Distribution of Volatile Compounds in Kaffir Lime (*Citrus hystrix*) Leaves Grown in Soilless Substrate Analyzed Using Electronic Nose

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DP and SK were involved in producing the plants scientifically in the green house. Author RR analyzed the samples using the electronic nose and compiling data and data analysis. All authors read and approved the final manuscript.

Article Information

Original Research Article

ABSTRACT

Objective: The key objective of this research is to investigate the aroma profile of Kaffir lime (*Citrus hystrix*) leaves grown under greenhouse and peatlite soilless substrate conditions using the electronic nose system.
Methodology: Fresh Citrus hystrix samples of recently matured (RML) and old (OL) leaves were analyzed using electronic nose. A total of 79 volatiles were identified and those equivalent to 90% were reported.
Results: The RML and OL leaves had similar volatiles such as Citronellal, N-Nonanal, Myrcene, Pentyl Octanoate and γ-Terpinene with no significant difference in the concentration. Citronellal was the major volatile found more than 20% in both recently matured and old leaves. Recently matured and old leaves also had dissimilar volatiles such as 5-Propyldihydro-2(3H)-Furanone(32.9%), β-Pinene (7.6%), Terpinen-4-ol (2.4%), 1-Hexanol (1.3%) and (Z)-3-Hexen-1-ol-Acetate (1.0%) were only found in the recently matured leaves, whereas 1-Nonanol (30.8%), 3-

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Methyl Butanoic Acid(4.6%), p-Methyl Acetophenone (1.5%), Trans-Hex-2-Enyl Acetate (1.4%) and Methyl Eugenol (1.1%) were detected only in OL.
Conclusion: The results are very useful in food and cosmetics industries to develop innovative products based on kaffirlime leaves/oil.

1. INTRODUCTION

Kaffir lime (Citrus hystrix) is native to Southeast Asia and used as spice for its aroma and to add flavor to food [1,2,3]. The aromatic volatiles are phytochemicals of primary or secondary metabolites [4]. Kaffir lime extracts are also used as a medicinal herb; possessing antioxidant [5], anticancer [6], and antimicrobial [7] properties. It is also used in hair and skin care products, perfumes, natural flavors, and as an insect repellent [8,9,10].

Kaffir lime volatiles have been extracted from the leaves, peel and the pulp of primarily in the form of oil [1,-3,6,9,11-13]. The detection and concentration of the volatiles vary depending on multiple factors including the extraction and analysis method, the plant species, fresh or dry tissue samples and amount of the tissue is sampled [5,11,13].

Irrespective of the tissue sampled (leaves, peel or pulp), more than 50% of the volatiles in Kaffir lime were similar [2,5,9]. However; the concentration of the volatiles varied. Evidence of various extraction methods (hydrodistillation and solvent) also yielded contrasting results. Kaffir lime leaf samples extracted from the hydrodistillation process, volatiles of Citronellal and l-citronellal was found in excess of 60% (65.4 and 61.7% respectively), followed by ß-citronellol (13.4%) and l-limonene (5.9%) [1, 11]. On the other hand, the peel was predominantly with ß-pinene (32.9%), sabinene (31.2%) and limonene (29.2%) [12,11]. Although, Citronellal (7.5%) was present in the peel, the concentration was significantly lower in comparison to the leaf.

Volatiles obtained by solvent extraction method had higer concentration of Cintronella ranging from 56.3% to 79.0%. Citronellal (74.8%), ß-citronellal (46.4%) were also considered primary volatiles [3,5]. Other significant volatiles Kaffir lime leaves were l-linalool (13.1%), ß-citronellol (11.0%), linalool (9.8%), citronellyl acetate (6.7%), caryophyllene (6.5%), 2,3-dihydrogeraniol (6.1%), sabinene (5.9%), squalene (5.4%), hexanal (3.1%), trans-ß-caryophyllene (3.3%), cis-Linalool oxide (1.8%), trans-ß-ocimene (1.5%), iso-pulegol (1.5%), terpinen-4-ol (1.5%), phenylacetaldehyde (1.4%), ß-pinene (1.2%), ß-myrcene (1.2%) and nerolidol (1.1%) [2,3,5,14]. In the case of Kaffir lime pulp, it consisted of 66.0% citronellal and the peel 34.5% terpinen-4-ol [9].

Due to the benefits of its bioactive ingredients, the distribution and concentration of the volatiles in Kaffir lime is critical. While studies focus on various aspects of tissue sampling and analysis of plant tissue using various methods, newer technologies such as the electronic nose (e-nose) may allow quicker results with greater accuracy. Fresh leaf sampling amongst recently matured leaves (RML) and old leaves (OL) may also have a significant impact on volatile distribution in Kaffir lime. Among the various analytical techniques, e-nose was found promising analytical to discriminate VOC from plant sources. VOC emitted by cucumber, pepper, and tomato leaves subjected to mechanical damage or pest and disease attacks compared with undamaged control leaves analyzed by e-nose, which could discriminate VOCs from undamaged leaves of the three tested plants [15].

