

One of the citrus species that is widely distributed in tropical Southeast Asian countries is kaffir lime (*Citrus hystrix* DC.). This species is known to have bifoliate leaves and strong aroma (Budiarto et al., 2021a; 2021b). The leaves of kaffir lime is popularly used for food spices and source of essential oil production (Budiarto et al., 2019c). As food spice, the quality of kaffir lime can be assessed through the consumer likeliness level that relies on aroma, color and texture (Budiarto et al., 2021c). In the international market, kaffir lime leaf are often found in dried, fresh or frozen form (Wongpornchai, 2019). In Indonesian local markets, kaffir lime leaf can be marketed as fresh leaves, leaf flour, and essential oil (Budiarto et al., 2019c). Leaf essential oil production highly relies on the amount of leaf biomass production.

Modification of the amount of light by shading (24% reduction of the incoming light) could potentially increase kaffir lime leaf biomass production (Budiarto et al., 2019b). Compared to a control/open sun condition, this light reduction process is reported to significantly improve leaf production to about 59% (Budiarto et al., 2019b). However, there is limited information available on the effect of shading during preharvest period on the yield and metabolite fingerprinting of the essential oils produced from kaffir lime leaves.

Metabolite fingerprinting could be the baseline data to evaluate the potential of *Citrus amblycarpa* as functional food (Budiarto et al., 2017). Metabolite composition and essential oil yield have been previously reported to be affected by shading in Mentha piperita (Costa et al., 2014), *Ocimum selloi* (Costa et al., 2010), Aloysia gratissima (Pinto et al., 2007), *Tetradenia riparia* (Araujo et al., 2018), *Salvia sclarea* (Kumar et al., 2013) and *Melissa officinalis* (Russo and Honermeier, 2017). There is a need to do similar evaluation specifically in kaffir lime, thus this study aimed to evaluate the yield and metabolite fingerprinting under different preharvest condition, i.e., mild shading, as compared to open sun condition.

Material and Methods

Study Site

This study was conducted on March 2019 at Pasir Kuda experimental field of IPB University, Bogor, Indonesia (GPS coordinates: 6.609042, 106.783605; altitude: 263 meters above sea level). One-year old kaffir lime plants were cultured under different preharvest conditions, i.e., open sun and mild shading (24% light reduction artificially created by using shading net). The extraction of essential oil was carried out in the Indonesian Medicinal and Aromatic Crops Research Institute (IMACRI or Balitro). The analysis of Gas Chromatography Mass Spectrometry (GCMS) instrument was carried out at the DKI Jakarta Provincial Health Laboratory, Indonesia.

Data Collection

A nested design with single factor (preharvest condition) with three replications was applied. There were 30 plants provided for each preharvest condition. Plants were cultured under optimal practice, i.e., application of organic fertilizer (0.5 kg per plant), chemical fertilizer (20 g N, 10 g P_2O_5,5 g K_2O), rainfed irrigation type, bimonthly hand weeding, 40 x 40 cm plant spacing and pesticide free. Each sample was prepared from ten plants to form pooled samples of 500 grams of fresh, fully developed and pest disease-free kaffir lime leaves that were harvested in the morning from both open sun and mild shading conditions. Harvested leaves were stored in a cooling box during the transfer to the distillation site. Essential oil was extracted from leaves by using water-steam distillation method for about 1.5 hours. Extracted essential oil was dried with anhydrous sodium sulfate, volumetrically measured and then stored in dark bottle for further analysis. Essential oil yield was expressed in percent fresh weight of kaffir lime leaves. The specific gravity was measured by using pycnometer at 20^{o}C, while refractive index was measured by using refractory method at 20^{o}C following Mahulette et al., (2020).

Kaffir lime leaves essential oil was also subjected to metabolite fingerprinting analysis using Gas Chromatography Mass Spectrometry (GCMS) instrument. Agilent 7890 GC/5975 MS-D (Agilent Technologies Inc, USA) was used with 1 µl injection volume, helium carrier gas (constant flow at 0.9 µm per minute), and Agilent 19091N-133HP-INNOWax Polyethylene Glyco column (size dimension 30 m x 250 µm x 0.25 µm). Column pressure was 7.06 psi and its regime was set in between 60-210^{o}C. The regime of injector was also set at 230^{o}C.

