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Changes in antioxidant properties and volatile compounds of kaffir lime leaf as affected by cooking processes

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Abstract

Kaffir lime leaf is one of important spices used in eastern cuisines especially in Thai food and that has been cooked in many ways. Our present study investigated the effect of cooking methods on phenolic compounds and antioxidant properties as well as volatile compounds of kaffir lime leaf. Six commonly used cooking methods including three hydrothermal processes, namely blanching, boiling and steaming, and three non-hydrothermal processes, namely frying, roasting and drying, were evaluated. The results showed steaming provided the highest values of total phenolic and total flavonoid contents along with antioxidant activities determined by in vitro DPPH and FRAP assays while drying with hot air gave the lowest values of those parameters tested. Critonella was the predominant volatile compound in fresh leaf (56%) which was enhanced by all non-hydrothermal processes however it was significantly decreased by all water involving processes. In contrast, beta-citronellol was increased by blanching and boiling but decreased by other cooking processes. Our findings suggest that different cooking methods caused various effects on the phenolic compounds and antioxidant properties of kaffir lime leaf. The presence of different volatile compounds in kaffir lime leaves cooked by each process may be a reason of a unique flavor to the food.

Introduction

Kaffir lime (*Citrus hystrix* DC.), a member of citrus family is a native lime fruit grown in Asia especially in tropical regions. Having distinctive aroma and flavor to food, kaffir lime leaves are precious to many dishes of Thai cuisine. It is a complement ingredient with lemon grass, galangal and lime juice in famous Thai soup, called Tom-Yam to give the soup its wholesome lemony essence, while the outer green peel (flavedo) of kaffir lime is commonly used in curry pastes (Lertworasirikul and Saetan, 2010). Additionally, kaffir lime has long been used as medicinal plant in folk medicine (Hutadilo-Towattana *et al*., 2006). Vegetables and spices have been associated with the prevention of degenerative diseases, such as cancer and cardiovascular diseases (Liu *et al*., 2008). Foods from plant origin usually contain natural antioxidants that can scavenge free radical (Alía *et al.*, 2003). Lawrence *et al*. (1971) reported that the volatile compounds found in kaffir lime leaf oil were citronellal, linalool, β-cubebene, β-pinene, myrcene, limonene, γ-terpinene, ρ-cymene, terpinolene, copaene, caryophyllene, citronellyl acetate, citronellol, geranyl acetate and δ-cadinene. Siripongvutikorn *et al*. (2005) reported that kaffir lime leaves was main sources of β-carotene and inhibited *Staphylococcus aureus* in Tom-Yum mix. In addition, kaffir lime leaves was found to be effective in inhibiting tumors in the digestive tract (Murakami *et al*., 1995) and had good antioxidant properties (Siriamornpun *et al*., 2014).

Most of the vegetables are cooked before consumed by various methods such as boiling, frying, steaming etc. These cooking processes may cause changes in physical characteristics and chemical composition (Rehmam *et al*., 2003; Zhang and Hamauzu, 2004) as well as bioactive compounds and antioxidant properties of vegetables (Oboh, 2005; Turkmen *et al*., 2005; Chuah *et al.*, 2008; Wachtel-Galor *et al*., 2008). Reports on the effects of cooking on the antioxidant compounds in vegetables

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have been conducted extensively (Turkmen *et al*., 2005; Chuah *et al*., 2008; Wachtel-Galor *et al.*, 2008). For example, Chuah *et al.* (2008) reported that thermal treatment an enhancement or no change in antioxidant activity of vegetables, while others have indicated a deterioration of activity after thermal treatments (Turkmen *et al*., 2005; Wachtel-Galor *et al.*, 2008). Zhang and Hamauzu (2004) found that antioxidants and phenolics in broccoli floret and stem decreased with duration of thermal processing, no matter whether the cooking was with conventional methods or microwave heating. Our previous study also showed that different drying methods (far-infrared radiation and low relative humidity drying) affected the content and composition of volatile compounds in kaffir lime leaf (Raksakantong *et al*., 2011a). However, very little information is available on the effect of other cooking methods on the volatile compounds and the antioxidant activities of kaffir lime leaves. Therefore, the purpose of this study was to investigate antioxidant properties and volatile compounds of kaffir lime leaf as affected by different cooking processes especially common cooking methods in Asian cuisines.

