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Evaluation of total phenolic and flavonoid contents, antioxidant and anti-inflammatory activities of aqueous extract from Keang-hleung paste extract and its ingredients

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Abstract

Physicians have recommended low calorie diets to Thais who are concerned about their body weight, high blood pressure and diabetes. For instance, sour curry dishes rather than coconut food dishes. Southern sour curry or Keang-hleung paste is claimed as a healthy food because of various spices used in the paste which include garlic, shallot, dried finger chillies, and turmeric. However, there is quite few information about functional properties of mixed ingredients as curry paste available in scientific database. Therefore, the antioxidant and anti-inflammatory properties of the used ingredients in the curry paste were studied. The paste showed very high level of total phenolic (14.20±0.04 mg GAE/g sample), and flavonoid (1.76±0.03 mg CE/g sample) contents. It also exhibited DPPH radical scavenging (11.81±0.14 mg GAE/g sample) and metal chelating activities (3.86±0.81 mg EDTA/g sample) but no FRAP activity in the individual ingredients. Total phenolic and total flavonoid contents, DPPH scavenging, FRAP and metal chelating activities of the paste decreased when heating time was increased to 100 °C and 121°C. In addition, turmeric had the best anti-inflammatory activity (IC_{50}=0.045 µg/ml) on macrophage RAW264.7 cell line, followed by dried finger chilies (IC_{50}=0.132 µg/ml) and the paste (IC_{50}=32.680 µg/ml).

Introduction

Recently, some compounds from herbs and/or spices and vegetables were accepted among food scientists, nutritionists and physicians as preventive or even curative compounds for many diseases. For example, daily consumption of vegetables, herbs and spices help to support human digestive system well. It is known that, spices and herbs contain various bioactive compounds providing antioxidant, preservative and antimicrobial properties in the food. In addition, a number of phenolic and flavonoid compounds found in various plants exhibited antioxidant, anti-inflammatory (Shobana and Akhilender Naidu, 2000; Makchuchit *et al.*, 2010; Settharaksa *et al.*, 2012), antimutagenic (Muralidhara and Narasimhamurthy, 1988; Jayaprakasha *et al.*, 2007), and anticarcinogenic activities (Menon *et al.*, 1999; Kaefer and Milner, 2011).

Southern sour curry or Keang-hleung paste is claimed as low calorie diet and popular food for people who aim to control their body weight, high blood pressure or diabetes. Though, this paste generally consists of garlic *(Allium sativum),* shallot (*Allium ascalonicum* L.), dried finger chillies *(Capsicum annuum)* and turmeric *(Curcuma longa),* the type and amount of ingredients used in the paste may differ from home to home and/or region to region (Siripongvutikorn *et al*., 2009). Dillon *et al*. (2003) reported that aged garlic extract (AGE) showed a potent antioxidant activity by eliminating superoxide ion and reducting lipid peroxide particularly when AGE was obtained through water extraction. In addition, antimicrobial activity of AGE was reported to inhibit Malassezia furfur (25 strains), *Candida albicans* (18 strains), other *Candida* sp. (12 strains) as well as 35 strains of various dermatophyte species (Shams-Ghahfarokhi *et al.*, 2006). The aqueous AGE extract also had been reported to reduce the enzymes in inflammatory processes (Dimitrov and Bennink, 2004). The inhibition of bacteria, yeast and fungi (Mahmoudabadi and Nasery, 2009) as well as lipid oxidation inhibition were also reported in shallot

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or onion (Leelarungrayub *et al.*, 2006). Recently, small yellow onion (*Allium flavum* L. subsp. *flavum,* Alliaceae) was reported to express a high anti-inflammatory activity, by inhibiting 12-lipoxygenase (12-LOX) activity (Simin *et al.,* 2013). Chili contains various bioactive compounds such as capsaicin, dihydrocapsaicin, nondihydrocapsaicin, hormocapsaicin and homodihydrocapsaicin (Surh *et al.*, 1998; Wangcharoen and Morasuk, 2007). Capsaicin could act as an antioxidant by attenuation of oxidants damage or lipid peroxidation in various organs of experimental animals (De and Ghosh, 1992; Toskulkao and Tekittipong, 1996; Surh, 2002). Moreover, capsaicin also showed the anti-inflammatory activity (Clementi *et al.*, 1994; Reddy and Lokesh, 1994; Savitha and Salimath, 1995). Curcumin, a bioactive compound, derived from turmeric rhizome, has a wide array of pharmacological and biological activities (Ammon and Wahl, 1991). It exhibited the antioxidant and anti-inflammatory effects in both *in vitro* and experimental animal systems (Surh, 1999).