The obtained metabolite fingerprinting displayed relative content of several metabolites that were qualitatively measured based on the peak area of certain metabolites compared to the total of peak area in the chromatogram. Not all metabolites from GC-MS chromatogram were displayed in present study due to the preliminary selection by eliminating certain metabolites with low similarity (lower than 80%). The exact content of citronellal was determined by using standard content for citronellal compound (Efendi et al., 2021).

Data Analysis

Metabolite fingerprinting results were described by qualitatively comparing the results obtained from the open sun and mild shading set ups. Oil yield, specific gravity, refractive index and citronellal content were subjected to analysis of variance. If there was any significant difference found, the analysis was then continued by Least Significant Different (LSD) at α 5% level. Statistical Tool for Agricultural Research (STAR) version 2.0.1 was used to analyze the quantitative data.

Results and Discussion

Yield of essential oil is the important variable to measure in essential oil agribusiness because of its role in determining profit (Budiarto et al., 2019c). The present experiment revealed that the yield of kaffir lime leaves essential oil was not significantly affected by mild shading condition, although it tended to decline. The yield of kaffir lime leaves essential oil ranged within 1.4-1.6%, with a mean of about 1.5% and 1.43% in open sun and mild shading conditions, respectively (Figure 1). In the current study, mild shading did not cause a significant reduction in the oil yield.

The oil yield was previously reported to have positive correlation to edaphic factors, such as soil C-organic status and soil pH (Efendi et al., 2021), while it seemed to decline under water deficit condition or summer season (Said-Al Ahl and Abdou, 2009; Garcia-Caparros, 2019; Rao et al., 1996). Earlier studies reported the success of oil yield improvement by applying manure on *Coriandrum sativum* L. (Darzi, 2012), *Allium cepa* L. (Yassen and Khalid, 2009), *Mentha piperita* L. (Ram and Kumar, 1997), *Melisa officinalis* L. (Santos et al., 2009), *Pogostemon cablin* (Singh et al., 2013), *Origanum vulgare* L. (Gerami et al., 2016) and *Ocimum basilicum* (Anwar et al., 2005).

In terms of visual appearance, there was a relatively similar color of kaffir lime leaves essential oil, from the two preharvest condition set ups. The color of kaffir lime leaves essential oil was transparent yellow (Figure 2). This similarity was presumably associated with the same plant stage development, i.e., vegetative growth. In contrast, earlier study showed the difference in essential oil color in response to different plant stages, i.e., flowering and fruiting (Atef et al., 2015). Freshly distilled oil was almost colorless to transparent yellow (Guenther, 1987), however, the non-optimal storage environment could stimulate the oxidation of essential oils leading to the formation of darker color. Therefore, to prevent that incidence in the present experiment, the obtained essential oil was stored in a dark glass bottle. It is important to understand the physical properties of the essential oil, because this variable was frequently used for quality assessment, which in turn influenced the commercial value of the oil (Ali et al., 2015; Rahimmaleka and Goli, 2013).

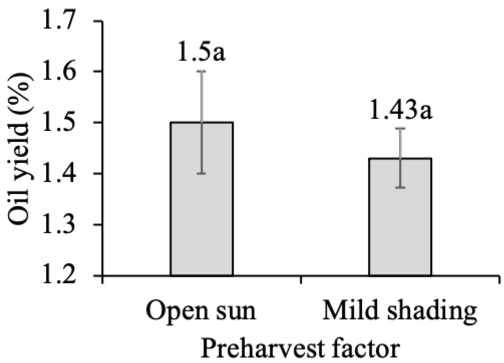

Figure 1. Yield of essential oil of kaffir lime leaves in response to open sun and mild shading. The same alphabet letters for mean values denotes no significant difference in oil yield based on the Least Significant Difference test at α=0.05; the error bar represents the standard deviation, with overlapping error bar representing no significant difference.

