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Original article

Effect of drying temperature on key odourants in kaffir lime (*Citrus hystrix* D.C., Rutaceae) leaves

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Summary Volatile compounds of fresh and dried kaffir lime leaves were analysed by gas chromatography–mass spectrometry (GC–MS). Fifty-four volatile compounds were identified in fresh kaffir lime leaf with citronellal being the most abundant, followed by L-linalool, hexanal, sabinene and β-citronellol. Aroma active compounds were determined by gas chromatography–olfactory (GC–O). Thirty-seven aroma active compounds were reported along with their odour descriptions and Log_3 FD. On the basis of high values of their Log_3 FD and odour characteristics together with odour activity value (OAV), the concentrations of selected eleven key odourants were compared between fresh and dried samples. Three drying temperatures at 50, 60 and 70 °C for 6 h were applied for hot air-drying. The concentrations of most compounds in 50 °C dried samples were not much different from those in fresh samples. Drying at 60 and 70 °C brought substantial losses of some key odourants. However, concentrations of citronellal and L-linalool, as key aroma compounds of kaffir lime leaves, in 60 and 70 °C dried kaffir lime leaves were not significantly different.

Keywords Drying temperature, GC–MS, GC–O, kaffir lime leaf, key odourants.

Introduction

Kaffir lime (*Citrus hystrix* D.C., Rutaceae) is a Southeast Asian citrus plant. They are also known as 'makrut' in Thailand and 'suwangi limau' or 'purut limau' in Malaysia. The most common product of the kaffir lime tree is its leaves that have hourglass shape and very strong citrus flavours. Kaffir lime leaf that contains about 0.08–0.66% essential oil (Phoungchandang *et al*., 2008; Waikedre *et al*., 2010) is used to give unique oriental flavour to 'tomyum' soup, curries, 'laksa' and many other Asian cuisines (Wijaya, 1995). Volatile compounds in kaffir lime leaf oil obtained by steam distillation have been reported. Citronellal was the main volatile compound found in the essential oil. Other volatile compounds detected in kaffir lime leaf oil were α-pinene, camphene, β-pinene, sabinene, myrcene, limonene, *trans*-ocimene, γ-terpinene, ρ-cymene, terpinolene, copaene, linalool, β-cubebene, isopulegol, caryophyllene, citronelly acetate, citronellol, geranyl acetate and δ-cadinene (Lawrence *et al*., 1971). Additionally, terpinen-4-ol was identified as the major compound in the essential oil of kaffir lime, which was prepared by hydrodistillation (Waikedre *et al*., 2010).

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Drying is a thermal process applying in commercial herbs and spice for extending their shelf life. Hot air-drying is one of the most popular methods used in dried herbs industry. The method was developed to overcome the problem of slow drying rate, microorganism growth and season dependence in traditional sun-drying. The advantages of hot air-drying over other drying methods are low cost and no requirement for special operation. In general, drying inhibits microorganism growth and forestalls certain biochemical changes. However, it can give rise to other alterations that affect the quality of the final product, such as changes in their appearance and odour. Raksakantong *et al*. (2012) found that drying methods affected some properties of kaffir lime leaf, including colour, fatty acid composition, antioxidant property and content of some volatile compounds. However, drying method is not the only crucial factor affecting quality of kaffir lime leaf, drying temperature can also cause certain changes to the herb. Phoungchandang *et al*. (2008) observed a reduction of citronellal content in kaffir lime leaf as drying temperature increased. Recently, the effect of drying methods on seventeen volatile compounds obtained from headspace method was investigated by Raksakantong *et al*. (2012). However, the effects on volatile compounds in food matrix phase

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and key odourants of kaffir lime leaf have not been reported.

The aim of this study was to identify key odourants of kaffir lime leaves and observe the effect of drying temperatures on the major aroma volatile compounds during hot air-drying.

Materials and methods

Sample preparation

Fresh kaffir lime leaves were purchased from a local market in Bangkok. They were cleaned with distilled water and wiped dry.

