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Shelf-life extension of dried kaffir lime leaves by low-impact drying: high flavor and color retention and physico-chemical properties

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Abstract

No research has yet focused on how the change of organoleptic properties and volatile compounds affected the shelf-life of dried herbs. This research determined the qualities of dried kaffir lime leaves (KL) treated by low impact drying (LTLT) and its effect on shelf-life extension. Samples were blanched then dried at 40°C for 10 hours (LTLT)before stored at accelerated conditions for 12 weeks. Physico-chemical and organoleptic properties, volatile compounds and microbial safety of samples were periodically compared with controls of fresh, commercial, and HTST-treated samples (blanching then drying at 60°C for 6 hours). LTLT samples significantly retained fresh-like color with less color differences (ΔE) than HTST (p<0.05). The volatile compounds of fresh KL was significantly the highest followed by LTLT-, HTST- and commercial-KL, respectively (p<0.05). Seven key volatile compounds were selected, and citronella, beta-citronella and linalool were three most abundant compounds. The flavor profile of LTLT-treated KL samples was the closest to freshKL. LTLT-treated samples had significantly higher citronella than HTST, resulting in strong, green-leave and citrus odor, whereas that of HTST-KL samples was dried KL leave and woody from nerolidol. During storage, all volatile compounds decreased causing changes in the flavor; however, LTLT-KL still had higher flavor retention than HTST. The moisture content and water activity of both samples slightly decreased but the color difference increased. Both samples still had the microbial safety within standard limit. Thus low impact dryinghelped extend the shelf-life of dried KL (48 weeks) while preserving great color and flavor retention of herbs.

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Introduction

Kaffir lime (*Citrus hystrix* DC.*)* is very popular in south Asia and southeastAsia due to its numbers of health benefits such as anti-inflammatory, antimicrobial and antioxidant properties (Kooltheat *et al.*, 2016). It has approximately three to five-meter height and mostly grows in India, china, Malaysia and Thailand. The unique citrus like aroma of these leaves make them to be used widely in cooking for many Thai dishes and soups especially in the famous *Tom Yum Kung* shrimp soup. This distinct aroma is due to the presence of volatile compounds in which citronella is the most abundant one. (Jirapakkul *et al.*, 2013).

Fresh leaves could only retain all their nutrition for only 3-4 days hence normally they are dried using hot air for shelf life extension(Raksakantong *et al.*, 2012). Drying is the most well-known technology that does not only provide products with microbiological stability but also reduces quality deterioration from biochemical reactions, gives a convenience in usage and transportation, and prolong the shelf life by minimizing the packaging requirements, enhancing the storage stability, and reduces the distribution and transportation costs (Bušić *et al.*, 2014; Ochoa-Martínez *et al.*, 2012). However,drying not always produce positive changes it may result negative changes in some physical and chemical parameters also. Such as Raksakantong *et al.* (2012) found changes in color, volatile compounds, fatty acid profile and some antioxidants in kaffir lime leaves due to the effect of drying.

A lot of publications in recent time have been made on the effects of drying on the volatile compounds of kaffir lime leaves; however, no data on the effects of drying treatments which causes the changing in the dried kaffir leaves during the shelf life is reported. For the shelf-life of dried herbs such as kaffir lime leaves, overall appearance, odor, flavor are crucial attributes of dried herbs as well as the microbial safety. Despite the shelf-life extension based on microbial safety by many researches, none has yet focused on how the change of organoleptic properties and key volatile compounds play a role in the shelf-life of dried herbs. Therefore, the objectives of this research were to compare the qualities of dried kaffir lime leaves (KL) treated by low impact drying to controls, and to determine if and how the shelf-life of samples were extended.

Material and method

Sample preparation

The fresh leaves of kaffir lime were purchased from a local market in Phitsanulok, Thailand. Visual sorting of samples was done and only those having same visual characters such as gloss, color and size were chosen. The herb leaves were divided into three equal portions among them one portion was used for the High Temperature Short Time (HTST) drying treatment the second was used for the Low Temperature Long Time (LTLT) drying treatment while the third was kept as it is in its original fresh form as a control to compare the results. It is pertinent to mention that before the drying treatments both the LTLT and HTST samples were pretreated according to the method written in patent no. TH12262 for the fixing of color and to preserve the aroma of leaves. All the analysis performed in this study were in triplicates and entirely the analytical results are reported on the dry weight basis of leaves. Before the analysis of all the samples they were stored at -20^{0}C minimum for 24 hour.

