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Antioxidant Properties of Kaffir Lime Oil as Affected by Hydrodistillation Process

Wulandari, W., Y[1], Darmadji, P[2]., and [3]Kurniawati, L
[1,3]Departement of Food Technology, Slamet Riyadi University
[2]Departement of Food and Agricultural Product Technology, Gadjah Mada University

Abstract:Kaffir lime oil is essential oil from kaffir lime leaves (*Citrus hystrix*. DC). That essential oil possesses some important bioactivities such as antioxidant, antileukemic, antitussive, antihemorrhage, antioxidative stress properties, and antibacterial properties. Nowdays, essential oil of kaffir lime is in demand as a fragrance in the food, perfumery and cosmetic industries our present study investigated the effect of leaves treatment by hydrodisstillation process on phenolic compounds and antioxidant properties as well as volatile compounds of kaffir lime leaf. The results showed milling provided the highest values of total phenolic (23.15 mg/GAE/g) and total flavonoid contents (12.85 mg/RE/g) along with antioxidant activities determined by in vitro DPPH assays. Our findings suggest that different treatment of leafs before hydrodisttilation process caused various effect on the phenolic compounds and antioxidant properties of kaffir lime oil.

Keywords : kaffir lime oil, phenolics, flavonoids, hydrodistillation

Introduction

Kaffir lime (*Citrus hystrix.* DC) is a Southeast Asian citrus plant with very strong fragrance (Tinjan *et al*., 2007). Kaffir lime leaves used a condiment in various Thai and Malaysian recipes to add a tangy flavor to soup and curries (Butryee *et al.*, 2009). Citrus hystric leaf is rich in vitamin E. Among 62 edible tropical analysed for α-tocopherol content citrus hystrix leaves (398.3 mg/kg edible portion) ranked second behind sauropus androgynus (426.8 mg/kg edible portion) (Ching and Mohamed, 2001).

Thirty-eight constituents were identified in the leaf essential oil of kaffir lime representing 89% of the total oil (Waikedre *et al.*, 2010).The oil was rich in monoterpenes (87%) with ß-pinene as major component (10%) and low limonene (4.7%). The essential oil of citrus hystric was characterized by high content of terpinen-4-ol (13.0%), α-terpineol (7.6%), 1,8-cineole (6.4%), and citronellol (6.0%). Twenty nine compounds were found in the essential oil of kaffir lime leaves (Loh *et al.,* 2011), ß-citronellal was the major compound amounting to 66.85% of total oil.

Kaffir lime leaf oil possesses some impotant bioactiveties such as antioxidant (Hutadilok *et al.*, 2009), antileukemic (Ampasavate *et al.*, 2010), antitussive, antihemorrhage, antioxidative stress properties (Laohavechvinch *et al.*, 2010), and antibacterial properties (Siripongvutikora *et al.*, 2005). Nowdays essential oil of kaffir lime is in demand as a fragrance in the food, perfumery, and cosmetic industries (Phoungchang *et al.*, 2008; Manosroi *et al*., 2005).

Hydrodistillation processing has been used for the isolation of essential oils (Gunther, 1987). It is known that conventional method used for extraction of essential oils from plant materials have some advantages such as simple technology and cheap so feasible commonly used to industrial extraction of essential oils. Most of the plant matrials are treatment before hydrodistillation process such as curing, drying, milling, reduction in particles size etc. The cooking processes of the vegetables may cause change in physical characteristics and chemical composition as well as bioactive compounds and antioxidant properties (Oboh, 2005; Turkam *et al*, 2005; Chuah *et al.*, 2008; Wachtel-Galor *et al.*, 2008).

Our previous study also showed that different drying methods affected the content and composition of volatile compounds in kaffir lime leaf (Raksakatong *et al.*, 2011). However, very little information is available compounds and the atioxidant activities of kaffir lime leaves. Therefore, the purpose of this study was to investigate antioxidant properties of kaffir lime oil as affected by some treatment on material plant before hydrodistillation process.

Material and Methods

Plant material and sample preparation

Sample lime leaves were colected from local orchid in Tulung Agung Central Java, located in Pulo Tondo. Upon arrival at the laboratory, mature leaves were sorted out. The mature leaves were washed under running tap water and dried by air at 3 day. All samples (fresh and treated) were stored at -20^{o}C prior to analysisi. All analyses were perfomed using triplicate samples and analytical results were expressed on dry material basis.

Hydrodistillation

Conventional hydrodistillation was carried out with a Clavenger apparatus. Dry plant material:water ratio of 1:10 was used and essential oil samples was collected at different process times. For each distillation time, experiments were conducted twice. The maximum distillation was 4 h since after time no more essential oil was obtained. The essential oil was collected in amber colored vials, dehydrated with anhydrous sodium sulfate, capped under nitrogen and kept at 4^{o}C until being analyzed.

