

Flavonoid, phenolic contents and antioxidant properties of Thai hot curry paste extract and its ingredients as affected of pH, solvent types and high temperature

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Abstract

Spices used in curry pastes containing phenolics and flavonoids have been reported to prevent oxidative stress related diseases. Thai hot curry, Kua-khling curry paste, a Thai traditional seasoning particular Thai-Muslim, was investigated as potential functional food. In the present study, the flavonoid, phenolic contents and antioxidant activities of extract from spices used in the paste were determined. The effect of pH, solvent type and temperature (100-121°C) were investigated after the samples were extracted. It was found that the extracts of spices presented flavonoid and phenolic contents in the range 0.04-11.18 mg catechin equivalent (CE)/100 g sample and 0.68-134.91 mg gallic acid equivalent (GAE)/100g sample, respectively. Antioxidant activities determined as DPPH radical scavenging activity, hydroxyl radical scavenging activity (HRSC) and ferric reducing antioxidant power (FRAP) of the extracts were 0.49-8,214.91 mg GAE/100g sample, 0.69-9.30 mg GAE/100g sample and 25.83-6,860.55 mg GAE/100 g sample, respectively. The best solvent for extraction was water since its yielded flavonoid and phenolic contents and DPPH activity as 61.03±6.22 CE/100 g, 964±79.13 GAE/100g and 1,740.84±13.57 GAE/100g, respectively. Flavonoid, phenolic contents and DPPH activity were decreased at pH lower and higher than pH 6. However, it was found that temperature at 100°C caused more depletion of flavonoids, phenolic contents and DPPH activity compared with heating 121°C. Therefore, thermally canned food may have a potential for health benefit as evaluated by antioxidant properties *in vitro* system.

Keywords

Hot curry paste
antioxidant activity
flavonoid content
phenolic content
spices

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Introduction

Spices and herbs have been effectively used in the indigenous systems of medicine and human food in India and also in other countries (Satia-Abouta, 2002). In addition, spices and herbs are reported to contain bioactive compounds imparting antioxidant, preservative and antimicrobial properties to the food. Many spices and herbs contain a number of phenolic and flavonoid compounds, having antioxidant (Shobana *et al.*, 2000) antiinflammatory (Ach *et al.*, 1994) antimutagenic (Muralidhara, 1998) and anticarcinogenic activities (Menon *et al.*, 1999). Some studies have been reported that phenolic compounds in spices and herbs significantly contributed to their antioxidant (Siripongvutikorn *et al.*, 2008; Saeh *et al.*, 2010) and pharmaceutical properties (Srinivasan *et al.*, 2005). Though, individual spices/herbs were addressed as food additives, few scientific data have been reported about combination of spices/herbs

as curry paste. Moreover, those results are usually difficult to directly compare, because of the season of plant samples, test methods and solvent used.

Typical phenolics that possess antioxidant activity are mainly phenolic acids and flavonoids. Phenolic acids are a major class of phenolic compounds, widely containing in the plant kingdom especially in fruits and vegetables. However, phenolic compounds in plant foods are presented in free and bound forms through ester, ether, or acetal bonds (Robbin, 2003). And it is assumed that many antioxidative phenolic compounds in plants are usually presented as a covalently bound form; therefore, some processing methods were employed to release them so as to enhance the antioxidant capacity (Seung-Cheol *et al.*, 2005). For example, it was reported that heat treatment may liberate some low molecular weight phenolic compounds and hence increase the antioxidant capacity of citrus peel (Seok-Moon *et al.*, 2004). However, the effect of heat treatment

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on citrus flavonoids was not involved, and no clear quantity relationships were elucidated. Then a further investigation was needed (Guihua *et al.*, 2007). The phenolic extracts of plants are always a mixture of different classes of phenols, which are selectively soluble in the solvents. The use of an alcoholic solution provides satisfactory results for the extraction process (Perv-Uzulanic *et al.*, 2006). The ethanol extract of lemon grass exhibited anti-mutagenic activity (Vinitketkumnue *et al.*, 1994).