Drying conditions:

The drying process was conducted using the hot-air oven (ABC, A728.002, France) with minimum output of 250W and a tray dimension of 34.5cm x 6 cm. Four round trays were used. Each tray contained 400g of pretreated leaves that were spread orderly as a thin layer onto the 935 cm^2 of drying surface. Two drying conditions were analyzed in this experiment 1) a low-impact drying using a low-temperature-long-time condition (LTLT) with temperature of 40^{0}C for 10 hours and 2) a relatively high-impact drying using high-temperature-short-time (HTST) with temperature of 60^{0}C only for 6 hours. The pretreatment of drying conditions to fix the color and flavor of samples were explained and protected in the

Patented No. TH12262. The results were compared with the fresh samples. The samples were kept at -20^{0}C until further analysis.

Determination of moisture content and water activity

For both the fresh and treated samples the moisture and water activity was analyzed using the method of Association of Official Analytical Chemistry AOAC (2000). For the purpose of moisture analysis the infrared moisture analyzer (MB 45, Ohaus, USA) was use while in determining the water activity, the water-activity meter (LabStart-aw, Novasina, Switzerland) was used. Three replications of all the samples were used in determining the final moisture and water activity results.

Determination of colorimetric parameters

The color measurements of all the samples were done by direct reading with the help of colorimeter (Model CR-300, Konica Minolta, Sensing, Inc. Osaka, Japan). The *L*, a*, b** color scale was used in which the *L* represents the lightness and darkness, *a* values determines the red-green color while the *b* values were used for the yellow-blue components of leaves. The values from *L,a,b* were used to determine ΔE, Chroma (C) and Hue angle (H^0) using equations (1-3). The colorimeter was calibrated against a white standard before use. For each treatment 10 measurements were taken individually and average value was calculated.

$$\Delta E=\sqrt{(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2} \quad (1)$$

$$C=\sqrt{(a^*)^2+(b^*)^2} \quad (2)$$

$$H^0=\tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (3)$$

Microbial analysis

The microbial safety of treated samples was assessed by the total plate count and the total yeast and mold count based on the standard of dried herb. Plate count agar was used for total plate count performance, and samples were incubated at 37 ^{o}C for 24-28 hours (AOAC, 1990). Total yeast and mold count was identified by a spread plate method using Rose Bengal agar, and samples were incubated at 25^{o}C for 3-5 days (AOAC, 1990). The microbial values were calculated as colony-forming unit (CFU)/g. All analyses were carried out in three replicates.

Extraction of volatile compounds

The volatile compounds from both fresh and treated leaves samples were extracted in same conditions by Simultaneous Distillation Extraction (SDE) method using a Likens and Nickerson's apparatus. 10-20g of sample was blended in a blender for 30s. The blend was put into a 1L round bottom flask (Neck size 24/40 scotch Germany) and 500mL of distilled water was added in it along with 200µL of 2-methyl-3-heptanone (concentration=7.0µg/µl), as internal standard. While 200mL dichloromethane was placed in a 500mL flask (Neck size 24/40 scotch Germany) of the Likens and Nickerson's apparatus. After which the simultaneous distillation extraction process continued for 3 hours. After extraction, dichloromethane possessing all the aroma compounds, was frozen at -20^{o}C for at least 1 hour to remove the residue water if present. The extract was purified under the stream of nitrogen gas at room temperature for 2 hours until only 20ml was left behind which was again frozen at -20^{0}C for 1 hour to make sure if any water droplets were present they got frozen. The 20mL extract was again purged with nitrogen gas at room temperature for further 30 minutes unless a final volume of 2ml was achieved. The final 2ml extract solution was passed through 2g of anhydrous sodium sulfate (Na_2SO_4)and was finally collected in 2.5ml amber color vial and stored at -20^{o}C before injection.

Determining the volatile compounds

The volatile compounds analysis was done with the help of GC-2010 chromatograph which was coupled with GC/MS-QP2010 (Shimadzu, Japan). For the separation of volatile compounds DB-5MS column was used (thickness 0.25µm, length 30.0m and

diameter 0.25µm). Scanning was performed from 35 to 350 m/z. Helium gas was used as a carrier gas with a column flow rate of 2.0 ml/min in a split ratio of 100.