Materials and Methods

Plant materials and sample preparation

Kaffir limes (*Citrus hystrix* DC.) leaves were purchased from a local market in Maha Sarakham Province, Thailand during July-September 2011. The samples were sorted visually for the same size. Kaffir limes leaves were selected on basis of dark green color with a glossy sheen and bright green fruits. The fresh leaves was taken and divided into seven portions (100 g for each application). One portion was retained raw, leaves were cooked using hydrothermal process (blanching, boiling and steaming) and non-hydrothermal process (deep frying, roasting and hot-air drying). The effect of cooking was compared with raw unprocessed (fresh). All analyses were performed using triplicate samples and analytical results were expressed on a dry matter basis. All samples (fresh and treated) were stored at –20ºC prior to analysis.

Hydrothermal process

In blanching process, fresh leaves (100 g) were blanched in 1000 ml of water at 85ºC in a stainless steel pan and cooked for 30 s. The samples were drained off and cooled rapidly on plenty of ice. For boiling, the water temperature was set at 80ºC prior to the samples were immersed and reached the boiling temperature. A 100 g portion of leaves were added to 1000 ml of water in stainless steel pan and cooked for 10 min. After cooking, the cooked tissues were drained and cooled rapidly on ice. For steaming, a 100 g portion of leaves were placed on tray in steam rack over boiling water in a closed water bath for 10 min under atmospheric pressure. The steamed leaves were removed and cooled on ice.

Non-hydrothermal process

In deep frying process, a minimum amount of cooking oil (100 ml) was placed in a non-stick frying pan (diameter of 20 cm) and heated at 180–185ºC on a hot plate (Sanyo IC-AI, 1200 W, 100 V) for 1 min. The samples (100 g) were placed in the frying pan for 2 min and drained using a wire mesh strainer. For roasting, a portion of leaves (100 g) were placed on stainless steel pan and roasted at 160ºC for 1 min until the fresh leaves were well cooked. In hot-air drying (HA) experiment, a laboratory scale dryer using in this study was developed in the Research Unit of Drying Technology for Agricultural Product, Faculty of Engineering, Mahasarakham University, Thailand. The sample tray (25.4 x 37 cm^2), the sample tray was placed midway between, and parallel to, the top and bottom heaters, and the distance between each set of heaters and a tray was fixed at 15 cm. One hundred grams of fresh leave were dried at 60ºC and air velocity at 1.5 m/s for 10 h.

Colorimetric parameters

Colour changes in samples were determined by a Minolta CR-300 Chroma Meter (Minolta, Japan) in *L, a, b* colour scale. Parameters *L, a, b* determine a three-dimensional color space, in which L represents brightness (on a lightness–darkness scale) whereas positive and negative a values determine the redness and greenness, and positive and negative b values determine yellowness and blueness, respectively. The instrument was calibrated against a white-standard. Measurements were individually taken for ten samples per treatment and the average of ten readings was calculated. The colour difference ΔE was calculated from the *L, a, b* parameters, using the Hunter-Scotfield equation:

Sample extraction

Each sample (1 g) was extracted with 10 ml of 80% ethanol at room temperature for 2 h on an orbital shaker set at 180 rpm. The mixture was centrifuged at 1400 × g for 20 min and the supernatant was decanted into a 30 ml vial. The pellet was re-extracted under identical conditions. Supernatant was combined and used for antioxidant activity, total phenolic and total flavonoid determinations.

Determination of total phenolic content

Total phenolic content (TPC) was determined using Folin–Ciocalteu reagent as followed by Bakar *et al*. (2009). Briefly, 300 µl of extract was mixed with 2.25 ml of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min; 2.25 ml of sodium carbonate (60 g/l) solution was added to the mixture. After incubation for 90 min at room temperature, absorbance was measured at 725 nm using spectrophotometer. Results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g).

Determination of total flavonoid content

Total flavonoid content (TFC) was determined using colorimetric method described followed by Bakar *et al.* (2009) with minor modifications. Briefly, 0.5 ml of the extract was mixed with 2.25 ml of distilled water in a test tube followed by addition of 0.15 ml of 5% $NaNO_2$ solution. After 6 min, 0.3 ml of a 10% $AlCl_3 \bullet 6H_2O$ solution was added and allowed to stand for another 5 min before 1.0 ml of 1M NaOH was added. The mixture was mixed by vortex mixer. The absorbance was measured immediately at 510 nm using spectrophotometer. Results were expressed as mg rutin equivalents in 1 g of dried sample (mg RE/g).