Thailand is claimed as kitchen of the world as a planet of animals and plants. Furthermore, Thais love to consume hot and spicy dish as curry or dip particularly in the southern and northeast part. However, ingredients composition of identical name of dish may differ from home to home or region to region (Siripongvutikorn *et al.*, 2008). Moreover, the difference of spices used in cooking style usually depend on protein base used as red meat, goat, sheep, beef or buffalo, or white or light meat as chicken and seafood. The hot and spicy curry paste named Kua-khling of Thai-Buddhist does not add some strong flavor spices as cumin, cinnamon, nutmeg and star anise while Thai-Muslim does. It is well known that sex hormone of goat has effect on its meat quality particularly odor attribute. Therefore, some aroma derived from some spices is selected to mask or change the negative smell. As mentioned above, Kua-khling curry paste consists of many spices and herbs may possess antioxidant activity and medicinal property. Therefore, the objective of this investigation was to study the effect of solvent, pH and temperature conditions on flavonoid, total phenolic contents and antioxidant properties of Kua-khling curry paste and its ingredients.

Materials and Methods

Plant materials and samples preparation

The Kua-khling paste consists of cayenne pepper (*Capsicum frutescens*), shallot (*Allium ascalonicum* L.), turmeric powder (*Curcuma longa*), garlic (*Allium sativum*), kaffir lime fruit peel (*Citrus hystrix*), lemon grass (*Cymbopogon citratus*), galangal (*Alpinia galangal* L.), cumin (*Cuminum cyminum* L.), pepper (*Piper nigrum* L.), cinnamon (*Cinnamomum bejolghota*), nutmeg (*Myristica fragrans*), star anise (*Illicium verum* Hook), coriander seed (*Coriandrum sativum* L.) and camphor seed (*Ammomum testaceum*). All ingredients were bought from grocery shop or fresh market in Hat-Yai, Songkhla Thailand. Each ingredient (100 g) was ground and homogenized in 50 ml of 50% (v/v) aqueous methanol with acidified 0.1% HCl and stirred for 12 h in dark room for extraction.

The mixture was centrifuged at 8000 x g for 20 min and supernatant was collected then defatted with hexane 3 times. The defatted supernatant was then evaporated at 50°C under reduce pressure to obtain solid matter.

Some flavonoid and phenolic compounds identification by HPLC analysis

Spices/herbs were extracted with 25 g of 50% (w/v) aqueous methanol acidified with 0.1% HCl. Homogenized samples were allowed to extract for 12 h to dissolve polyphenolics. Samples were further aided following acid hydrolysis into aglycones using 2 N HCl in 50% methanol and heating at 90°C for 90 min.

Flavonoid and phenolic contents were characterized and quantified by HPLC using modified chromatographic conditions of (Talcott *et al.*, 2003). Separations were performed on a Zorbax Eclipse XDB-C18 column (250mmx4.6mm) with a C18 guard column. Mobile phases consisted of water (Phase A) and 60% methanol (Phase B) both adjusted to pH 2.4 with o-phosphoric acid. A gradient solvent program ran phase B from 0% to 30% in 3min, 30% to 50% in 5 min, 50% to 70% in 17 min, 70% to 80% in 5 min, and 80% to 100% in 5 min, and held for 10 min all at 0.8 mL/min (Pozo-Insfran *et al.*, 2006).

Effect of solvent types

Spices/herbs (100g) were ground and homogenized in 50 ml of water, 25%, 50%, 75% and 99.99% (v/v) ethanol without acidified 0.1% HCl, then stirred for 12 h in dark room for extraction. The mixtures were then brought to centrifuge, defat and evaporate to obtain solid sample as mentioned above. Each dried sample extracted with various solvent types was subjected to analyze.

Effect of pH

Spices/herbs (100 g) were ground and homogenized in 100 ml of acetate phosphate buffer (100 mM, pH 2-10) and stirred for 2 h then extracted with the solvent, which exhibited the highest flavonoid, total phenolic contents and DPPH radical scavenging activity. The mixtures were then stirred, centrifuged, defatted and evaporated as mentioned above to obtain the dried samples.

Effect of temperature

Spices/herbs (100 g) were ground and homogenized in 100 ml of water and heated in water bath at 100 °C and 121 °C in oil bath for 1, 3, 5, 10, 20 and 30 min. The mixtures were brought to extract with selected solvent as mentioned before.

The extracts were brought to defat, evaporate as mentioned above.

Chemical analyses

Determination of total polyphenolic compounds

Total phenolic content was determined by the Folin-Ciocalteu method (Slink and Singleton, 1997). The extracted samples (12.5 µl) were mixed with 50 µl of water, 12.5 µl of Folin-Ciocalteu reagent for 6 min, 125 µl of 7% sodium carbonate (NaCO₃) and 100 µl of water then added. The absorbance was measure at 760 nm after 90 min of incubation at room temperature. Results were express as µl/ml of gallic acid equivalents (GAE) per 100 g sample. All determinations were performed in triplicates.