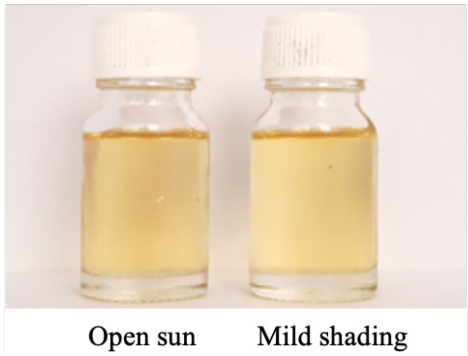

Figure 2. Visual appearance of essential oil from kaffir lime leaves in response to open sun and mild shading.

Other observed physical characteristics of essential oil in the present experiment were specific gravity and refractive index. Specific gravity was measured based on the ratio between the weight of the essential oil and the weight of water at the same volume and temperature. Refractive index was measured based on the angle of refraction of the essential oil that was maintained at a constant temperature condition. The specific gravity (Figure 3) and refractive index (Figure 4) were not significantly different for KLLEO from open sun and mild shading conditions. The specific gravity varied in between 0.8688-0.9102, with the mean in open sun and mild shading of about 0.8773 and 0.8937, respectively. The variation in refractive index was 1.4526 – 1.4590, with the mean in open sun and mild shading for about 1.4543 and 1.4574, respectively. The specific gravity and refractive index were two important variables to assess the quality of clove essential oil (Mahulette et al., 2020).

Essential oil of kaffir lime leaves is composed of various metabolites that simultaneously influence the production of aroma. Previous studies (Nor, 1999; Jantan et al., 1996; Othman et al., 2016) have displayed the metabolite fingerprinting of kaffir lime essential oil, however, there was still some variation noticed presumably in response to different genotype, growing location and method of analysis. Thus, there was an urgency to look at the metabolite fingerprinting in response to mild shading preharvest condition.

Most of the metabolites obtained from the present study are classified as monoterpenes (e.g., citronellal, citronellol, sabinene, linalool, limonene, isopulegol) and a small portion of sesquiterpenes (e.g., caryophyllene, caryophyllene oxide). Metabolite fingerprinting results are shown in Table 1. There was variation of composition and relative content of metabolites in response to preharvest condition. Caryophyllene was absent in KLLEO under mild shading. Compared to open sun, mild shading preharvest treatment is reported to (i) decrease the relative percentage of citronellal, lavanduol, and sabinene; and (ii) increase the relative percentage of bergamal, caryophyllene oxide, citronellol, citronellic acid, isopulegol, isopulegyl formate, limonene, linalool and linalool oxide trans). The results of this preliminary study require follow up investigation, i.e., (i) quantitative analysis of metabolites by using standard compound to accurately justify the effect of mild shading compared to open sun; and (ii) hedonic test to reveal the possibility of aromatic preference.

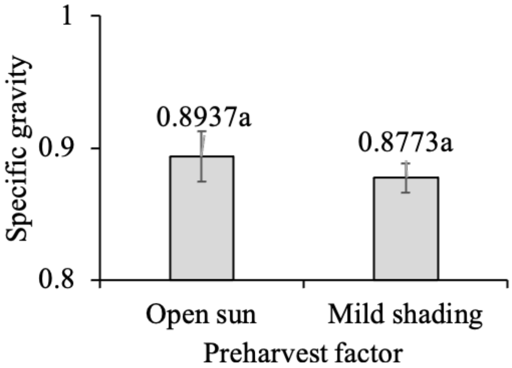

Figure 3. Specific gravity of essential oil from kaffir lime leaves in response to open sun and mild shading. The same alphabet letters for mean values denotes no significant difference in specific gravity based on the Least Significant Difference test at α= 0.05; the error bar represents the standard deviation, with overlapping error bar representing no significant difference.