The oven temperature was held was 40^{0}C for 5 minutes and then increased to 225^{0}C with the following rate and was held for 10 minutes.

1. Rate of 0.0^{o}C min^{-1} from 40^{o}C hold for 5 min;
2. Rate of 2.0^{o}C min^{-1} from 40 to 100^{o}C;
3. Rate of 5.0^{o}Cmin^{-1}from 100 to 225^{o}C and hold for 10 min;

The injector and detector were launched at 225^{0}C and 250^{o}C, respectively. 1µl of sample was injected every time. The relative concentrations of volatile compounds were calculated with the help of area of internal standard (2-methyl 3-heptanone). For the identification of volatile compounds the mass spectrum was compared with the Wiley 7 library and also the peaks comparison was done with the pure authentic standards purchased from Sigma Aldrich. The Odor activity values (OAVs) was calculated by dividing the relative concentration of that volatile compound in fresh leaves with its odor threshold level in water.

Determination of Shelf-life of dried KL leaves

All treated Kaffir lime leaves samples were wrapped in the edible film (Patent no. TH12262) and packed in the aluminum foil bag. All treated samples and control (fresh) were kept under two storage temperatures, which were 25 ^{o}C and 45 ^{o}C (accelerated storage temperature) for at least 12 weeks unless the end of shelf-life was reached.

The criteria determining the shelf-life were either products exceeded the standard microbial safety of dried herbs or products were deemed undesirable. During storage, treated samples were randomly taken and tested on weekly basis till the 4th week and then after an interval of four weeks they were again tested until the 12th week. The qualities of treated samples were compared with frozen duplicates kept at 0^{o}C as control samples using sensory evaluation by 5 trained panelists (Different-from-Control-Test). Samples were deemed different from control when differences of two units of each sensory attribute were observed. The accelerated storage temperature was selected based on the protocol of "Product Crash Test" used in the food industry. The estimated shelf-life of dried KL leaves was then calculated based on the Arrhenius law.

Statistical analysis

All data collected from each experiment was statistically analyzed by using the analysis of variance (ANOVA) test using SPSS version 17.0 (IBM Corporation, New York, NY, USA). The difference amongst samples was analyzed by Duncan's multiple range tests (DMRT) at 95% ($p < 0.05$) confidential level. All analysis was done at least in triplicates and was averaged.

Results and discussion

Changes in moisture content and water activity of dried KL leaves during accelerated storage

In Table 1, the changes in moisture content of KL leaves samples during accelerated storage conditions are illustrated. It showed that the moisture content of treated samples decreased as the storage period went on. The moisture content of HTST-treated KL leaves sample stored under accelerated storage condition in the first three weeks significantly decreased while no significant difference was found after that. On the other hand, there was no significant difference amongst all LTLT-treated KL leaves during the storage period of 12 weeks. Niamnuy *et al.* (2014), also observed a similar trend in the *Orthosiphon stamineus* dried herbal leaves in which the initial moisture content was 13% and during the storage time of 40 days it dropped to 11.73% only. Likewise, the changes of water activity (a_w) values of all treated KL leaves during accelerated storage conditions are presented in Table 2. As the storage time increased, the water activity values of samples decreased with the same trend of moisture content results.

Table 1. Changes in moisture content of dried kaffir lime leaves during accelerated storage temperature.

	Moisture content (%)						
Sample	Storage time (weeks)						
	0	1	2	3	4	8	12
LTLT-treated KL leaves	10.21±1.36^{a}	8.80±0.67^{b}	10.60±0.32^{a}	10.41±0.45^{a}	10.19±0.21^{a}	10.18±0.16^{a}	9.39±0.14^{ab}
HTST-treated KL leaves	9.75±0.28^{bc}	8.38±0.51^{d}	9.48±0.43^{c}	8.84±0.18^{d}	10.81±0.20^{a}	10.12±0.09^{b}	10.19±0.11^{b}

Values are expressed as means ± standard deviation. Mean with different letters in the same column were significantly different at $p<0.05$.

The water activity of a product is a critical indicator which influence the shelf-life of dry products as most bacteria are unable to grow below water activity of 0.91 (Syamaladevi *et al.*, 2016). The changes of a_w illustrated the relationship with moisture content, and reduction of both a_w and moisture content of dried kaffir lime leaves would suppress the microorganisms growth and also prolonged the shelf life. Similar trends in results were reported by Speckhahn *et al.* (2010) . Water activity also plays an important role in enzyme activity and vitamin identification in foods which are also essential factors affecting the color, taste and aroma of dried products. However, dried foods at $a_w \leq 0.1$ tend to have fast auto-oxidation whereas at a_w of 0.3, the oxidation rate seems to be the lowest, then rises again when the a_w increases from 0.55-0.85 because of catalyst mobilization or oxygen (Nawar *et al.*, 1996).