Determination of flavonoid compounds

The content of flavonoids was determined by Chen and Li (2007). The extracted samples (25 µl) were mixed with 125 µl of water and 10 µl of 5% sodium nitrate (NaNO₃) for 6 min. Then 15 µl of 10% aluminum chloride was added and stand for 5 min before 50 µl of 1 M sodium hydroxide was added sequentially. The absorbance of the solution at a wavelength of 510 nm was detected. The total flavonoid content in each spice extract was then calculated using a standard curve prepared with catechin and expressed as µl/ml catechin equivalents (CE) per 100 g sample. All determinations were performed in triplicates.

Determination of antioxidant activities

DPPH radical-scavenging activity

The DPPH radical-scavenging activity was determined by the method of Brand-Williams *et al.* (1999). The extracted samples (100 µl) were mixed with 0.0039 g of 2, 2-diphenyl-1-picrylhydrazyl which added 50 ml of absolute ethanol to obtain 200 µM. (The mixture of DPPH reagent was shaken vigorously and allowed to stand at ambient temperature in the dark for 3 h.) The absorbance was measured at 517 nm after 30 min of incubation at room temperature. Results were expressed as µl/ml of gallic acid equivalents (GAE) per 100 g sample. All determinations were performed in triplicates.

Hydroxyl radical-scavenging assay

The assay was determined by the method of Sminoff and Cunbes (1994). The reaction mixture contained 100 µl of ferric sulphate (0.5 mM) and 75 µl of 3% hydrogen peroxide was incubated in the dark room for 5 min. 50 µl of 0.435 mM brilliant green reagent was added into the mixture and subsequently incubated for 10 min. The reagent was

mixed with sample solutions (25 µl). The absorbance was measured at 624 nm after incubation for 10 min in the dark room. Results were expressed as µl/ml of gallic acid equivalents (GAE) per 100 g sample. All determinations were performed in triplicates.

Ferric reducing power (FRAP) assay

A potential antioxidant will reduce the ferric ion (Fe³⁺) to the ferrous ion (Fe²⁺); the latter forms a blue complex (FE/TPTZ), which increases the absorption at 595 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 µM, pH 3.6), a solution of 10 µM TPTZ in 40 µM HCl, and 20 µM FeCl₃ at 10:1:1 (v/v/v). The reagent (270 µl) was mixed with sample solutions (30 µl) before subjected to measure absorbance at 595 nm after the mixture was incubated for 30 min in the dark room. Standard curve was prepared using different concentration. Results were expressed as µl/ml of trolox equivalents (TE) per 100 g sample. All determinations were performed in triplicates (Benzie and Strain, 1996).

Results and Discussion

Total phenolic, flavonoid contents and antioxidant activities

Flavonoid, total phenolic contents and antioxidant activities of each ingredient extracted with methanol and acidified with HCl are shown in Table 1. Surprising, the result showed that Kua-khling curry paste was highest in total flavonoid as 81.62±0.03 mg CE/100 g sample. This may be due to suitable of solvent to mixture extractability compared with individual material. In general, extractability of any component depends on degree of polarity and ratio of

Table 1 Flavonoid, total phenolic contents and antioxidant activities of spice and herb extracts