Although phytochemicals in individual spice and herb have been shown the antioxidant and anti-inflammatory activities, the investigation of these functional activities have not been well addressed in a food matrix as mixed paste. In addition, to simulate the pH in the digestive system and human body, as well as heating process used in household and industrial plant, the effect of pH ranged from 2-8 and heating temperatures at 100°C and 121°C were determined. The results were also aimed at supporting the health benefits/claims of Keang-hleung paste, and this is essential for further investigation in animal models.

Materials and Methods

Materials and chemicals

Garlic *(Allium sativum),* shallot (*Allium ascalonicum* L.), dried finger chillies *(Capsicum annuum),* and turmeric *(Curcuma longa)* were purchased from a local market in Hat Yai, Songkhla, Thailand. Roswell Park Memorial Institute (RPMI) 1640 Medium was obtained from LifeTechnologies (Grand Island, NY, USA). Fetal bovine serum and penicillin-streptomycin were obtained from Invitrogen (Grand Island, NY, USA). Lipopolysaccharides from Salmonella enterica (LPS) and Nω-Nitro-L-arginine (L-NA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals and reagents were of analytical grade.

Keang-hleung paste preparation

The basic Keang-hleung paste consisted of a mixture of garlic, shallot, dried finger chilies, turmeric as 15, 35, 35 and 15%, respectively. All ingredients were sorted and washed thoroughly to remove dust and dirt. They were drained overnight at room temperature before blending into a fine paste of 20-40 mesh.

Extraction procedure

Each sample including used spices/herbs and the mixed as the paste (50 g) was extracted with 100 ml of distilled water, and stirred with a magnetic stirrer for 12 hr in dark at room temperature. The extracts were filtered through cheesecloth, and centrifuged at 4,000 x *g* for 30 mins to discharge the sediment. The supernatant was incubated in water bath at 50°C until the dried material was obtained, and then kept at -20°C for further study.

Effect of pH

Each sample as mentioned in extraction procedure (50 g) was homogenized with 100 ml of 100 mM acetate phosphate buffer (pH 2-8) and stirred for 2 hr. This was then centrifuged to obtain supernatant and dried as mentioned above to obtain the solid sample.

Effect of temperature

The sample (50 g) was homogenized with 100 ml of distilled water and heated in water bath at 100°C and 121°C in oil bath for 0, 5, 10, 20, and 30 mins before turning into extract as mentioned above in extraction procedure.

Chemical analyses

Determination of total polyphenolic compounds

The total phenolic content of the extracted sample was determined using the Folin-Ciocalteau assay following the method of Settharaksa *et al.* (2012). Gallic acid was used as antioxidant standard, and reported as mg of garlic acid equivalents (GAE) per g sample. All experiments were performed in triplicates.

Determination of flavonoid compounds

A volume of 25 µl of the extracted sample (0-10 mg/ml), 125 µl of water and 10 µl of 5% sodium nitrate ($NaNO_2$) were placed in 96-wells plates and allowed to stand for 6 mins at room temperature. Then 15 µl of 10% aluminium chloride was added and left for 5 mins. Thereafter, 50 µl of 1 M sodium hydroxide (NaOH) was added. The absorbance of the solution was measured at a wavelength of 510 nm. Results were expressed as mg of catechin equivalents (CE) per g sample. All experiments were performed

in triplicates.

Determination of antioxidant activities

DPPH radical scavenging activity

DPPH radical scavenging activity was determined using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay (Settharaksa *et al.*, 2012). The results were expressed as mg of GAE equivalents per g sample. All experiments were carried out in triplicates.

Ferric reducing power (FRAP) assay

The FRAP assay was done according to Settharaksa *et al.* (2012). The results were reported as mg of GAE equivalents per g sample. All experiments were performed in triplicates.

Metal chelating ability

The metal chelating activity of the extracted sample was determined by the method of Dinis *et al.,* 1994. Ethylenediaminetetraacetic acid disodium salt (EDTA) was used as reference standard. All measurements were performed in triplicates. The results were reported as mg of EDTA per g sample.

Growth and activation of cells

Murine macrophage RAW264.7 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 Medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml of penicillin and 100 µg/ml of streptomycin and incubated at 37°C in humidified atmosphere of 5% CO_2/95% air.