Table 2. Changes in water activity of dried kaffir lime leaves during accelerated storage temperature.

	Water activity (a_w)						
Sample	Storage time (weeks)						
	0	1	2	3	4	8	12
LTLT-treated KL leaves	0.42±0.00^{c}	0.37±0.00^{e}	0.44±0.00^{b}	0.41±0.00^{c}	0.41±0.00^{c}	0.44±0.44^{a}	0.42±0.00^{c}
HTST-treated KL leaves	0.42±0.005^{b}	0.33±0.005^{e}	0.39±0.005^{c}	0.37±0.00^{d}	0.44±0.005^{a}	0.42±0.01^{b}	0.43±0.00^{b}

Values are expressed as means ± standard deviation. Mean with different letters in the same column were significantly different at $p<0.05$.

Changes in colors of dried KL leaves during accelerated storage

Among other physico-chemical properties color is one of the most important parameter for the consumer attraction. During heating changes in the color parameters of kaffir lime leaves takes place (Ratseewo *et al.*, 2016).The color values of kaffir lime leaves are depicted in Table 3. The lightness (L*) of dried kaffir lime leaves of low temperature long time (LTLT)and high temperature short time (HTST) continuously increased during accelerated storage time. For the greenness (a*) values of all treated samples, they tended to increase during storage time whereas the yellowness (b*) and chroma values of treated kaffir lime leaves significantly decreased for both LTLT and HTST drying conditions during storage time when compared with the control. However, the overall color depicted as hue angle of both LTLT- and HTST-treated kaffir lime leaves still remained green, and was not significantly*($p>0.05$)* different from control. The changes were perhaps due to the destruction of color pigments because of heating or the non-enzymatic browning reaction could also be a factor for the color change (Raksakantong *et al.*, 2012; Wanyo *et al.*, 2011).

Changes in microbial safety of dried KL leaves during accelerated storage

For the microbial changes of both LTLT and HTST drying conditions, the total bacterial count and the total yeast and mold count are shown in Table 4-5. According to the Thai Community product standard of dried herbs (TPCS 480/2547), the total plate count of the sample must not to exceed $5x10^5$ colonies per

gram in order to consider the product safe for human consumption (TPCS 2004). As shown in Table 4, none of the samples treated by two drying conditions (LTLT & HTST) showed the total plate count values that were over the standard limitation during the accelerated storage of 12 weeks. For the results shown in Table 5, the total yeast and mold count values slightly decreased during storage time, and they did not exceeded the standard for total yeast and mold count of 100 colonies per gram in samples.

Table 1. Parametersof dried kaffir lime leaves during accelerated storage temperature.