Herbs/Spices	Flavonoid content mg CE/100g sample [□]	Total phenolic content mgGAE/100g sample [□]	DPPH radical scavenging activity mgGAE/100g sample [□]	Hydroxyl radical scavenging assay mgGAE/100g sample [□]	Ferric reducing antioxidant powder mgGAE/100g sample [□]
1. Garlic	3.55±0.04 ^a	3.73±0.17 ^{def}	1,131.81±102.18 ^e	9.30±0.19 ^a	198.61±22.38 ^b
2. Turmeric powder	2.17±0.12 ^f 0.33±0.15 ^h	0.68±0.00 ^g	0.49±0.03 ^f	1.07±0.002 ^g	25.83±1.86
3. Galangal	^b	1.53±0.05 ^h	3,630.12±54.02 ^b	1.92±0.006 ^d	41.61±0.46 ^c
4. Nutmeg	1.35±0.01 ^c	6.64±0.97 ^{cd}	337.59±7.05 ^d	1.92±0.009 ^d	786.08±21.94 ^e
5. Lemon grass	4.63±0.17 ^g	5.96±0.23 ^d	85.19±2.60 ^f	0.69±0.03 ^b	326.74±2.50 ^f
6. Cumin	3.31±0.13 ^g	5.67±0.12 ^d	408.87±6.56 ^d	1.53±0.04 ^c	560.45±3.37 ^d
7. Star anise	4.44±0.15 ^g	2.87±0.17 ^{ef}	1,736.03±88.28 ^e	0.74±0.03 ^b	1,876.88±4.46 ^e
8. Cayenne pepper	2.57±0.03 ^g 0.56±0.07 ^h	9.80±0.19 ^e	606.63±3.64 ^d	2.29±0.004 ^c	418.83±6.74 ^e
9. Camphor seed	^b	1.27±0.01 ^h	3,462.35±21.39 ^b	1.13±0.04 ^c	49.53±1.01 ^f
10. Coriander seed	0.04±0.009 ⁱ	3.12±0.11 ^{ef}	8,214.91±402.26 ^e	0.96±0.01 ^b	328.96±2.50 ^f
11. Shallot	1.60±0.16 ^c 11.18±0.52 ^b	4.87±0.03 ^{de}	1,016.46±5.61 ^c	5.95±0.08 ^b	114.06±2.72 ^f
12. Cinnamon	^b	134.91±5.47 ^a	724.14±29.71 ^d	1.36±0.01 ^f	6,860.55±91.70 ^e
13. Kaffir lime fruit peel	0.31±0.03 ^h	^b	3.01±0.11 ^{def}	1.35±0.007 ^f	467.37±18.82 ^e
14. Kua-khling paste	81.62±0.03 ^a	34.02±3.26 ^a	745.76±25.15 ^d	5.94±0.39 ^b	1,443.90±29.47 ^e

Values are means of three replicate determination ±standard deviations

^{ab} means with a column with the different letters are significantly different

Table 2 Effect of solvent type on flavonoid, total phenolic contents and DPPH radical scavenging activity of the paste

Sample	Flavonoid content (mgCE/100g sample)	Total phenolic content (mgGAE/100g sample)	DPPH radical scavenging activity (mgGAE/100g sample)
Methanol+0.1% HCl	81.62±0.03 ^a	34.02±3.26 ^f	745.76±25.15 ^d
Water	61.03±6.22 ^b	964.44±79.13 ^a	1,740.84±13.57 ^a
Ethanol 25%	2.03±0.74 ^f	57.32±9.39 ^e	1,478.59±9.18 ^b
Ethanol 50%	35.85±1.11 ^d	98.384±7.14 ^d	849.45±54.14 ^c
Ethanol 75%	15.56±0.10 ^e	173.87±14.09 ^c	1,546.44±85.23 ^b
Absolute ethanol	49.11±3.89 ^c	201.46±11.62 ^b	594.44±47.26 ^a

Values are means of three replicate determination ±standard deviations
^{a-f} means with a column with the different letters are significantly different

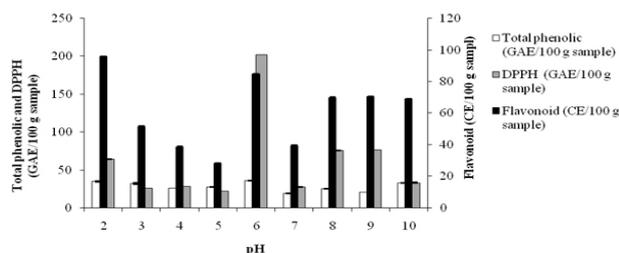


Figure 1 Effect of pH on flavonoid, total phenolic contents and DPPH radical scavenging activity on the paste

solute and solvent. It meant that combination of the ingredients as the paste reached or closed to polarity of the solvent used, methanol acidified with HCl. Kallithraka *et al.* (1995) reported that the methanolic solvent was better for catechin, epicatechin and epigallocatechin extraction.