Drying methods

The fresh kaffir lime leaves were dried in a laboratory-scale hot air oven at three different temperatures (50, 60 and 70 °C) for 6 h. The whole leaf of fresh sample was uniformly spread on two stacks of aluminium tray. Dried kaffir lime leaves were stored in laminated film (aluminium foil/PE) bags with nitrogen gas purging. The samples were kept at −40 °C until analysis.

Determination of moisture content

The moisture contents of the fresh and dried samples were determined according to Association of Official Analytical Chemistry (AOAC) (2000). The analysis of each sample was carried out in triplicate.

Volatile compound extraction

Twenty grams of trimmed fresh kaffir lime leaves were blended with 200 cm^3 of liquid nitrogen and 20 g of NaCl in a Waring blender for 20 s. The blend was extracted two times with 80 cm^3 of dichloromethane and two times with 80 cm^3 of pentane, respectively. Solvents were HPLC grade. The extracts were pooled, and 0.0005 cm^3 of internal standards (2-methyl-3-heptanone, *tert*-butyl benzene and 2-undecanol) were added. The mixture was concentrated to 50 cm^3 under the stream of N_2 before distillation under 1.34×10^{-4} Pa at room temperature for 2 h. Then, the extract was dried over 2 g of anhydrous Na_2SO_4 and purged to 1 cm^3 under a steam of N_2 gas. Volatile compounds in dried kaffir lime leaves were extracted under the same condition used for the fresh ones.

Determination of volatile compounds

The concentrated extract (0.0001 cm^3) was analysed on a Hewlett-Packard model HP 6890 GC (Hewlett-Packard, Palo Alto, CA, USA) equipped with a mass selective detector model HP 5973. Capillary column, HP-5 (60 m length, 0.25 mm ID and 0.25 μm film thickness) was used. The carrier gas was helium with a flow rate of 2.2 cm^3 min^{-1}. The injector was operated in a split mode (50:1). The oven temperature was initiated at 35 °C for 1 min, raised at a rate of 10 °C min^{-1} to 200 °C and held at this temperature for 10 min (Tinjan & Jirapakkul, 2007). Identity of volatile compounds was facilitated by comparing mass spectrum with Wiley 275 library and NIST 98 library, by matching the retention indices (RI) and by comparing to authentic compounds. Areas of the internal standards (tert-butyl benzene for monoterpene, 2-undecanol for L-linalool and 2-methyl-3-heptanone for others) were used to calculate relative concentration of volatile compounds. Odour activity values (OAVs) were calculated from the relative concentrations of the volatile compounds divided by their odour threshold in water.

Aroma extract dilution analysis

The odour characteristics of volatile compounds in fresh kaffir lime leaf were analysed on a GC equipped with FID (HP6890 II plus; Hewlett-Packard) and an olfactory detector (SGE Analytical Science, Victoria, Australia). Operating condition was the same as for GC–MS method. Splitless mode was used with capillary column, HP-5 (30 m length, 0.32 mm ID and 0.25 μm film thickness). Aroma extract dilution analysis (AEDA) approach was used. Concentrated extract was diluted in a series of 1:3, 1:9, 1:81 with a mixture of pentane:dichloromethane (1:1). Each extract was analysed by two trained panels in random sequence of samples. The last dilution that the panel was able to detect odour of that compound was reported as its Log_3 FD value.

Statistical analysis

Volatile concentrations of fresh and dried kaffir lime leaves were subjected to analysis of variance (ANOVA) using SPSS version 12 (IBM Corporation, New York, NY, USA). In case of significant differences, Duncan's multiple range test (DMRT) at $P \leq 0.05$ was used. Three replications of volatile extraction were investigated.

Results and discussion

Volatile compounds of fresh kaffir lime leaves

The solvent-direct extraction technique was used to isolate volatile compounds in fresh kaffir lime leaf because this technique had been used to isolate the volatile compounds in food matrix. Not only volatile compounds perceived in orthonasal stimulation, retronasal stimulation could also be recovered by this extraction technique. Fifty-four volatile compounds were positively identified by GC–MS, as aldehydes (78.59%), hydrocarbons (12.09%), alcohols (6.40%) and esters (2.56%) (Table 1). Citronellal (74.82%) was the most abundant compound found in fresh kaffir lime leaves, in accordance with those previously reported (Lawrence *et al.*, 1971; Wijaya, 1995; Pudil *et al.*, 1998). The other compounds detected at high relative concentrations were L-linalool (3.64%), hexanal (3.18%), *trans*-β-caryophyllene (3.36%), sabinene (2.11%) and β-citronellol (2.01%). These compounds were also presented in the leaves of other citrus species such as sour orange (*C. aurantium* L.) (Lota *et al.*, 2001), lemon (*C. limon*) (Vekiari *et al.*, 2002) and Page Mandarin (Darjazi, 2011).