Determination of antioxidant activity

DPPH radical scavenging activity (DPPH) and Ferric reducing/antioxidant power (FRAP) were used for determination of antioxidant activity. The DPPH radical scavenging activity was conducted according to the method described by Gulluce *et al*. (2007). Briefly, 0.1 ml of aqueous extract was added to 3 ml of a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percent inhibition activity was calculated as $[(Ao - Ae)/Ao] \times 100$ (Ao = absorbance without extract; Ae = absorbance with extract). For FRAP assay, the FRAP value of each sample was determined using the method of Butsat and Siriamornpun (2010). The FRAP solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3$ solution. The fresh working solution was warmed at 37ºC before using. Kaffir lime leaves extracts (100 µl) were allowed to react with 1.9 ml of the FRAP solution. The absorbance at 593 nm of the mixture was measured after 60 min of reaction. The results were calculated by standard curves prepared with known concentrations of $FeSO_4$ and were expressed as µmol $FeSO_4$/g dry sample.

Determination of volatile compounds

The samples were ground and 0.2 g was put in vials. The vials were sealed with an aluminium–rubber septum (Supelco, Bellefonte, PA, USA) and analysed by the headspace sampling technique (Barcarolo and Casson, 1997). GC-MS analysis was carried out using a GC-2010 chromatograph coupled to a GC/MS-QP2010 (Shimadzu, Japan). Samples were analyzed on a fused-silica capillary column Rtx-5Ms (5% diphenyl 95% dimethyl polysiloxane, 30 m length, 0.25 mm internal diameter, 0.25 µm film thickness; Restek, PA, USA) and Rtx-5 (5% diphenyl 95% dimethyl polysiloxane, 30 m length, 0.25 mm internal diameter, 0.25 µm film thickness; Restek, PA, USA). Carrier gas, helium; constant pressure, 134.2 kPa; injector temperature, 250°C; split ratio, 1:5; temperature program, 80 to 250°C at 10 °C/min then held isothermal (2 min) at 250°C; ion source temperature, 200°C; transfer line temperature, 250°C; ionization energy, 70 eV; electron ionization mass spectra were acquired over the mass range 35-550 u.

Statistical analysis

Analysis of variance (ANOVA) in a completely randomized design, Duncan's multiple range tests was performed to compare the data. All determinations were done at least in triplicate and all were averaged. The confidence limits used in this study were based on 95% ($p < 0.05$).

Results and Discussion

Uses of kaffir lime leaf in Thai style cooking and examples of cuisines containing kaffir lime leaf are presented in Table 1. It can be seen that kaffir lime leaf is extensively used in a number of dishes and make different food flavor to each dish. The main reason is that kaffir leaf brings about desirable and unique flavor to the food. However the chemical properties such as antioxidant properties can be varied from one to another depending on the cooking processes.

Effect of cooking methods on the total phenolic content (TPC)

TPC values of kaffir lime leaves, before and after cooking, are shown in Table 2. When comparing the effect of among six cooking methods, the contents of total phenolic were significantly ($p < 0.05$) increased in steaming and roasting methods compared to that of fresh sample while others showed adverse results. The highest level of TPC in the kaffir lime leaves after cooking was found in steamed leaves (22.18 mg GAE/g dry sample), followed by roasted (20.58 mg

Table 1. Uses of kaffir lime leaf in Thai style cooking

Type of cooking	Examples of cuisines
Fresh leaf	Use whole leaf or chopped leaf as decoration ingredient on top of the meals such as red curry, green curry, spicy meat salad, etc.
Hydrothermal process	
Blanching	Blanched meat, sea food, fish
Boiling	Soup: Tom yum (hot and sour soup) ,Tom kha (mild soup with coconut milk) Curry: green curry, red curry, light curry (curry without coconut milk)
Steaming	Steamed fish, chicken, beef, pork, steamed insect, steamed shells
Non-hydrothermal process	
Deep frying	Use whole deep fried leaf as decoration ingredient and eaten together with the meal
Drying	Use as ingredient for making all types of chilli paste
Roasting	The use is similar to those of deep fired leaf depending on the preference

GAE/g dry sample), while the lowest was in hot air dried (15.11 mg GAE/g dry sample).

