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Note

Phenolic Content and Radical Scavenging Capacity of Kaffir Lime Fruit Peel Extracts Obtained by Pressurized Hot Water Extraction

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The effects of the pressurized hot water extraction (PHWE) parameters (extraction temperature: 100, 150 and 200°C and extraction time: 5, 10 and 15 min) on the total phenolic content and DPPH radical scavenging capacity of the kaffir lime (*Citrus hystrix*) fruit peel extracts were investigated. Both indices increased as the extraction temperature increased. The extraction time only slightly affected the values. This study also demonstrated that the PHWE produced extracts with a higher phenolic content and radical scavenging capacity than that obtained by a conventional extraction method (water and 60% methanol at 50°C, 1 h).

Keywords: Kaffir lime; pressurized hot water extraction; subcritical water extraction; DPPH scavenging capacity; antioxidant activity; response surface

Introduction

Kaffir lime (*Citrus hystrix*) is a regional spice in the Asian countries including Thailand. The fruit peels and leaves of this spice are often added to Thai foods to give them a characteristic flavor. The methanolic extract of the kaffir lime peel was found to exert a strong effect on the production of the hydroxyl radical (Hutadilok-Towatana *et al.*, 2006).

Pressurized hot water extraction (PHWE) (so-called subcritical water extraction) is receiving much interest as an environmentally friendly technique for the extraction of valuable substances from various materials (Ayala *et al.*, 2001; Smith, 2002). One problem associated with this method is that high operating temperatures, which are, in most cases, 100 to 250°C, may degrade some thermally labile components. For example, Ju *et al.* (2005) showed that during the extraction of phenolic compounds from grape skin using PHWE (100 to 160°C, 40 s), the total phenolics and other individual phenolics began to decrease at 110°C or higher. On the other hand, Rodriguez-Meizoso *et al.* (2006) indicated that not all antioxidant compounds would be degraded or oxidized at such temperatures.

Current knowledge regarding the antioxidant from the kaffir lime peel is very limited. In this study, we investigated the effects of the PHWE parameters (temperature and time) on the total phenolic content and the radical scavenging capacity of the kaffir lime peel extract.

Materials and Methods

Materials Fresh kaffir lime fruits were purchased from a local market in Nakhon Pathom, Thailand, and were peeled using a knife. The collected peels were dried in a hot-air oven at 50°C for 10 h. The dried peels were then ground in a blender and stored at -20°C until used.

Distilled water used for the extraction was purged with nitrogen gas for 3 h before use. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (Germany). Folin-Ciocalteu reagent and gallic acid were purchased from Fluka (Switzerland).

Pressurized hot water extraction The batch-type extraction vessel used in this study was fabricated by the Taiatsu Techno Corporation (Osaka, Japan) with the net volume of 10.8 mL. A detailed structure of the vessel was described by Khuwijitjaru *et al*. (2004). The extraction was performed as

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follows: distilled water (8.0 g) and ground kaffir lime peels (0.2 g) were added to the vessel. The vessel was closed and heated to the desired temperature using a ribbon heater. The extraction was performed at a constant temperature (100, 150 and 200°C). The extraction time (5, 10 and 15 min) was counted after the temperature inside the vessel reached the specified temperature. Times needed to heat the vessel were 8 min for 100°C, 14 min for 150°C and 20 min for 200°C. After the extraction, the vessel was immediately cooled to room temperature using running tap water. The mixture was filtered through a Whatman No. 4 filter paper and the liquid was centrifuged at 3,000 rpm for 5 min. The volume of the obtained supernatant was adjusted with distilled water to 10 mL. All the extractions were performed in triplicate with a completely randomized design.

Conventional solvent extractions The sample of ground peels (0.2 g) was mixed with 8.0 g of water or 60% ethanol in test tubes equipped with a cap and the mixture was shaken in a water bath at 50°C for 60 min. The mixture was then filtered through Whatman No. 4 filter paper and the liquid was centrifuged at 3,000 rpm for 5 min. The volume of the obtained supernatant was adjusted with distilled water to 10 mL. All extractions were performed in triplicate.

Total phenolic content The extract (200 µL) was mixed with Folin-Cicalteu reagent (1,000 µL) and then 0.8 mL of 7.5% (w/v) Na_2CO_3 was added to the mixture. The mixture was kept in the dark for 30 min before the absorbance was determined at 765 nm. The total phenolic content was calculated from the calibration curve prepared using gallic acid as the standard.