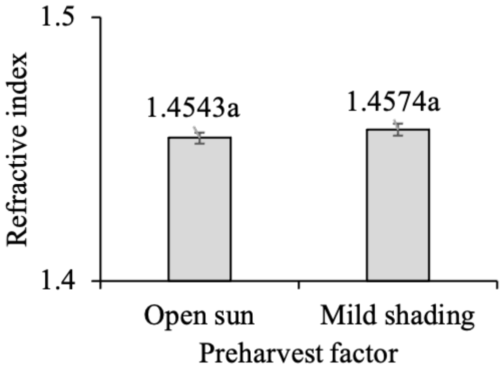

Figure 4. Refractive index of essential oil from kaffir lime leaves in response to open sun and mild shading. The same alphabet letters for mean values denotes no significant difference in refractive index based on the Least Significant Difference test at α=0.05; the error bar represents the standard deviation, with overlapping error bar representing no significant difference.

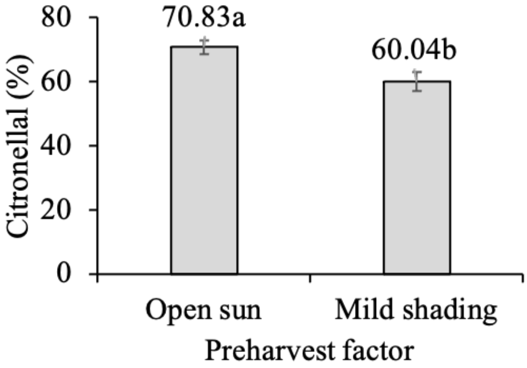

Figure 5. The absolute content of citronellal (%) of the essential oil from kaffir lime leaves in response to open sun and mild shading. The same alphabet letters for mean values denotes no significant difference in citronellal absolute content based on the Least Significant Difference test at α=0.05; the error bar represents the standard deviation, with overlapping error bar representing no significant difference.

The main metabolite that was quantitatively measured in the current study is citronellal. Citronellal ($C_{10}H_{18}O$) is a monoterpene metabolite with an economically important value for perfume synthesis (Lenardao et al., 2007). This metabolite is also known for its pharmacological properties, such as antifungal (Rammanee and Hongpattarakere 2011) and anti-inflammatory effects (Lota et al., 2002; Jabalpurwala et al., 2009). Therefore, the content of citronellal in essential oil is worthy to measure. This metabolite was frequently found in large proportions, i.e., 69.12-79.56% (Table 1), 61.7–74.8% (Nor, 1999; Jantan et al., 1996; Waikedre et al., 2010; Ratseewo et al., 2016; Tinjan and Jirapakkul 2007). Additionally, quantitative analysis revealed that the absolute content of citronellal was significantly decreased in mild shading condition, as compared to open sun (Figure 5). The reduction of citronellal was around 10%, as the effect of mild shading during preharvest period. This finding should serve as additional consideration in further development of mild shading technology for kaffir lime production. Further studies on the modification of mild shading combined with cultural treatments to increase the citronellal content are worth pursuing.

Conclusion

Varying the preharvest factor (mild shading vs. open sun) did not significantly affect the yield and physical characteristics (color, specific gravity and refractive index) of kaffir lime leaf essential oil (KLLEO). However, compared to open sun, mild shading significantly affected the metabolite fingerprints

Table 1. Metabolite fingerprinting of essential oil from kaffir lime leaves in response to open sun and mild shading.

No	Metabolite	Relative percentage (%)	
		Open sun	Mild shading
1	Bergamal	0.61	2.91
2	Caryophyllene	0.91	0.00
3	Caryophyllene oxide	0.67	1.40
4	Citronellal	79.56	69.12
5	Citronellol	2.95	3.19
6	Citronellic acid	1.04	1.83
7	Isopulegol	1.30	1.80
8	Isopulegyl formate	0.50	0.73
9	Lavandulol	1.61	1.49
10	Limonene	1.12	2.97
11	Linalool	4.67	6.04
12	Linalool oxide, trans	0.64	2.50
13	Sabinene	2.30	2.00

of kaffir lime by causing an increase in the relative percentage of bergamal, citronellol, caryophyllene oxide, citronellic acid, isopulegol, isopulegyl formate, limonene, linalool, linalool oxide. Mild shading also decreases the relative percentage of sabinene, lavanduol, and citronellal. The amount of citronellal, the main component of KLLEO, was significantly reduced by 10% with mild shading.

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