Aroma active compounds of fresh kaffir lime leaves

The aroma active compounds were differentiated from volatile compounds by their ability to perceive by human sense. OAVs and AEDA were used as tools to identify key odourant in food samples. High Log_3 FD values indicate the importance of these aroma compounds. In Table 2, Log_3 FD factors of aroma compounds in fresh kaffir lime leaf were in the range of 1–4. Of the thirty-seven aroma compounds obtained, one was left unidentified. Citronellal and L-linalool whose aroma characteristics contributed to kaffir lime odour had the highest Log_3 FD (=4). These two compounds could be considered key odourants of kaffir lime leaf, because they had the highest FD factors and were often related to the top note of the citrus aroma. Citronellal and L-linalool have previously been reported as aroma active compounds in citrus peel oil (Choi, 2003; Minh Tu *et al.*, 2003) and grapefruit oil (Lin & Russell, 2001). The odour description of hydrocarbon group with the range of Log_3 FD values of 1–3 could also contribute to spice, woody, citrus and kaffir lime leaf odour. According to Table 2, sabinene, β-myrcene, *trans*-β-ocimene, *trans*-β-caryophyllene and γ-cadinene were dominant hydrocarbons with high Log_3 FD values (2–3) and associated with citrus or kaffir lime leaf–like odour. β-Citronellol and *trans*-geraniol were considered as key odourants of fresh kaffir lime leaf with Log_3 FD values of 3. These compounds might relate to fresh, sweet and floral sense of kaffir lime leaf. Nerolidol was also in the alcohol group with Log_3 FD values of 3 but its characteristic odour was represented as dried kaffir lime leaf and woody odour. Hexanal and *trans*-2-hexenal have been reported as the oxidation products of linoleic and linolenic acids, respectively (Karmas *et al.*, 1994). Their odour descriptions with green and grassy aroma were also perceived by AEDA. According to the results in

Table 1 Volatile compounds of fresh kaffir lime leaves

Compound	$RI_{HP\text{-}5}$	Relative concentration (ppm)
Alcohols		
1-penten-3-ol	>700	2.60
cis-2-pentenol	769	4.50
Octanol	1078	1.10
L-linalool	1104	197.10
Isopulegol	1175	3.00
neoiso(iso)pulegol	1179	2.50
α-terpinen-4-ol	1191	Trace
α-terpineol	1204	0.90
β-citronellol	1232	108.80
trans-geraniol	1259	14.20
2-(2-hydroxyl-2propyl) -5-methyl-cyclohexanol	1352	Trace
Nerolidol	1572	11.70
Ketone		
1-penten-3-one	>700	3.70
Aldehydes		
4-pentenal	745	1.70
Hexanal	800	172.10
trans-2-hexenal	855	25.10
Citronellal	1164	4047.40
Decanal	1210	1.00
Neral	1251	0.60
Geranial	1278	3.10
Hydrocarbons		
α-thujene	934	0.60
α-pinene	943	5.30
Sabinene	982	114.00
β-pinene	988	6.40
β-myrcene	994	49.20
α-phellandrene	1013	0.70
δ-3-carene	1020	1.60
l-limonene	1038	9.70
cis-β-ocimene	1044	1.10
trans-β-ocimene	1052	30.50
γ-terpinene	1061	10.00
α-terpinolene	1090	2.30
trans-4,8-dimethyl-1,3,7-nonatriene	1120	3.40
Cycloisosativene	1392	1.80
α-copaene	1397	38.50
β-cubebene	1410	32.30
trans-β-caryophyllene	1448	181.80
epi-bicyclosesquiphellandrene	1455	3.10
α-guaiene	1460	1.50
trans-β-farnerene	1463	1.50
α-humulene	1483	21.70
Aromadendrene	1491	0.70
γ-cadinene	1500	4.50
Germacrene D	1508	19.30
trans,trans-α-farnesene	1514	28.60
Bicyclogermacrene	1524	52.60
δ-guaiene	1529	0.70
δ-cadinene	1544	32.60