The objective of this study was to identify and quantify the major volatile constituents of fresh Kaffir lime plant leaves using an electronic nose (e-nose) grown in peatlite soilless substrate under control nutrient regime. In addition to that, this study also focused on the distribution and concentration of volatiles in RML and OL, allowing the designation of physiological age of leaf development for its unique aromatic volatiles and desirables.

2. MATERIALS AND METHODS

Kaffir lime (*Citrus hystrix*) plants one year old were grown in a peatlite soilless substrate (peat 70% and perlite 30% mix) and were fertigated as needed with 3.57 mM of a water-soluble fertilizer (20N-4.4P-8.3K) for six weeks in a glass greenhouse at Tennessee State University (36.1668° N, 86.8276° W) in Nashville, TN. The

greenhouse temperatures were set at 15-18°C night and 21-23°C day. To determine the distribution and concentration of volatiles, composite samples were collected on November 27, 2018, from the RML and the OL. The leaves were rinsed with deionized water and blotted dry to remove any leaf surface pollutants. Four replications consisting of eight leaf disk samples per replication were taken using a cork borer (diameter 1.5 cm) from the RML and OL (Fig. 1). The leaf disk samples were sliced and immediately transferred into a glass vial and capped for measurement of volatiles using the e-nose.

2.1 Electronic Nose Analysis

An ultrafast gas chromatography electronic nose (HERACLES II from Alpha MOS Toulouse, France) was used to characterize the volatiles from the Kaffir lime leaves. The e-nose was equipped with a sorption trap, an injection module for gas / liquid samples, dedicated autosampler, a set of independent columns of different polarity (10 m long nonpolar MXT-5 and medium polar MXT-1701 columns), 2 FID detectors, AlphaSoft V12 software package for HERACLES II with implemented modules for chromatographic data analysis and chemical / sensory characterization of detected volatile compounds, and AroChemBase V4 HERACLES V12 library. The autosampler loaded with samples was incubated at 40°C for 1200 s, while the syringe temperature was kept at 100°C. Leaf

sample weighed of 0.25 g was placed in a 20 mL airtight vials equilibrated 15 s at the injector temperature of 200°C and the flow rate of carrier gas equaled 30 ml/min. The sample was retained in the sorption trap for 20 s at 40°C. The initial temperature of analysis was set to 40°C and kept for 2 s. Next, the temperature was raised to 270°C and kept for 18 s. Hydrogen gas was used as the carrier.

3. RESULTS

The e-nose results of the kaffirlime leaves indicated that there were more than 75 volatiles identified using the AromaChembase software; however, only those equivalent to 90% concentration were identified and reported here. Volatiles such as Citronellal, N-Nonanal, Myrcene, Pentyl Octanoate and γ-Terpinene were identified in the RML and OL, but there was no significant difference in concentration amongst the volatiles.

The major volatiles in the RML were 5-Propyldihydro-2(3H)-Furanone (32.9%), Citronellal (21.2%), N-Nonanal (13.4%), β-Pinene (7.6%), Myrcene (5.9%), Pentyl Octanoate (3.0%), Terpinen-4-ol (2.4%), γ-Terpinene (1.7%), 1-Hexanol (1.3%) and (Z)-3-Hexen-1-ol-Acetate (1.0%) (Table 1). A chromatogram of aromatic volatiles of Kaffir lime RML and OL were shown in Fig. 2 and 3, respectively.

Fig. 1. a) Aerial and b) Front view of Kaffir lime plant grown in peatlite soilless substrate and the position of the sampled leave

Table 1. The detection of volatiles equivalent to 90% found in RML of Kaffir lime