Samples	Storage time	Color parameters					ΔE
	(week)	*L**	*a**	*b**	chroma (C*)	hue angle (h*)	
	0	40.88 ± 0.18^{d}	(-8.81 ± 0.11^{f})	26.94 ±0.23^{b}	28.35 ± 0.19^{a}	108 ± 0.36^{a}	N/A
	1	42.69 ± 0.57^{c}	(-1.07 ± 0.87^{e})	28.05 ± 0.50^{a}	28.07 ± 0.50^{a}	92.18 ± 0.15^{b}	17.74
HTST-treated	2	43.70 ± 0.35^{ab}	1.60 ±0.12^{d}	26.84 ± 0.32^{b}	26.89 ± 0.31^{b}	86.59 ± 0.29^{c}	24.02
KL leaves	3	45.39 ± 0.19^{ab}	1.53 ±0.06^{d}	27.34 ± 0.14^{b}	27.39 ± 0.13^{b}	86.79 ±0.13^{c}	24.05
	4	46.84 ± 0.17^{a}	2.58 ± 0.07^{b}	27.14 ±0.09^{b}	27.27 ±0.09^{b}	84.55 ± 0.17^{d}	26.76
	8	45.11 ± 0.46b^{c}	2.11 ± 0.09^{c}	23.36 ±0.38^{d}	27.45 ±0.37^{d}	84.81 ± 0.29^{d}	26.24
	12	45.50 ± 0.35^{c}	3.04 ± 0.09^{a}	24.48 ± 0.22^{c}	27.66 ± 0.21^{c}	82.91 ± 0.28^{e}	28.25
	0	41.52 ± 0.33^{c}	(-8.8 ± 0.09^{g})	28.65 ± 0.20^{b}	29.97 ± 0.21^{a}	107.07 ± 0.83^{a}	N/A
	1	42.50 ± 0.15^{b}	(-0.34 ± 0.01^{e})	27.05 ± 0.10d^{e}	27.05 ± 0.10^{c}	90.71 ±0.04^{c}	18.74
LTLT-treated	2	42.06 ± 0.30^{b}	0.04 ± 0.08^{d}	27.25 ± 0.29^{cd}	27.25 ± 0.29^{c}	89.90 ± 0.17^{d}	19.56
KL leaves	3	43.17 ± 0.33^{a}	(-3.01 ± 0.10^{f})	29.89 ± 0.45^{a}	30.04 ± 0.45^{a}	97.75 ± 0.12^{b}	11.16
	4	42.11 ± 0.18^{b}	0.66 ± 0.07^{b}	26.75 ± 0.14^{e}	26.87 ± 0.14^{c}	86.32 ± 0.16^{f}	23.10
	8	43.41 ± 0.23^{a}	2.13 ± 0.01^{c}	27.06 ± 0.33^{de}	27.14 ± 0.33^{c}	86.48 ± 0.06^{e}	23.61
	12	45.34 ± 0.16^{a}	2.93 ± 0.05^{a}	27.57 ± 0.049^{c}	27.73 ± 0.05^{b}	85.93 ± 0.11^{g}	24.60

Values are expressed as means ± standard deviation. Mean with different letters in the same column were significantly different at $p<0.05$.

Therefore, the dried kaffir lime leaves samples from two drying conditions, after being kept under accelerated temperature for twelve weeks, still maintained their microbial safety to be safe for consumption based on the microbial standard of dried herb (Thai Industrial Standard Institute, 2004). The antimicrobial properties of kaffir lime leaves have long been reported as the oil and extract of the leaves has the tendency to suppress the growth of microbes (Nanasombat & Lohasupthawee, 2005).

Table 2. Total bacterial count in dried kaffir lime leave samples from various drying conditions during accelerated storage.

Sample	Total plate count (cfu/g)						
	Storage time (weeks)						
	0	1	2	3	4	8	12
HTST-treated KL leaves	12×10^{3}	5×10^{3}	2×10^{3}	19×10^{2}	29×10^{2}	4×10^{2}	720
LTLT-treated KL leaves	16×10^{3}	58×10^{2}	66×10^{2}	32×10^{2}	98×10^{2}	640	1×10^{3}

The odor profiles and their changing trends in samples during accelerated storage

Seven key volatile compounds were selected from twenty-eight compounds on the basis of their perceived odor concentrations and Odor Activity Values (OAVs) of dominant compounds for KL leaves. Besides the concentration amounts of each compound, the OAV value of each volatile compound provided an indication of how much that compound is perceived and how it contributes to overall

odor/flavor of products. Since the OAV relates to the threshold of volatile compound, it is used more frequently to characterize the overall odor/flavor than the concentrations of compounds. Therefore, the higher OAV value leads to more contributing portion of the compound in the final make up of odor profile. Three most abundant compounds for KL leaves were citronella, beta-citronella and linalool which agreed with previous published result (Ratseewo *et al.*, 2016).Thus they were considered as key odorants as well as important flavor note compounds for kaffir lime leaves such as the dl-limonene and nerolidol which were also selected due to their high odor activity values and good perceived aroma characteristics of KL or citrus.

Table 5. Total yeast and mold in dried kaffir lime leave samples from various drying conditions during accelerated storage.

Sample	Total yeast and mold count (cfu/g)						
	Storage time (weeks)						
	0	1	2	3	4	8	12
HTST-treated KL leaves	30	<10	30	<10	<10	<10	<10
LTLT-treated KL leaves	50	<10	<10	<10	13	<10	<10

The OAV values with the odor description and the concentrations of selected volatile compounds in LTLT-treated samples were compared with controls including fresh KL, HTST-samples and commercial (Table 6-7). Citronella, nerolidol and linalool were on the top three highest OAVs where citronella, beta-citronella and linalool were the three highest concentrations. As described earlier that OAVs of volatile compounds were better tool to characterize the overall odor of product rather than the concentrations. Thus, based on OAVs, nerolidol contributed more on the composition of perceiving odor in dried KL samples than beta-citronella and linalool, resulting in samples having dried-kaffir-lime-leaf and woody odor.