Table 1 *Continued*

Compound	RI_{HP-5}	Relative concentration (ppm)
Esters		
Citronellyl acetate	1355	103.20
Geranyl acetate	1367	5.40
Neryl acetate	1386	28.90
Diethyl phthalate	1617	1.20
Others		
trans-sabinene hydrate	1078	7.00
1H-Indole	1309	6.60

RI, retention index from capillary column, HP-5 (60 m length, 0.25 mm ID and 0.25 μm film thickness).

Tables 1 and 2, not all of the components identified by GC–MS contributed to the aroma in kaffir lime leaf because of their low relative concentration or their high threshold. Thus, the effect of drying temperature would be focused on selected eleven compounds that were considered to be key odourants in kaffir lime leaf (Table 3). Aroma compounds that had Log_3 FD values of 3–4 and their odour descriptions related to kaffir lime leaf odour were selected. In addition, hexanal, limonene and citronellyl acetate were also selected because their high OAVs were noticed. As mentioned above, high value in Log_3 FD, related odour description and OAV could imply the potential of being key odourants.

Table 2 Aroma active compounds of fresh kaffir lime leaves identified by GC–O

RI_{HP-5}	Compound	Odour description	Log_3 FD	Identification[a]
>800	Hexenol	Green, fresh leaf	1	RI, Odour
800	Hexanal	Green, grassy	<1	AC, MS, RI, Odour
855	*trans*-2-hexenal	Green, grassy	2	MS, RI, Odour
859	*trans*-2-hexenol	Camphoraceous, green	3	RI, Odour
872	Unknown	Dry, woody	3	–
934	α-thujene	Green, spice	2	MS, RI, Odour
943	α-pinene	Pine, woody	3	MS, RI, Odour
982	sabinene	Woody, terpinic, citrus	3	MS, RI, Odour
988	β-pinene	Pine, lemon, wood	2	MS, RI, Odour
994	β-myrcene	Spice, kaffir lime leaf, citrus	3	MS, RI, Odour
1051	α-terpinene	Lemon, kaffir lime leaf, citrus	<1	RI, Odour
1052	*trans*-β-ocimene	Spice, sweet	2	MS, RI, Odour
1061	*trans*-2-octenal	Green, sweet, fresh	2	RI, Odour
1078	*trans*-sabinene hydrate	Spice, dry, woody	3	MS, RI, Odour
1104	L-linalool	Floral, sweet	4	AC, MS, RI, Odour
1164	Citronellal	Strong citrus, green, kaffir lime leaf, citrus	4	AC, MS, RI, Odour
1204	Decanal	Kaffir lime leaf, citrus	2	MS, RI, Odour
1210	2,4-nonadienal	Kaffir lime leaf, citrus	2	RI, Odour
1232	β-citronellol	Fresh kaffir lime leaf, citrus	3	AC, MS, RI, Odour
1261	*trans*-geraniol	Floral, sweet	3	AC, MS, RI, Odour
1278	Geranial	Lemon, kaffir lime leaf	2	AC, MS, RI, Odour
1309	Methyl geranate	Dried kaffir lime leaf, citrus	2	RI, Odour
1372	Undecanol	Lemon, dry, kaffir lime leaf	1	RI, Odour
1355	Citronellyl acetate	Lemon, kaffir lime leaf, sweet	2	MS, RI, Odour
1386	Neryl acetate	Kaffir lime leaf, citrus	2	MS, RI, Odour
1397	α-copaene	Spice	2	MS, RI, Odour
1410	β-cubebene	Kaffir lime leaf, citrus	1	MS, RI, Odour
1448	*trans*-β-caryophyllene	Kaffir lime leaf, citrus, spice	3	AC, MS, RI, Odour
1473	Linalyl isovalerate	Kaffir lime leaf, citrus	3	RI, Odour
1483	α-humulene	Dried kaffir lime leaf, woody	1	MS, RI, Odour
1491	Aromadendrene	Dried kaffir lime leaf, woody	2	MS, RI, Odour
1514	*trans,trans*-α-farnesene	Dried kaffir lime leaf, woody	2	MS
1524	Bicyclogermacrene	Very little lemon-like, woody	3	MS, RI, Odour
1529	δ-guaiene	Lemon, woody, kaffir lime leaf	2	MS
1544	γ-cadinene	Dried kaffir lime leaf, woody	2	MS, RI, Odour
1572	Nerolidol	Dried kaffir lime leaf, woody	3	AC, MS, RI, Odour
1584	Hexyl octanoate	Lemon-like	2	RI, Odour