Peak #	Recently matured leaves (RML)					
	Volatile name	Surface Percent (%)	Volatile groups /Total percent	Sensory Descriptors	Retention Time (s)	Kovat's Index(*)
9	1-Hexanol	1.35±0.28	**Alcohol** 1.35±0.28	Dry, Floral, Fruity, Grassy, Green, herbaceous, Woody, Mild Woody, Resinous, Sweet, Toasty	50.44	867
3	N-Nonanal	13.47±1.15	**Aldehyde** 13.47± 1.15	Chlorine, Citrus, Fatty, Floral, Fruity, Gaseous, Gravy, Green, Lavender, Melon, Soapy, Sweet, Tallow, Waxy	68.43	1111
10	(Z)-3-Hexen-1-ol-Acetate	1.01 ± 0.26	**Ester** 1.01± 0.26	Fruity, Green, Sharp, Sweet, Banana	62.06	1010
1	5-Propyldihydro-2(3H)-Furanone	32.98±8.29	**Furans** 32.98± 8.29	Fatty, Fruity, Nutty	71.85	1173
6	Pentyl Octanoate	3.08 ± 0.91	**Ketone** 3.08 ± 0.91	Orris	85.98	1470
5	Myrcene	1.25 ± 0.93	**Terpenes** 34.3 ± 5.93	Balsamic, Fruity, Ethereal, Geranium, Lemon, Metallic, Musty, Soapy, Spicy, Sweet, Woody		
8	γ-Terpinene	1.72 ± 0.16		Citrus, Fruity, Gasoline, Herbaceous, Sweet, Terpenic, Turpentine	65.01	1056
2	Citronellal	21.24± 0.49		Citrus, Fatty, Floral, Fruity, Green, Lemon, Rose	75.53	1243
4	β-Pinene	7.65 ± 3.73		Terpenic	60.7	991
7	Terpinen-4-ol	2.44 ± 0.62		Fruity, Herbaceous, Licorice, Moldy, Musty, Nutmeg, Pine, Spicy, Sweet, Terpenic, Turpentine, Woody	72.86	1191
	Total	**90.92 ± 0.38**				

Retention Index - Calculated

Volatiles on indentified in the RML were 5-Propyldihydro-2(3H)-Furanone, β-Pinene, Terpinen-4-ol1-Hexanol and (Z)-3-Hexen-1-ol-Acetate. On the hand, the volatiles in the OL consisted of 1-Nonanol (30.8%) as the abundant volatile followed by Citronellal (22.4%), N-Nonanal (12.7%), Myrcene (12.0%), 3-Methyl Butanoic Acid (4.6%), γ-Terpinene (2.5%), Pentyl Octanoate (1.8%), p-Methyl Acetophenone (1.5%), Trans-Hex-2-Enyl Acetate (1.4%) and Methyl Eugenol (1.1%) (Table 2).

4. DISCUSSION

The plant growth and development phase have a direct impact on the major volatiles identified in Kaffir lime, which is similar to previous studies [16,17]. Citronellal responsible for the characteristic aroma in Kaffir lime has been reported more than 70% [2]. Although, Citronellal was a major volatile, it accounted for less than 25% irrespective of the physiological age of leaf. The differences could be attributed to several factors including the sample particle size, the temperature, the position and physiological age of sampled plant tissue [13]. Smaller particle size resulted in an increased the concentration of the volatiles. This could be due to an increased in in the surface area of the tissue sampled, or the loss of volatiles due to volatilization.

Temperature plays a major role in the detection and concentration of volatiles during measurement. Drying Kaffir lime leaves could possibly emit some of the volatiles because of the low molecular weights, which causes rapid loss [2]. Similarly, high temperature released during grinding leaves could also significantly affect the loss of key volatiles [13]. There were instances where Kaffir lime peel sampled under various temperatures, some of the volatiles lost due to incomplete extraction process or thermal degradation [12]. On the hand, in this study, the aforementioned factors were considered before sampling. This includes: 1) the fresh leaf samples were obtained from live plants, and 2) samples were immediately capped to prevent evaporation of such volatiles with low molecular weights and less stability. In spite of that, volatiles common to Kaffir lime such as sabinene and limonene weren't detected as major volatiles. Also, samples of fresh peels and peel oil of Kaffir lime contain higher concentration of these volatiles as opposed to the leaves [1,9]. This could be due to the position and physiological of age of the plant tissue chosen for sampling.

Major volatiles such as 5-Propyldihydro-2(3H)-Furanone, β-Pinene, Terpinen-4-ol1-Hexanol and (Z)-3-Hexen-1-ol-Acetate was restricted to the RML, while 1-Nonanol, 3-Methyl Butanoic Acid, p-Methyl Acetophenone, Trans-Hex-2-Enyl Acetate and Methyl Eugenol were only detected in the OL. This could be due to the physiological age of tissue growth and development. The physiological age of the plant tissue sampled has a significant effect on the synthesis and accumulation of volatiles. For example, volatiles ranged from traces to 10% in initial flowering phases, to 50-70% in full flowering phases [18]. The synthesis of volatiles during various phases of plant growth and development could also possibly have a significant impact on volatile
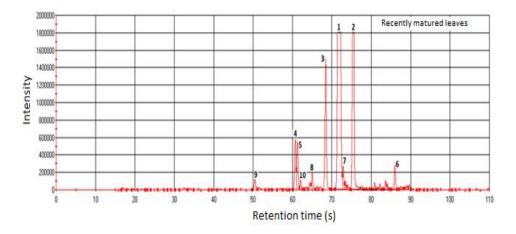