Table 6. Comparison of the OAV and perceived odors of key volatile compounds from kaffir lime leaves treated at various drying conditions.

Compounds	RI_{DB-5}	Threshold in water (ppm)	OAV					Odor description
			Fresh KL leaves	Commercial KL leaves	LTLT-dried KL leaves	HTST-dried KL Leaves		
beta-myrcene	988	13.07[1]	162.25	1.98	76.10	73.02		Spice Kaffir Lime leaf citrus*
dl-limonene	1026	10[1]	36.82	0.51	20.52	15.80		Orange, citrus
linalool	1114	6[2]	1369.63	45.55	304.68	333.21		Floral, sweet**
citronella	1163	25[3]	6859.60	150.57	2835.11	434.97		Strong citrus, green, kaffir lime leave, citrus**
beta-citronella	1238	40[3]	1178.66	7.87	106.86	92.26		Fresh kaffir lime leaf, citrus**
cytronellyl acetate	1359	250[3]	15.65	0.39	25.63	0.38		Lemon Kaffir Lime leaf sweet*
Nerolidol	1564	0.25[4]	4836.00	0.00	1662.61	8922.24		Dried Kaffir Lime leaf woody**

RI= retention index from capillary column DB-5 (thickness 0.25µm, length 30.0m and diameter 0.25µm), *(Rychlik *et al.*, 1998),**(Jirapakkul *et al.*, 2013), [1](Buttery *et al.*, 1971), [2](Guadagni *et al.*, 1966), [3](Yamamoto *et al.*, 2004), [4](Miyazawa *et al.*, 2015).

This was different from previous research because the others normally considered the concentrations and neglected the importance of OAVs (Ratseewo *et al.*, 2016). Therefore, LTLT-treated KL samples had the closest flavor profile to fresh leave followed by those of HTST-treated KL and commercial leaves. According to the OAVs, LTLT-KL samples had strong, green-leave and citrus odor from significantly higher value of citronella whereas the odor of HTST-KL samples were dominated by nerolidol, resulting in

samples being perceived more woody and dried leave. However, HTST-treated control samples still had better odor profile than the commercial samples which barely contained perceivable good notes for KL even though their expiration date was not met yet. This may be due to the fact that HTST, despite its relatively higher temperature, the samples were blanched which may help retain the color and flavor. On the other hand, the drying condition of commercial samples was unknown however high temperature was definitely used without blanching as drying pretreatment. The reason was because blanching consumed resources and time in the process of drying herbs therefore manufacturers may not use this method.

Table 7. Comparison of the amounts of key volatile compounds from kaffir lime leaves treated at various drying conditions.

Compounds	Odor description	Concentration (ppm)			
		Fresh KL leaves	Commercial KL leaves	LTLT-dried KL leaves	HTST-dried KL Leaves
beta-myrcene	Spice Kaffir Lime leaf citrus*	2120.65	25.84	994.67	954.41
dl-limonene	Orange, citrus	368.21	5.13	205.22	158.01
linalool	Floral, sweet**	8217.81	273.31	1828.05	1999.26
citronella	Strong citrus, green, kaffir lime leave, citrus**	171489.98	3764.33	70877.71	10874.26
beta-citronella	Fresh kaffir lime leaf, citrus**	47146.58	314.64	4274.34	3690.52
cytronellyl acetate	Lemon Kaffir Lime leaf sweet*	3912.58	98.33	6408.21	96.22
Nerolidol	Dried Kaffir Lime leaf woody**	1209.00	0.00	415.65	2230.56

*(Rychlik *et al.*, 1998),**(Jirapakkul *et al.*, 2013).