Cinnamon powder possessed the highest of total phenolic content and FRAP as 134.91±5.47 and 6,860.55±91.70 mg GAE/100 g sample respectively while coriander seed extract showed highest the DPPH radical scavenging activity. Dorman *et al.* (2000) reported the cinnamon has the structural feature containing an electron repelling group in *ortho* position to the phenolic group required for a strong free radical-scavenging activity. Several studies revealed those flavonoids, isoflavones, flavones, anthocyanin, catechin and other phenolics were the majority of the antioxidant activity of plant material (Kahkonen *et al.*, 1999). However, there was difficult to get correlation between neither flavonoid nor phenolic and antioxidant activities (Table 1). Furthermore, each ingredient possessed different antioxidant property. This may be the reason for ancient wisdom of using many ingredients in folk medicine or cooking paste to serve the optimum purpose. Therefore, if some ingredients were increased or decreased the holistic characters of the active ingredients might be changed. As basic knowledge, the DPPH (1,1-diphenyl-2-picrylhydrazyl)- assay is complexity of the analyzed substrates, often mixtures of dozens of compounds with different functional groups, polarity, and chemical behavior. In addition DPPH is a stable free radical because of its spare electron

delocalization over the whole molecule (Szabo *et al.*, 2007). Generally, DPPH radical scavenging activity has been used as antioxidant activity assay as its diversity, frequently employed and accurate method. Therefore, DPPH radical scavenging activity was selected to study the effect of solvent type, pH and heat treatment on antioxidant activity in the curry paste.

HPLC analyses

In this present work, the RP-HPLC peaks of the paste extract were identical with the peak of ferulic acid, quercetin, coumalic acid, caffeic acid, catechin, gallic acid and catechuic as 0.17±0.006, 8.55±0.007, 0.49±0.007, 4.49±0.007, 0.29±0.007, 27.23±0.007 and 4.57±0.045 µg/g, respectively. It pointed out that gallic acid and quercetin were major active component in this curry paste. The amount of polyphenols was also dependent on the extraction method (Kim and Lee, 2004). Caffeic acid was found to have high antioxidant quality, comparable to that of ferulic acid, quercetin, coumalic acid and catechin. Ferulic acid was reported to inhibit the photo-peroxidation of linolic acid at high concentrations (Wang, 2003).

Effect of solvent type

Flavonoid, total phenolic contents and DPPH radical scavenging activity of the curry paste extracts as effect of solvent types are shown in Table 2. It was found that using water as extracted solvent was better than ethanolic solvents in term of flavonoids and total phenolic content but was lower than methanol plus HCl as flavonoids. In general, flavonoid structures are more less polarity compared with phenolic acid. Moreover, DPPH radical scavenging activity obtaining from water extraction was also equally high when compared with ethanol and methanol plus HCl extraction. Using water as solvent extract made DPPH radical scavenging activity of Kua-khling curry paste correlated with flavonoid and total phenolic content. A similar characteristic had been reported for antioxidant activity of sage, where water extraction was found to be the most effective extraction procedure compare with 70% ethanol and methanol (Ollanketo *et al.*, 2002). While, Koffi *et al.* (2010) reported that water extraction of plant organs leaves a large amount of residual polyphenols that only an appropriate combination of solvents would extract. It appears that the vast majority of polyphenols are not water soluble. Therefore, to be assured of obtaining fractions rich in polyphenols manufacturers would have to use extraction solvents with a mixture of suitable solvents.