There have been many studies conducted on changes of phenolics in various cooking processes (Turkmen *et al.*, 2005; Wachtel-Galor *et al*., 2008). Turkmen *et al.* (2005) reported that total phenolic content of green vegetables was increased after boiling, steaming and microwaving. Similarly, steaming was showed to retain and enhance the brassica vegetable followed by boiling and then microwaving (Wachtel-Galor *et al*., 2008). Thermal treatment has an effect on a range of characteristics of the plant including texture, colour, structure, nutritional value as well as antioxidant properties (Rechkemmer, 2007; Wachtel-Galor *et al.*, 2008; Wanyo *et al*., 2011). It is assumed that food processes, in some cases, might accelerate more bound phenolic compounds releasing from the breakdown of cellular constituents (Raksakantong *et al.*, 2011b). This is due to the inactivation of the polyphenol oxidase enzyme during cooking, leading to the inhibition of polyphenols degradation (Yamaguchi *et al.*, 2003). On the other hand, losses of polyphenols upon blanching have been reported in some tropical green leafy vegetable (Oboh, 2005), broccoli (Zhang and Hamauzu, 2004), coloured peppers, hot air drying of Thai kaffir lime (Raksakantong *et al*., 2011a) and hot air dried mulberry leaves (Wanyo *et al*., 2011). Ismail *et al.* (2004) reported a decrease in antioxidant capacity of shallots, spinach, cabbage and kale after 1 min of cooking. Our present study found that steaming gave the highest TPC, the explanation could be that during steaming, the temperature were lower than in the other methods and therefore did not affect the phenolic content as much however blanching with a very short time (30 s at 85°C) might not sufficient to inactivate polyphenol oxidase hence resulting in a decrease of TPC.

Effect of cooking methods on the total flavonoid content (TFC)

Flavonoids are the most important natural phenolics known as one of the most diverse and wide spread group of natural compounds (Prasad *et al*., 2009). Besides their antioxidant activity, flavonoids have been demonstrated a wide range of biochemical and pharmacological effect including anti-inflammatory, anti-viral, anti-allergenic, anti-carcinogenic, anti-aging activity (Orak, 2007). TFC value was expressed as mg of rutin equivalent/g dry sample and is presented in Table 2. Similar to TPC, steaming significantly increased the amount of total flavonoids while other cooking methods significantly decreased. The effect of thermal processes on changes in TFC has been reported in different kinds of vegetables and fruits. Wanyo *et al*. (2011) demonstrated that TFC was decreased by far-infrared radiation with hot-air convection (FIR–HA) and hot-air (HA) (60°C) drying methods in mulberry leaves. The results are also in agreement with the findings by Raksakantong *et al.* (2011b) that drying kaprow khao samples with hot-air (HA) decreased the content of total flavonoids. Flavonoids are present as ester or glycosides so they can be partially hydrolysed during boiling and this hydrolytical changes influence both their distribution between lipidic and aqueous phases and their reaction with free radicals. This phenomenon may result in loss of some nutrients or microchemical components (Pokorny, 2003). However, some flavonoid compound could be enhanced by hydrothermal treatment (blanching, boiling and steaming) such as kaempferol (Yao and Ren, 2011).When we compare between two cooking methods; blanching and boiling which both are hydrothermal treatments it was found that boiling provided higher TFC and TPC than boiling. The reason could be that -for blanching, the heat is less intensive and cooking time is shorter than in boiling, some oxiductase or polyphenoloxidase may be still activated, causing greater decreases of TPC, TFC and FRAP values.