Fig. 2. GC chromatogram of Kaffir lime RML. Peak number coincides with the volatiles listed under Table 1

Table 2. The detection of volatiles equivalent to 90% found in OL of Kaffir lime

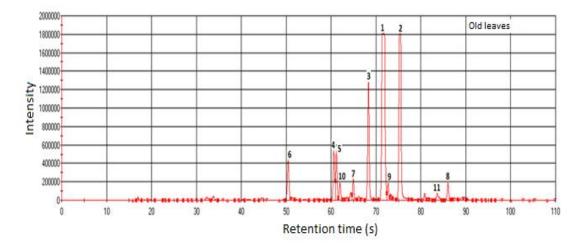| Peak #	Old leaves (OL)					
	Volatile name	Surface Percent (%)	Volatile groups /Total percent	Sensory Descriptors	Retention Time (s)	Kovat's Index
6*	3-Methyl Butanoic Acid	4.60 ± 0.29	**Acids** 4.60 ± 0.29	Acidic, Cheese, Rancid, Sweaty	50.45	868
1	1-Nonanol	30.81 ± 5.76	**Alcohol**	Fatty, Floral, Fruity, Green	71.52	1167
11*	Methyl Eugenol	1.13 ± 0.29		Clove, Spicy	83.63	1009
3*	N-Nonanal	12.75 ± 1.95	**Aldehyde** 12.75 ± 1.95	Chlorine, Citrus, Fatty, Floral, Fruity, Gaseous, Gravy, Green, Lavender, Melon, Soapy, Sweet, Tallow, Waxy	68.32	1109
10*	Trans-Hex-2-Enyl Acetate	1.49 ± 0.15	**Ester** 1.49 ± 0.15	Green	61.99	1009
8	Pentyl Octanoate	1.85 ± 0.19	**Ketone**	Orris	86.02	1471
9*	p-Methyl Acetophenone	1.59 ± 0.11	3.44 ± 0.30	Almond, Bitter Almond, Floral, Harsh, Hay, Sweet	72.68	1188
4	Myrcene	7.24 ± 3.60	**Monoterpene** 32.22 ± 7.04	Balsamic, Fruity, Etheral, Geranium, Lemon, Metallic, Musty, Soapy, Spicy Sweet, Woody	60.62	990
7	γ-Terpinene	2.55 ± 1.13		Citrus, Fruity, Gasoline, Herbaceous, Sweet, Terpenic, Turpentine	64.93	1055
2	Citronellal	22.43 ± 2.31		Citrus, Fatty, Floral, Fruity, Green, Lemon, Rose	75.51	1243
5	Unidentified	4.85 ± .20	Unidentified 4.85 ± 0.20		61.17	997
	Total	**91.28 ± 0.60**				

detected only in the OL

Fig. 3. GC chromatogram of Kaffir lime OL. Peak No. coincides with the volatiles listed in Table 2
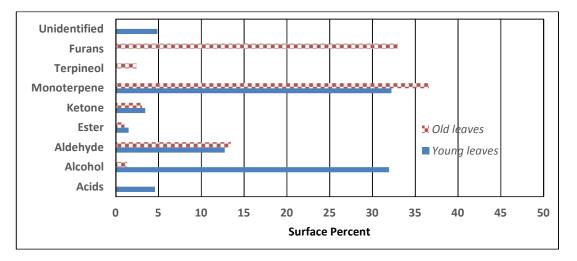

Fig. 4. Comparison of volatile groups for recently matured and old leaves of Kaffir lime samples

concentrations. Among the different volatile groups, monoterpenes were the major volatile group followed by furans and alcohols. However, alcohol group was found more in young leaves while furans in old laves. Acid volatiles were not detected in old leaves and found in low levels in young leaves. On the other hand, terpineol volatiles detected at low levels in the old leaves.

5. CONCLUSION

This study explored and identified the volatile volatiles of RML vs. OL in Kaffir lime using the e-nose. The volatile Citronellal was found consistently across the RML and OL. The 5-Propyldihydro-2(3H)-Furanone and N-Nonanal were also major components in the RML, while 1-Nonanol and N-Nonanal were major constituents in the OL. Plant physiological age, growing environmental factors may have a significant impact on the synthesis, distribution and accumulation of volatiles at various phases of plant growth development. The results of volatiles detected by in leaf tissue using e-nose could potentially influence the harvesting techniques in many industries such as medicinal, food and cosmetic. However, additional research studies are needed to better understand the synthesis and accumulation of various significance of volatiles for the sustainability of the Kaffir lime industry. Kaffirlime have a great

potential in research and commercialization. The usage of kaffirlime covers in aromatherapy and spa practices, insect repellent making, shampoo and beauty products.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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