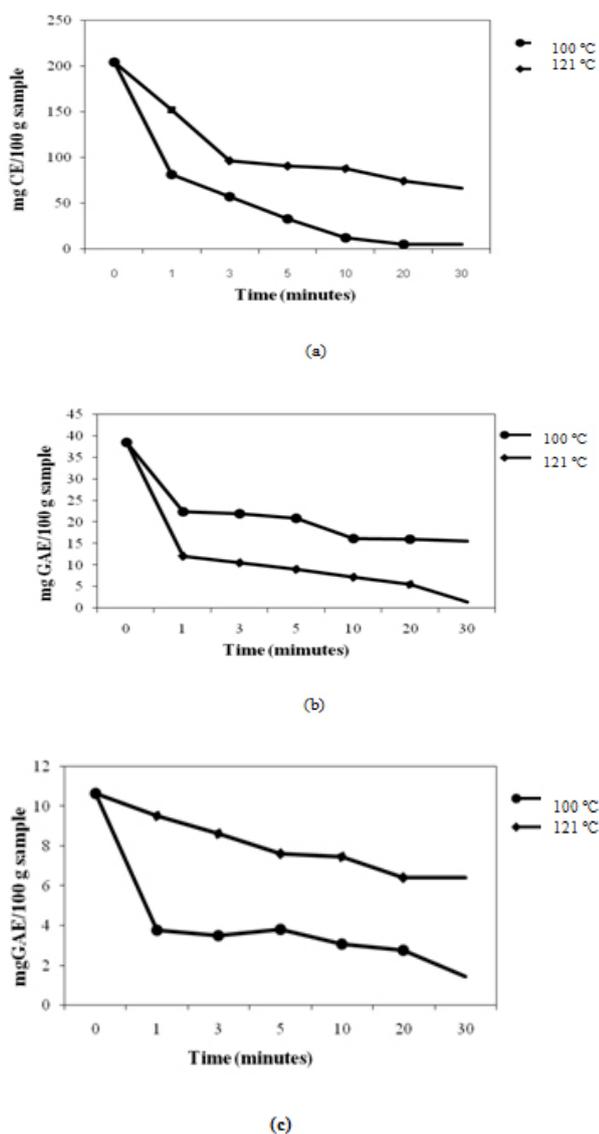


Figure 2 Effect of heating temperature and time on flavonoid content (a), total phenolic content (b) and DPPH radical scavenging activity (c) of the paste

Effect of pH

The flavonoid, total phenolic contents and antioxidant activity of extracts as effect of pH are shown in Figure 1. The result indicated that at slightly acidic pH 6, flavonoid, total phenolic content and DPPH radical scavenging activity were highest. It meant that these active compounds derived from the paste were easily destroyed by high acidic and basic condition. However, it confirmed that flavonoid and total phenolic content in the curry paste play an important role for DPPH radical scavenging activity as mentioned above. The antioxidant activities of different extracts indicate strong dependence of DPPH radical scavenging activity on the pH of the system. Yen and Duh (1993) reported that a methanol extract from peanut hulls had a higher DPPH radical scavenging activity at neutral and acid pH. The DPPH radical scavenging activity of different extracts

from cocoa by products was higher at alkaline pH (Azizah *et al.*, 1999). These differences may be due to different samples used and various compounds being extracted in each case. As our knowledge, phytochemical components in each plant depends on whatever that it is waxy or non waxy species part, fresh or dried from sample, and used parts as leaf, fruit or bark, as well as the selected compound such as carotenoids, non polar molecule and simple phenolics, polar compounds, are also determinant factors for difference in phytochemical compounds.

Effect of temperature

Effect of heating temperature and time on flavonoid, total phenolic contents and antioxidant activity are presented in Figure 2. It was found that increased heating time reduced flavonoids, total phenolic contents and DPPH radical scavenging activity. It meant that the active compounds were heat labile or easily destroyed fragment by heat. Surprising, heating temperature at 121°C reduced less flavonoid than heating at 100°C. Moreover, DPPH radical scavenging activity of heated extract paste at 121°C was higher. Therefore, DPPH radical scavenging activity of this curry paste depended on flavonoid content not phenolic content. In addition, the flavonoid content and antioxidant activity at 121°C was higher than heating at 100°C. Similar to the result of Tomaino *et al.* (2005) who reported that DPPH radical scavenging activity in basil heating at 120°C was higher than heating at 80°C and 100°C. Kim *et al.* (2006) concluded that the total phenolic content in whole grape seed extract (WGSE) and powdered grape seed extract (PGSE) were significantly increased by heat treatment, however, heating at 200°C decreased the total phenolic content of WGSE and PGSE significantly ($p < 0.05$). On the other hand, Xu *et al.* (2007) reported that the total amount of phenolic acids in huyou peel extract decreased after heat treatment, which indicated that some phenolic acids probably were destroyed by heat treatment and converted insoluble phenolic compounds to soluble phenolics. This indicated that the phenolic compounds of plants may be present in different bound forms depending on the species. Therefore, the effective processing step to liberate antioxidant compounds from different plant species may not be similar (Kim *et al.*, 2006).

Conclusions

All ingredients used in the Kua-khling curry paste possessed flavonoid, total phenolic contents and antioxidant activities in different amounts. Using

water as extracted solvent was a good promising for those contents and DPPH radical scavenging activity. However, if pH of testing system was higher than 6, it reduced more flavonoid, total phenolic contents and DPPH radical scavenging activity. Even, thermal processing also degraded the curry paste quality in term of flavonoid, total phenolic contents and DPPH radical scavenging activity. However, retaining of both contents and DPPH radical scavenging activity in the paste were found after thermal process. Therefore, consuming this curry paste has a potential for health benefit.

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