Effect of cooking methods on the antioxidant activity

DPPH radical scavenging activity

DPPH radical is a free radical compound which has been widely used to test free-radical scavenging ability (Sakanaka *et al*., 2005). The DPPH radical-scavenging activities expressed as % inhibition are given in Table 2. The values of cooked samples were in a wide range of 44% by hot-air drying to 56% by steaming and boiling. The percentage inhibition of steamed and boiled leaves significantly ($p < 0.05$)

Table 2. Total phenolic content, Total flavonoid content, FRAP and DPPH value of kaffir lime leaves cooked by different methods

Samples	Total phenolic content (mg GAE/g)	Total flavonoid content (mg RE/ g)	FRAP (μmol $FeSO_4$/g)
Fresh	20.10±0.14c	10.52±0.03b	447±9.01c
Hydrothermal			
Blanched	17.80±0.24e	8.39±0.06d	404±10.54d
Boiled	19.08±0.14d	9.54±0.04c	583±18.17b
Steamed	22.18±0.06a	11.84±0.02a	628±27.02a
Non-hydrothermal			
Deep fried	17.94± 0.88e	8.25±0.74d	445±10.03c
Hot air dried	15.11± 0.30f	8.78± 0.56d	386±11.49d
Roasted	20.58± 0.11b	9.51±0.12c	441±14.31c

Values are expressed as means ± standard deviation (n = 3). Means with different letters in the same column were significantly different at the level $p < 0.05$.

increased when compared with the values for fresh ones. This finding is the same with TPC and TFC which steaming has shown to provide the highest values in treated samples. Turkmen *et al.* (2005) showed that there was a significant difference in the contents of antioxidant activity during boiling steaming and microwaving cooking in samples (pepper, squash, green beans, leek, broccoli and spinach). According to Wachtel-Galor *et al.* (2008) reported that steaming for 5 and 10 minutes caused significantly enhance of DPPH radical scavenging activities in cauliflower, cabbage, broccoli and choy sum. This effect is perhaps due to production of redox-active secondary plant metabolites or breakdown products. In addition, modification and changes of intracellular molecules, cell wall structure and matrix during food preparation and process may release more antioxidants (Rechkemmer, 2007).

Ferric reducing activity based on FRAP assay

The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex and producing a coloured ferrous tripyridyltriazine (Fe^{2+}–TPTZ) (Benzie and Strain, 1996). FRAP values of the kaffir lime leaves are shown in Table 2. The mean FRAP values of the studied cooking method showed that steaming > boiling > fresh ≈ deep frying ≈ roasting > blanching ≈ hot-air drying. Similar results to DPPH, TPC and TFC, steaming gave the highest values of FRAP (628 μmol $FeSO_4$/g) (Table 3). Our findings support the results from previous study of Wachtel-Galor *et al.*, (2008) that steaming cooking increases the antioxidant activity of selected vegetables in their study. For thermal process, heat is transferred more slowly by hot air than by hot water because of differences in heat conductivity. In some cases, decomposition of antioxidants during heating is partially compensated by formation of Maillard products, which also possess antioxidant activity, partially due to their chelating capacity (Pokorny, 2003).

Percentage losses or gains for antioxidant activity, total flavonoid and total phenolic compounds

Percentage losses or gains for TPC, TFC FRAP and DPPH of kaffir lime leaves by different cooking methods compared to fresh leaves are demonstrated in Figure 1. TPC, TFC, FRAP and DPPH values were increased by steaming (TPC: 10%, TFC: 13%, FRAP; 40% and DPPH 21%, respectively). Boiling enhanced FRAP and DPPH (30 and 21%, respectively) but loss in TPC and TFC (5 and 9%, respectively). While, HA drying decreased TPC, TFC, FRAP and DPPH value (25, 16, 13 and 4%, respectively), compared to those of fresh leaves. Our results were similar to the data from previous study by Wachtel-Galor *et al.* (2008) that total antioxidant activity (FRAP) of cauliflower was increased by 88 and 160%, respectively after cooking by boiling and steaming for 10 min. The properties of naturally occurring antioxidants were improved or induced the formation of new compounds with antioxidant properties by food processing, so that the overall antioxidant activity increases or remains unchanged (Tomaino *et al.*, 2005). Therefore degradation of polyphenol compounds by thermal process may result in releasing antioxidant compounds which have different chemical and biological properties (Tsai *et al.*, 2002).