Radical scavenging capacity The radical scavenging capacity of the kaffir lime peel extract was determined from the ability to reduce the DPPH radical. The extract (100 µL) was added to 6.0×10^{-5} mol/L DPPH solution (3,900 µL) and the mixture was kept in the dark for 2 h. Absorbance of the mixture at 515 nm was then measured. The % inhibition was calculated by

$$\% \text{ inhibition} = (A_0 - A_{\text{extract}})) / A_0 \qquad (1)$$

where A_0 is the absorbance of freshly prepared DPPH solution and A_{extract} is the absorbance of the DPPH solution with the extract after 2 h.

Statistical analysis One-way ANOVA was used for comparing the phenolic content and radical scavenging capacity of the extracts obtained from conventional methods and PHWE method. This analysis was performed using the statistic software R (version 2.4.1) (R Development Core Team, 2006). A response surface model was used to describe the relation of each response as a function of the extraction temperature and extraction time. The analysis was performed using the software Design Expert (version 6.0.5, Stat-Ease, Inc., MN, USA).

Results and Discussion

Appearance of the extracts The kaffir lime peel extracts at 100 and 150°C were clear, pale yellow liquids and were similar to those obtained by the conventional solvent extraction. However, the extract at 200°C was a light brown-yellow color.

Total phenolic compounds The total phenolic compounds in the extracts are shown in Table 1. The highest value was obtained for the extract prepared at the highest temperature and longest time in this study (200°C, 15 min). The second order polynomial model (Figure 1) gave an R^2 of 0.97 and a non-significant lack-of-fit ($P > 0.3$) which indicated that the model sufficiently fitted the data. Increasing the temperature from 100 to 200°C increased the total phenolic compound in the extracts and it should be noted that the increase is greater at the higher temperature. These results were in contrast to some studies that reported a decrease in the phenolic compounds at a high temperature extractions with PHWE (110°C) (Ju *et al.*, 2005) or with pressurized solvent (130°C) (Piñeiro *et al.*, 2004) due to the degradation of the substances. Rodriguez-Meizoso *et al.* (2006) reported that the total phenolic compounds of the oregano leave extract were not significantly different in the extraction temperature range from 25 to 200°C. We found that the effect of time arose only at the higher temperature (200°C), but still much less than the effect of temperature. Comparing the PHWE with the conventional solvent extractions, we found that both water and 60%

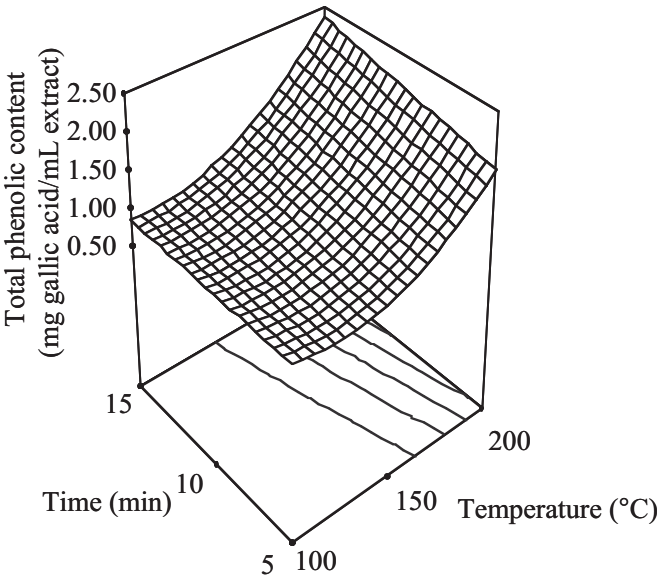

Fig. 1. Second order polynomial response surface showing the total phenolic content as a function of extraction temperature and extraction time.