The concentration of selected main volatile compounds of LTLT- and HTST-treated samples during accelerated storage were compared in Table 8. During accelerated storage, significant differences were found in volatile compounds of samples ($p<0.05$). As storage time increased, overall flavor retention decreased. Citronellal was the most abundant with the highest concentration for both drying conditions followed by beta-citronellol, citronellyl acetate, linalool, nerolidol, beta-myrcene and dl-limonene. The concentrations of these compounds in both LTLT- and HTST-treated samples kept under accelerated storage temperature gradually decreased during the first four weeks however, their values sharply dropped at the storage time of 8th and 12th week, respectively. The reason was because at accelerated storage temperature, the biochemical changes in the product rapidly occurred with significantly higher changing rate depending upon the degree of accelerated temperature. However, previous studies showed that the volatile compounds could be lost during storage at room temperature as well. Some fluctuating in the concentration amounts observed during storage time in this study also agreed with others. The observation of the increasing instead of decreasing in the concentration of some of the volatile compounds was due to the heating application increase that altered the odor profile (Díaz-Maroto *et al.*, 2002). Likewise, the concentration of thymol in thyme was increased because of the heating Venskutonis (1997).At the 12th week the LTLT-treated samples still retained some volatile compounds with good fresh-citrus like notes with more concentrations than those of HTST samples. According to the OAVs, LTLT-treated samples still had floral and sweet odor from linalool and citronella whereas HTST-treated samples was more dried and woody from nerolidol. However, according to the sensory data of different-from-control-test, even though the color and odor retention of both LTLT- and HTST-samples at the 12th week were less but they were not noticeably different from control therefore, they were still acceptable (data not shown).

Table 8. Changes in selected volatile compounds concentration of dried KL leaves during storage at accelerated storage conditions.

Volatile Compound	Volatile Concentration (µg/g)													
	Accelerated Storage time (weeks)													
	0		1		2		3		4		8		12	
	LTLT	HTST	LTLT	HTST	LTLT	HTST	LTLT	HTST	LTLT	HTST	LTLT	HTST	LTLT	HTST
beta-myrcene	60.2	45.4	41.7	38.3	30.6	28.1	31.0	6.5	8.3	2.9	4.7	2.0	11.9	n.d.
dl-limonene	19.1	17.3	6.8	6.8	13.0	9.0	5.3	1.0	4.6	3.0	4.3	2.9	n.d	n.d
linalool	334.1	182.7	235.2	113.9	151.3	80.5	146.1	65.1	124.0	40.8	135.0	97.5	68.1	44.8
Citronella	12843.4	9212.1	3534.6	3036.7	4857.6	1123.2	2091.6	375.2	1449.1	468.6	427.2	276.3	53.1	32.1
Beta- citronellol	459.3	269.0	236.2	205.9	158.1	131.3	209.8	96.0	115.9	59.5	92.2	51.3	34.0	28.9
cytronellyl acetate	450.2	311.0	374.3	280.4	150.7	93.6	195.0	112.3	90.1	63.3	67.3	55.3	54.8	49.4
Nerolidol	193.1	275.3	88.0	98.4	60.2	58.6	n.d	28.1	1.8	9.9	n.d	4.7	n.d	13.6

n.d: not detected.

After taken all parameters evaluated during accelerated shelf-life storage into consideration, all treated samples could be kept at 25°C and 45°C for at least 12 weeks while the product safety and acceptable qualities were maintained. Therefore, according to the Arrhenius equation, the estimated shelf-life of both LTLT-treated and HTST-treated kaffir lime leaves would be about 48 weeks at regular room temperature of 25°C where LTLT-samples maintained better appearance and flavor retention than HTST-samples.

Conclusion

Dried KL leaves treated by low impact drying condition (LTLT) showed significantly greater improvement in physico-chemical properties, color and flavor from key volatile compounds than those of HTST and commercial KL leaves as compared to fresh leaves ($p<0.05$). During the accelerated shelf life storage, no significant difference was found in the water activity and moisture content values of both LTLT and HTST samples ($p>0.05$). At the accelerated storage time of 12th week which estimated calculated to be 48 weeks, both the samples maintained the microbial count below the standard limits thus their food safety requirements were met. For volatile compound, the LTLT-treated samples still had floral and sweet odor from linalool and citronella whereas HTST-treated samples was more dried and woody from nerolidol. In summary, LTLT-samples maintained better appearance and flavor retention than HTST-samples, and the samples had estimated shelf-life of 48 weeks. In summary, low impact drying method is a promising drying method which provides dried herbs with high color and flavor retention as well as shelf-life extension. The data from this study could be used to predict the shelf life of kaffir lime leaves using different drying conditions, and it would benefit in predicting the impact of shelf-life on the qualities of dried herbs as well.

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