Overall, many factors influence changes in the antioxidant functionality such as heat transfer medium (water air or radiation), temperature, food composition, time and light access. When comparing different studies, it should be noted that the effect of cooking is likely to depend on several factors such as

Table 3. Volatile compounds (% area) of kaffir lime leaves cooked by different methods

No	RT	Volatile compounds	Fresh	*Hydrothermal*			*Non-hydrothermal*		
				Blanched	Boiled	Steamed	Deep fried	Roasted	Hot air dried
1	3.144	Phenylacetaldehyde	1.40±0.02a	nd	nd	nd	nd	nd	nd
2	3.608	Linalool	9.76±0.44d	3.02±0.22c	nd	nd	3.23±0.04b	nd	3.72±0.31a
3	4.207	Citronella	56.36±7.93d	4.21±0.42e	nd	nd	86.74±1.43a	82.86±1.01b	77.26±9.01c
4	4.218	3,7-Dimethyl-6-octenal	nd	nd	4.09±0.03b	51.77±7.21a	nd	nd	nd
5	5.089	2,3 -dihydrogeraniol	6.14±0.76a	nd	nd	nd	nd	nd	nd
6	5.091	Beta-citronellol	4.72±0.39d	57.15±6.31b	76.05±2.00a	nd	1.53±0.02f	1.63±0.01e	4.92±1.00c
7	6.64	Citronellylacitate	nd	5±0.12a	1.46±0.08f	3.3±0.68b	2.42±0.07d	3.09±0.12c	1.86±0.02e
8	7.047	Copaene	nd	2.05±0.04a	nd	nd	nd	nd	nd
9	7.05	Alpha-copaene	nd	nd	1.99±0.01b	3.55±0.42a	nd	nd	nd
10	7.048	Alpha-cubebene	nd	nd	nd	nd	1.47±0.01a	nd	nd
11	7.214	Germacrene D	nd	1.13±0.01b	nd	nd	1.18±0.02a	nd	nd
12	7.623	Caryophyllene	6.53±0.16c	nd	9.18±0.05b	26.6±2.67a	2.63±0.05f	nd	3.32±0.10d
13	7.625	Trans-caryophyllene	nd	12.44±0.52a	nd	nd	nd	4.32±0.24b	nd
14	8.256	Dihydroionone	nd	nd	nd	nd	nd	2.89±0.11	nd
15	8.568	Bicyclogermacrene	nd	2.8±0.10d	4.85±0.01b	5.16±0.04a	0.8±0.01	2.72±0.13e	2.91±±0.03c
16	8.846	Delta-cadinene	nd	nd	nd	2.3±0.84a	nd	nd	nd
17	8.849	Cadinene	nd	nd	nd	nd	nd	1.07±0.01a	nd
18	9.354	Sqalene	5.44±0.28a	nd	nd	nd	nd	nd	nd
19	14.002	Alpha-cedrol	nd	nd	nd	nd	nd	nd	1.3±0.01a
20	14.941	Phytol	nd	3.22±0.06c	2.38±0.03d	6.26±0.44a	nd	1.42±0.03e	4.73±0.19b
21	22.6	4,4-Dimethyl-2-oxo-tetrahydrofu	nd	nd	nd	1.07±0.05a	nd	nd	nd

Values are expressed as means ± standard deviation (n = 3). Means with different letters in the same row were significantly different at the level $p < 0.05$. nd = not detected

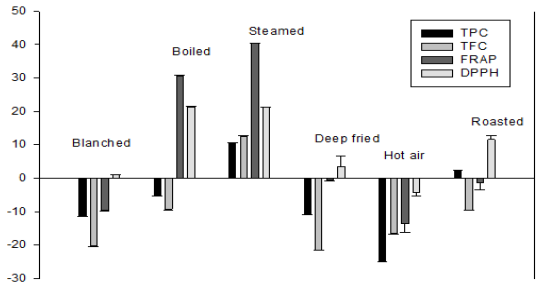

Figure 1. Percentage loss or gain of total phenolic content (TPC), total flavonoid content (TFC) ferric reducing/ antioxidant power (FRAP) and DPPH radical scavenging (DPPH) for kaffir lime leaf samples after different processing methods compared to fresh leaves. Different letters on different bars indicate significant differences between treatments ($p < 0.05$) for TPC, TFC and FRAP

the cooking procedure, degree of heating, leaching into the cooking medium, solvent used for extraction, pH and surface area exposed to water and oxygen (Wachtel-Galor *et al.*, 2008). In addition, different plants contain various components which might be thermally unstable but some might be not so the same cooking method may have different effects on different types of plants (Bernhardt and Schlich, 2006). Results of our present study have shown that besides colour changes, cooking methods had both positive and negative effects on phenolic and flavonoid contents as well as antioxidants properties. Steaming was the only method that enhances the contents total phenolics compounds (TPC and TFC) and antioxidant activities of kaffir lime leaves.