Table 1. Total phenolic content (mg gallic acid/mL extract) and DPPH scavenging capacity (% inhibition) of kaffir lime fruit peel extracts as affected by extraction conditions.*

Temperature (°C)	Time (min)	Total phenolic content **	% Inhibition **
Pressurized hot water			
100	5	0.82 ± 0.04 ^{ab}	36.76 ± 1.33 ^{ab}
	10	0.87 ± 0.02 ^{ab}	40.18 ± 3.36 ^{abc}
	15	0.86 ± 0.04 ^{ab}	41.46 ± 1.78 ^{bc}
150	5	0.95 ± 0.04 ^{abc}	45.30 ± 5.72 ^{cd}
	10	0.99 ± 0.07 ^{bc}	51.41 ± 0.60 ^{d}
	15	1.15 ± 0.07 ^{c}	60.43 ± 0.12 ^{e}
200	5	1.75 ± 0.12 ^{d}	90.15 ± 0.12 ^{f}
	10	2.08 ± 0.14 ^{e}	90.08 ± 0.36 ^{f}
	15	2.37 ± 0.17 ^{f}	90.34 ± 0.23 ^{f}
Conventional extraction (50°C)			
Water	60	0.73 ± 0.06 ^{a}	33.75 ± 1.90 ^{a}
60% methanol	60	1.03 ± 0.06 ^{bc}	40.28 ± 3.45 ^{abc}

* Data expressed as mean ± s.d.
** Different letters in the same column indicate that the mean values significantly differ (Tukey's HSD, $P < 0.05$).

ethanol were not effective to extract the phenolic compounds from the kaffir lime peel.

Radical scavenging capacity The % inhibition values for the kaffir lime peel extracts are shown in Table 1. The second order polynomial model gave a significant lack-of-fit ($P < 0.001$), thus the model was not adequate. Because the % inhibition values were bounded between lower and upper limit, therefore the data were transformed using Logit function:

$$\text{Logit (\% inhibition)} = \ln[(\text{\% inhibition} - 34.22)/(91.60 - \text{\% inhibition})] \quad (2)$$

The new model gave satisfactory fit to the data ($R^2 = 0.98$, non significant lack-of-fit with $P > 0.4$) (Figure 2). The % inhibition values also increased as the temperature increased. The highest % inhibition was equally about 90% for all the extracts at 200°C. This would be ascribed to the limitation of the DPPH concentration used in this experiment. We expected that the radical scavenging capacity might increase as the extraction time increased because more phenolic compounds were extracted and/or possibly the Maillard reaction products that possess the radical scavenging capacity may be formed. Ju *et al.* (2005) observed that beside the decrease in the total phenolic contents in the grape skin extracts at 110°C or higher, the antioxidant activity (reported as ORAC value) still increased with temperature (up to 200°C). Rodriguez-Meizoso *et al.* (2006) also reported that the highest antioxidant activity (reported as DPPH scavenging capacity) of the oregano extracts was observed at the highest extraction temperature (200°C). Ibanez *et al.* (2003) demonstrated that the temperature affected the selectivity of the extraction and some antioxidant compounds could be further extracted at higher temperature (for example, as high as 200°C).

As mentioned above, both the total phenolic content and

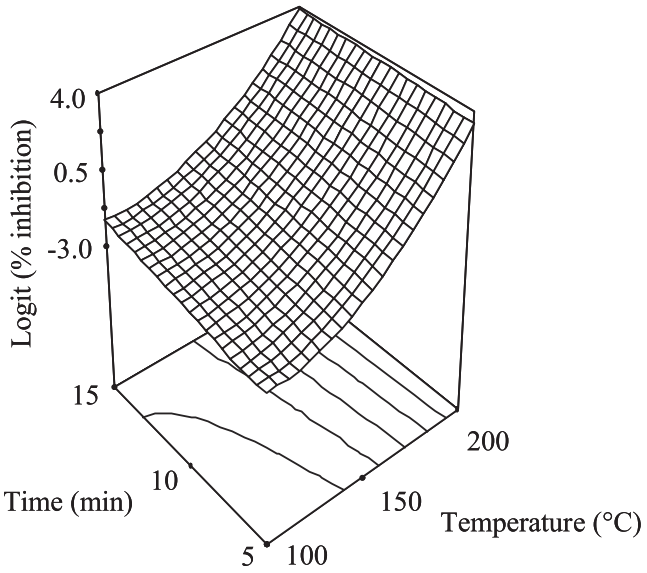

Fig. 2. Second order polynomial response surface showing Logit (% inhibition) as a function of extraction temperature and extraction time.

the DPPH scavenging capacity of the kaffir lime fruit peel extracts by the PHWE were higher at the higher extraction temperatures and longer extraction times. Because the DPPH scavenging capacity was proportional to the total phenolic content, the phenolic compounds would be responsible for the antioxidant activity of the extract.

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