Screening test for NO production

Screening test for NO production was performed as described previously (Matsuda *et al*., 2003; Rao *et al*., 2005). Briefly, 1x106 RAW264.7 cells were seeded in 96-well flat-bottomed plates and incubated at 37°C to allow macrophages adherence. After two hours, the non-adherent cells and medium were removed and the adherent cells were cultured in fresh medium containing 0. 5 µg/ml LPS and various concentrations of the extracts for 24 hr. NO production in each well was assessed by measuring the accumulation of nitrite (NO_2^-) in the culture medium using Griess reagent.

Cytotoxicity was determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) assay. Briefly, after 24 h incubation with the extracts, MTT solution (10 µl, 5 mg/ml in PBS) was added to the wells. After a further 2 hr in culture, the solution was removed and dimethylsulfoxide solution (DMSO) was then added to dissolve the formazan crystals in the cells. The optical density (O.D.) of the formazan solution was measured with a microplate reader at 570 nm. When O.D. of extract-treated group reduced below 80% of O.D. in the control group, the test compound was considered to exhibit cytotoxic effect. L-NA was used as reference compounds. Inhibition (%) was calculated by the following equation (1) and IC_{50} was determined graphically (Matsuda *et al*., 2003).

$$\text{NO Inhibition (\%)} = \frac{(OD_C - OD_{Bc}) - (OD_S - OD_{Bs})}{(OD_C - OD_{Bc})} \times 100 \quad \text{.....equation (1)}$$

Where C = Control (RPMI+ LPS)
Bc = Blank of control (RPMI)
S = Sample (Sample + LPS)
Bs = Blank of sample (RPMI + Sample)

Results and Discussion

Total phenolic and flavonoid contents and antioxidant activities of the paste and its ingredients

The total phenolic, flavonoid contents and antioxidant activity of the paste and its ingredients are shown in Table 1. The results showed that the amount of total phenolic and flavonoid contents of the paste were higher compared with its individual ingredient. In addition, the result showed that the majority of the bioactive compounds found in the paste were in the group of phenolic compounds not flavonoids. This is in agreement with other research groups (Pietta, 2000; Maisuthisakul *et al.*, 2007). An increase in total phenolic and flavonoid contents in the paste may be due to chemical reaction during mixing and blending process as reported by Seah *et al*. (2010). This result also confirmed the study of other researchers (Seah *et al*., 2010; Seah *et al*., 2011; Settharaksa *et al.*, 2012). From observation, it was noticed that the texture, color and flavor of the mixed ingredient significantly changed during the production of the paste, and thus confirmed the chemical reaction occurrence. Compared with in individual ingredients, the highest level of total phenolic and flavonoid contents were found in garlic followed by dried finger chilies, turmeric and shallot in order. Moreover, the sample contained a higher phenolic and flavonoid contents.

The antioxidant activity determined as DPPH radical scavenging, ferric reducing power and metal chelating activity of the paste and its ingredients were addressed in Table 1. As expected, antioxidant activities determined by DPPH and metal chelating assays of the paste were higher than those of the individual ingredients, particularly DPPH radical scavenging activity. Some researchers inferred that the mixed spices showed higher antioxidant activity

Table 1. Total phenolic, flavonoid contents and antioxidant activities of each ingredient and paste

Samples	Total phenolic content (mg GAE/g sample)	Flavonoid contents (mg CE/g sample)	DPPH radical scavenging activity (mg GAE/g sample)	FRAP (mg GAE/ g sample)	Metal chelating (mg EDTA/ g sample)
Dried finger chilies	1.03±0.04^{c}	0.52±0.02^{c}	2.14±0.73^{b}	2.44 ±0.08^{a}	3.17±0.01^{a}
Garlic	1.91 ±0.06^{b}	0.77±0.02^{b}	0.08±0.04^{c}	0.20±0.01^{d}	1.43 ±0.26^{b}
Turmeric	0.30±0.05^{d}	0.18±0.02^{d}	0.13±0.04^{c}	0.19±0.01^{d}	3.59 ±0.15^{a}
Shallot	0.06±0.01^{e}	0.06±0.006^{e}	0.03±0.01^{c}	0.27±0.03^{c}	1.3±0.01^{b}
The paste	14.20±0.04^{a}	1.76±0.03^{a}	11.81±0.01^{a}	2.27±0.04^{b}	3.86±0.81^{a}

Each value is expressed as a mean ± SD (n = 3)
$^{a-e}$ means with a column with the different letters are significantly different