Determination of total phenolic content

Total phenolic content (TPC) was determined using Folin-Ciocalteu reagent as fallowed by Bakar *et al.* (2009). Briefly, 300µl 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 temperatur 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 spectrohptometer. Results were expressed as mg gallic acid equevalents in 1 g sample (mg GAE/g).

Determination of total flavonoid content

Total flavonoid content (TPC) was determined using colorimetric method discribed fallowed by Bakar *et al* (2009) with minor modification. Briefly, 0.5 ml of the extract was mixed with 2.25 ml of distilled water in a test tube followed by addition of 0.15ml of 5% $NaNO_2$ solution. After 6 min, 0.3 ml of a 10% $AlCl_3.6H_2O$ solution was added and allowed to stand for another 5 min before 1.0 ml of 1 M 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

Determination of antioxidant activity

DPPH radical scavanging activity (DPPH) was 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

$$= \left(\frac{Ao - Ae}{Ao} \right) x100$$

(*Ao* = absorbance without extract; *Ae* = absorbance with extract)

Statistical analysis

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

Results and Discussion

Essential oil yield

Kaffir lime leaf were phisical treatment before distillation process such as cutting into particle sice of 1 cm and milling at 3 minutes. The maximum yields obtained were affected by a kind of treatment are presented in Table 1. When comparing the effect of treatments, the yield of essential oil were significantly ($p < 0.05$) increased in cutting and milling methods compared to that whole sample while others showed adverse results. The highest level of yield after hydrodistillation process was found in milled leaves (0.65 mL oil/g dry samples), fallowed by cutted (0.75 mL oil/ g dry samples), while the lowest was in whole leaves (0.58 mL/ g dry samples). It can therefore, be concluded that milled treatment before distillation process offers higher essential oil yield from kaffir lime in comparison with cutted and no treatment. It was shown from the matrix particle size of the sample was milled, as the reduction in the particle size will increase the surface area and may weaken the cell walls and increase the percentage of broken cells, and will lead to the reduction in the mass transfer resistence. Thus the extracted oil will be more accessible to the hydrodistillation process, as a result, increasing the essential oil yield.

Tabel 1. Percetage yields of kaffir lime leaf oils by hydrodistillation

Samples	Yield (%)± SD
Fresh	0.58 ± 0.03
Milled	0.85 ± 0.02
Cutted	0.65 ± 0.03

Citrus hystrix leaf rich in vitamin E. Among 62 edible troplical plants analysed for α-tocopherol content *Citrus hystrix* leaves (398.3 mg/kg edible portion) ranked second behind *Sauropus androgynus* leaves (Ching and Mohamed, 2001). Thirty-eight constituents were identified in the leaf essential oil of Kaffir lime representing 89% of the essential oil (Waikedre *et al.*, 2010). The oil was rich in monoterpenes (87%) with ß-pinene as the major component (10%) and low limonene (4.7%). The essential oil of *C.hystrix* was characterized by high content of terpinen-4-ol (13.0%), α-terpeniol (7.6%), 1,8-cineole (6.4%), and citronellol (6.0%). The oil was found inactive against bacteria.

The component of volatile compounds in kaffir lime leaves from this research were analyzed using GC-MS and the results are presented in Figure 1 and Table 2. Citronellal was dominant volatile compound. The compositions of the essential oil extracted by using hydrodistillation with milled treatment for the leaves.

Tabel 2. Concentrations of the compounds present in the essential oil of kaffir lime leaf oils by hydrodistillation

No	Compounds	Retention Time	Concentration (%)
1	Sabinene	13.945	3.21
2	Linalool	19.155	3.02
3	Citronellal	21.060	80.86
4	Isocaryophyllene	29.037	1.33
5	Methyl-trans 9-octadecenoate	44.022	11.58