Changes in volatile compounds as affected by cooking process

The component of volatile compounds in kaffir lime leaves cooked by different methods namely blanching, hot-air drying, deep frying, and roasting leaves were analyzed using GC-MS and the results are presented in Table 3. Cooking brought about changes in the concentration of certain volatile compounds in regards to cooking methods. For example, critonella was the predominant volatile compound which was enhanced by deep frying, roasting and hot air drying citronella content was increased by deep frying, roasting and hot air drying (87, 83 and 77%, respectively) however was decreased by water involving process such as blanching and totally destroyed by boiling and steaming. On the other hand, sqalene was found only in fresh leaves and totally destroyed by all means of cooking whereas citronellylacitate and bicyclogermacrene were not detected in fresh leaves but could be enhanced by all means of cooking.

Changes in the concentrations of the volatile compounds during processing depend on several factors such as cooking method and parameters that are characteristic of the product subjected to cooking (Venskutonis, 1997). For drying, in some cases increases in the quantities of some of the

Table 4. Color parameters of kaffir lime leaves cooked by different methods.

samples	*L*	*a*	*b*	*ΔE*
Fresh	42.44±0.56c	-9.49±0.42d	11.82±0.31e	-
Hydrothermal process				
Blanched	40.41±0.38d	-6.71±0.82c	7.05±0.69bc	5.63±0.21d
Boiled	41.41±0.53d	-1.98±0.45a	9.69±0.37d	7.91±0.24c
Steamed	42.52±0.71c	-3.38±0.26b	11.41±0.96e	5.91±0.36d
Non-hydrothermal process				
Deep fried	49.51±0.73b	-3.32±0.47b	4.58±0.28a	11.12±65a
Hot air dried	51.39±0.26a	-3.40±0.78b	7.61±0.36c	11.82±0.24a
Roasted	39.30±0.32e	-2.41±0.65a	6.28±0.25b	9.58±0.33b

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

components characteristic of a given spice have been observed (Baritaux *et al*., 1992; Bartley and Jacobs, 2000) or the formation of new compounds after drying has been recorded, probably as a consequence of oxidation reactions, hydrolysis of glycosylated forms, or the release of compounds by the rupture of cell walls (Huopalahti *et al.*, 1985)

Colour parameters

Colour is one of important psychological characteristics of food products that influence the consumer attraction. Color degradation of food product occurs during cooking is a result of heating (Lozano and Ibarz, 1997). Colour parameters of cooked samples compared to fresh kaffir lime leaves are shown in Table 4. Overall, significant changes in *L* value of the all cooked leaves were observed, when compared with the fresh leaves. The total colour difference *ΔE*, which is a combination of parameters *L, a, b* values, is a colorimetric parameter extensively used to characterize the variation of colors in food during processing. The results presented in this work suggest that the changes in *ΔE* of blanched, steamed, boiled and roasted leaves were smaller as compared to those of deep fried and HA drying. As the colour of blanching, steaming and boiling of kaffir lime, appeared to be more like fresh leaf, these may imply that these cooking methods could better preserve bioactive compounds and activities. The colour changes in kaffir lime leaves caused by the thermal may be due the non-enzymatic browning reaction, but also to the destruction of pigments present in the leaf (Wanyo *et al*., 2011; Raksakantong *et al*, 2011a).

Conclusion

The results presented here clearly demonstrate that cooking methods influenced the antioxidant capacity and volatile compounds during processing of kaffir lime leaf. Favorable effect on phenolic compounds and antioxidant activities was achieved by steaming and whereas other cooking method studied provided adverse results except for boiling which increased antioxidant capacities but not TPC and TFC. Changes in the concentrations composition of the volatile compounds as affected by different means of cooking were also observed. We have demonstrated the importance of how cooking methods influence antioxidant properties and bioactive components of plants. This would be valuable for process design or cooking plans to preserve the bioactive compounds and their health benefits of kaffir lime leaf.

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