[a]Identified by: AC-authentic compound, MS-mass spectrometry, RI- retention index, Odour-odour description.
RI, retention index from capillary column, HP-5 (30 m length, 0.32 mm ID and 0.25 μm film thickness).

Table 3 Selected key odourants of fresh kaffir lime leaves

RI_{HP-5}	Compound	Characteristic odour	Log_3 FD	Concentration (ppm)	Threshold (ppb)	OAV
800	Hexanal	Green, grassy[a]	<1	168.57	4.5[f]	37 460.00
982	Sabinene	Woody, terpinic, citrus[a]	3	124.71	n.a.	n.a.
994	β-myrcene	Spice, kaffir lime leaf, citrus[a]	3	52.6	13.0[d]	404.60
1038	l-limonene	Orange, citrus[b]	–	11.17	10.0[f]	11 170.00
1104	L-linalool	Floral, sweet[a]	4	220.34	6.0[d]	36 723.30
1164	Citronellal	Strong citrus, green, kaffir lime leaf, citrus[a]	4	4467.44	25.0[c]	178 697.60
1232	β-citronellol	Fresh kaffir lime leaf, citrus[a]	3	72.87	40.0[c]	1821.80
1259	*trans*-geraniol	Floral, sweet[a]	3	1.14	40.0[e]	28.50
1355	citronellyl acetate	Lemon, kaffir lime leaf, sweet[a]	2	63.56	250.0[c]	254.24
1448	*trans*-β-caryophyllene	Kaffir lime leaf, citrus, spice[a]	3	171.47	64.0[d]	2079.20
1572	Nerolidol	Dried kaffir lime leaf, woody[a]	3	4.12	n.a.	n.a.

[a]from AEDA approach.
[b]from Leffingwell (2004).
[c]Yamamoto *et al.* (2004).
[d]Guadagni *et al.* (1966).
[e]Takeoka *et al.* (1990).
[f]Buttery *et al.* (1971).
OAV (Odour Activity Value) = concentration of compound/threshold of compound.
RI = retention index from capillary column, HP-5 (60 m length, 0.25 mm ID and 0.25 μm film thickness); n.a., not available.

Effect of drying temperature on key odourants in kaffir lime leaves

The moisture content of fresh sample was 57.65% (wet basis). Three drying temperatures 50, 60 and 70 °C were conducted for 6 h to reduce the moisture content of fresh kaffir lime leaves to lower than 12% in wet basis (TISI, 2004;. From our preliminary study, moisture content of some commercial dried kaffir lime leaf products was varied in the range of 3.23–5.45% (wet basis). Moisture contents of 50, 60 and 70 °C dried samples were 8.39%, 3.85% and 3.49%, respectively. Thus, different moisture contents of all three dried samples were followed with the standard. Therefore, key odourants of kaffir lime leaves with different drying temperatures were studied. For solvent extraction, the weight of the dried samples was calculated to obtain the same amount of solid content as in the fresh sample. Concentrations of eleven key odourants are shown in Table 4. Most dried samples had lower concentration of volatile compounds than the fresh kaffir lime leaf. Aroma compounds could be driven out during the drying process, because they have low or medium molecular weight, which are easily evaporated. However, thermal degradation and oxidation reaction could be another possible way of volatiles reduction. In 50 °C dried sample, concentrations of L-linalool, *trans*-geraniol, nerolidol and terpenes were not significantly different from those in the fresh kaffir lime leaf. Increasing in the quantities of some compounds such as β-citronellol and citronellyl acetate had been detected as compared to those in the fresh kaffir lime leaves. This could probably occur as a consequence of oxidation reactions or the release of substances after the cell wall was ruptured (Diaz-Maroto *et al.*, 2004). Citronellal was reported as precursor of β-citronellol and citronellyl acetate via hydrogenation and oxidation reactions, respectively (Maki-Arvela *et al.*, 2003). On the other hand, an increase in acidity