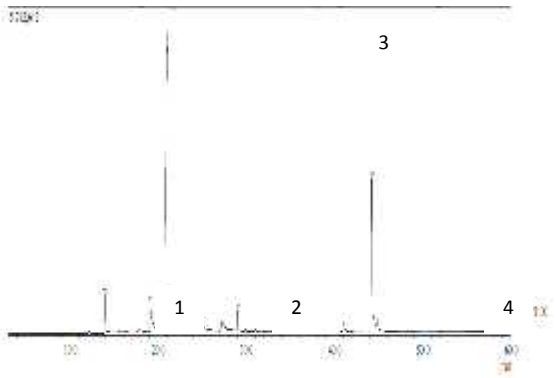

Figure 1. Chromatogram of the kaffir lime leaf extracted by hydrodistillation

DPPH radical scavenging activity

The DPPH radical scavenging activities expressed as % inhibition are given in Tabel 3. DPPH radical is a free radical compound which has been widely used to test-radical scavenging ability (Sakanaka *et.al.*, 2005). The value of distillated samples were in wide range 40,59% in whole to 65,56% by milled and cutted. The percetage inhibition of milled and cutted leaves significantly ($p< 0.05$) increased when compared with the values for fresh ones. This finding is the same with TPC and TFC which milling has shown the highest values in treated samples. This effect is perhaps due to production of redox-active secondary plant metabolites or breakdown products. In addition, modification and changes of intracellullar molecules, cell wall structure and matrix during food preparation and process may release more antioxidants (Rechkemmer, 2007).

Total phenolic content and total flanonoids from this research in kaffir lime leaves in fresh and after hydrodistillation process, are shown in Table 3. When comparing the effect of hydrodistillation process, the contents of total phenolic and total flavonoids were significally ($p< 0.05$) increased in cutting and milling methods compared to that fresh sample while others showed adverse results. The highest value of TPC in the kaffir lime leaves after hydrodistillation was found in milled leaves (23.15 mg GAE/g dry sample), followed by cutted (20.03 mg GAE/g dry sample), while the lowest was in whole leaves (18.72 mg GAE/g dry sample). There have been many studies conducted on changes of phenolics in various cooking process . Turkmen *et al.* (2005) reported that total phenolics 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 treatments has an effect on a range of characteristics of plant including texture, structure, colour, nutritional value as well as antioxidant properties (Rechkemmer, 2007; Wachtel-Galor *et al.*, 2008; Wanyo *et al.*, 2011).

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). Flavonoids are the most important natural phenolic known as one of the most diverse and wide spread group of natural compounds (Prasad *et al.*, 2009). The effect of thermal processes on change in total flavonoid content (TFC) has been reported in defferent kinds of vegetables and fruits. Wanyo *et al.* (2011), demostrated that TFC ws decreased by far-infrared radiation with hot-air convection and hot-air in mulberry leaves. This phenomenon may result in loss of some nutrients or microchemical components (Pokorny, 2003). However, some flavonoid compound could be enhanced by hydrothermal treatment such as kaempferol (Yao and Ren, 2011). When we compare between two treatment milling and cutting in hydrodistillation processes it was found that milling provided higher TFC and TPC than cutting.

Table 3. Total phenolic content, total flavonoid content, and DPPH value of kaffir lime ol by hydrodistillation processes

Samples	Total Phenolic Content (mg/GAE/g)	Total Phenolic Content (mg/GAE/g)	DPPH % inhibition
Fresh	21.10 ± 0.14^{a}	11.56 ± 0.05^{b}	44.89 ± 0.08^{a}
Hydrodistillation			
Whole	18.72 ± 0.21^{a}	9.40 ± 0.04^{d}	41.52 ± 10.24^{d}
Cutted	20.03 ± 0.13^{c}	10.54 ± 0.03^{c}	55.34 ± 19.25^{b}
Milled	23.15 ± 0.05^{a}	12.85 ± 0.01^{a}	58.30 ± 25.01^{a}

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

In another study it was found that the yield of phenolics from *Citrus hystrix* leaves, total phenolic content (TPC) and DPPH-IC_{50} obtained were 5.06%, 116.53 mg GAE/g extract and IC_{50} of 0.063 mg/ml, recpectively (Jamilah *et.al.*, 2011). Both DPPH radical scavenging activity assay and linoleic acid model syatem assays followed the decreasing order of : *Curcuma longa>Murraya koenigii>Citrus hystrix>Pandanus amaryllifolius* (Indris *et.al.,* 2008). 2.000 ppm was chosen as the optimum concentration to be used in deep frying experiment. Extracts of *Citrus hystrix* exhibited protective activity towards RBD (refenid, leached, and deodorised) palm olein that was improving and also maintaining the sensories characteristics of French fries. The French fries treated with kaffir lime extracts were acceptable by panelists until day 5 of frying. The natural antioxidants significantly lowered the rate of oil oxidation during deep-fat frying and maintaining the quality of Frech fries. Studies indicated that the antooxidative activity of extract Kaffir lime contains antioxidants and has very good potensial to be explored as sources of natural antioxidants.

Conclusion

The results presented that pretreatments of samples in hydrodistillation processes influenced the antiokxidant capacity and volatile compounds of kaffir lime oil. Favorable effect on phenolic compounds and antioxidant activities was acheived by milling. different treatment of leafs before hydrodisttilation process caused various effect on the phenolic compounds and antioxidant properties of kaffir lime oil.

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