Table 4 Effect of drying temperatures on key odourants of kaffir lime leaves

		Concentration (ppm)			
RI_{HP-5}	Compound	Fresh	50 °C	60 °C	70 °C
800	Hexanal	168.57[a]	1.72[b]	0.00[b]	0.00[b]
982	Sabinene	124.71[a]	103.94[a]	57.08[b]	38.60[b]
994	β-myrcene	52.60[a]	56.54[a]	27.65[b]	22.03[b]
1038	l-limonene	11.17[ns]	11.94[ns]	6.48[ns]	8.16[ns]
1104	L-linalool	220.34[a]	205.74[a]	50.35[b]	41.92[b]
1164	Citronellal	4674.44[a]	4126.28[b]	1699.77[c]	1376.73[c]
1232	β-citronellol	72.87[b]	130.22[a]	107.64[a]	133.04[a]
1259	*trans*-geraniol	1.14[a]	1.52[a]	1.20[a]	0.00[b]
1355	Citronellyl acetate	63.56[b]	114.20[a]	91.74[a]	44.83[b]
1448	*trans*-β-caryophyllene	171.47[a]	206.65[a]	106.08[b]	68.76[b]
1572	Nerolidol	4.12[a]	4.00[a]	4.25[a]	1.60[b]

Values with different letters in row were significantly different ($P \leq 0.05$).
ns, not significant; RI, retention index from capillary column, HP-5 column (60 m length, 0.25 mm ID and 0.25 μm film thickness).

because of the loss of water content during the drying process might give rise to hydrolysis of the glycosides from which aglycones were released (De Torres *et al.*, 2010) resulting in concentration increase. In overall, concentrations of key odourants of 50 °C dried samples were close to those of the fresh kaffir lime leaf implying the most similar odour to fresh kaffir lime leaf than other dried samples. Drying at 60 and 70 °C for 6 h brought about substantial losses of some aroma compounds. Thermal loss, thermal degradation and oxidation reaction might be accelerated at these temperatures and were considered as a possible way of some aroma compounds reduction. Sesquiterpene could be degraded to monoterpene as found in convectional dried ginger (Kubra & Rao, 2012). In this work, *trans*-β-caryophyllene reduction was noticed after drying at 60 and 70 °C. However, concentrations of monoterpenes (sabinene and β-myrcene) in 60 and 70 °C dried kaffir lime leaves showed no increase, as it might be lost during the drying period. Significant loss of hexanal was detected in all dried samples. This reduction might reduce a sensation of freshness and grassy odour of dried kaffir lime leaves. Among eleven key odourants, six aroma compounds showed no significant difference between 50 and 60 °C dried samples. Meanwhile, concentrations of citronella and L-linalool of 60 and 70 °C dried samples also were not significantly different. The results might be useful as a primary data for selecting suitable temperature of hot air-drying. Optimisation of drying time and operating condition should be considered as cofactors in further study.

Conclusions

This research described the volatile, aroma compounds and key odourants of fresh and dried kaffir lime leaves identified by GC–MS and GC–O. Citronellal and L-linalool were significantly considered as key odourants because of their highest value of Log_3 FD value and their characteristic odour of kaffir lime leave. For drying temperature, hot air-drying at 50 ° C for 6 h showed no significant effect on most key odourants compared to fresh kaffir lime leaf. At higher drying temperatures of 60 and 70 °C, a significant reduction on some key odourants was evident. However, concentration of most selected key odourants, particularly citronellal and L-linalool in 60 and 70 °C dried kaffir lime leaves were not significantly different.

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