Table 2. Effect of pH on total phenolic, flavonoid contents and antioxidant activities of Keang-hleung paste

pH	Total phenolic content (mg GAE/g sample)	Flavonoid contents (mg CE/g sample)	DPPH radical scavenging activity (mg GAE/g sample)	FRAP (mg TE/ g sample)	Metal chelating (mg EDTA/ g sample)
2	15.95±0.64^{a}	0.46±0.02^{de}	1.03±0.12^{bc}	2.29±0.05^{b}	3.65±0.25^{a}
3	10.35±1.29^{bc}	1.32±0.39^{b}	0.96±0.10^{cd}	2.09±0.03^{d}	3.45±0.24^{a}
4	11.44±0.28^{b}	0.36±0.01^{e}	1.19±0.04^{b}	2.36±0.07^{b}	3.07±0.08^{b}
5	5.83±0.61^{cd}	1.07±0.35^{bc}	0.83±0.05^{de}	1.84±0.06^{e}	3.10±0.19^{b}
6	8.47±0.82^{bc}	0.56±0.23^{d}	1.06±0.08^{bc}	2.20±0.02^{c}	3.67±0.15^{a}
7	8.32 ±0.72^{bc}	0.85±0.02^{cd}	1.49±0.12^{a}	2.46±0.02^{a}	3.55±0.06^{a}
8	3.58±0.80^{d}	2.55±0.16^{a}	0.77±0.05^{e}	1.81±0.01^{e}	2.92±0.04^{b}

Each value is expressed as a mean ± SD (n = 3)
$^{a-e}$ means with a column with the different letters are significantly different

due to synergistic effect of compounds derived from individual ones (Seah *et al*., 2010). The result also showed that antioxidant activities were in positively correlated with total phenolic and flavonoid contents, which was similar to the finding of Maisuthisakul *et al*. (2007). Moreover, it was found that DPPH radical scavenging activity of this experiment had a strong relationship with total phenolic content.

FRAP assay measures antioxidant ability by reducing Fe(III) to be Fe(II). It implies that FRAP is used on the premise of electron donating ability of an antioxidant agent. Therefore, 2,4,6-trypyridyl-s-triazine-Fe(III) or TPTZ-Fe(III) complex was changed to 2,4,6-trypyridyls-triazine-Fe(II) or TPTZ-Fe(III) complex. The FRAP value of the paste and its ingredient is shown in the range of 0.19±0.01 to 2.44±0.08 mg GAE/g sample (Table 1). The highest value of FRAP was found in dried finger chilies followed by the paste (Table 1), while the lowest was in garlic and turmeric. This result displayed that making the paste with these ingredients did not confer substantial FRAP activity of the paste compared to DPPH activity. This result is similar to the finding of Seah *et al.* (2010) who reported that the lower FRAP value of the paste might be due to its active compounds and not flavonoids which provide electron transfer. Although the antioxidants are reducing agents because of their ability to donate a single electron or hydrogen atom, not all reducing agents are antioxidants (Dini *et al.*, 2008). Moreover, the ferric reducing activity might depend on the degree of hydroxylation and extent of conjugation of the phenolic compounds. Consequently, if some ingredients were increased or decreased, the holistic characters of the active ingredients might be changed (Settharaksa *et al.*, 2012).

One of the mechanisms of antioxidant action is chelation of transition metals, which can prevent catalysis of hydroperoxide decomposition (Gordon, 1990; Aparadh *et al.*, 2012). The red color of complex formed by the interaction of ferrozine with Fe^{2+} ions is decreased by the action of metal chelating compounds that exist in the reaction mixtures (Geckil *et al.*, 2005). Results obtained showed that the paste had the highest chelating activity as 3.86±0.81mg EDTA/g sample (Table 1) followed by turmeric, dried finger chilies, garlic and shallot with 3.59 ±0.15, 3.17±0.1, 1.43 ±0.26 and 1.3±0.01 mg EDTA/g sample, respectively. It was observed that the metal chelating activity of the paste concurred with its total phenolic and flavonoid contents. Several studies revealed that phenolic compounds exhibited redox properties, such as reducing agents, hydrogen donators and singlet oxygen quenchers, and were responsible of Fe-chelating activity (Heim *et al.*, 2002; Soong and Barlow, 2004; Balasundram *et al*., 2006). Recently, Loizzo *et al.* (2012) reported the strong relationship between total flavonoids and Fe-chelating ability of peel and pulp extracts from cherimoya. However, the metal chelating activity of each individual ingredient showed irrelevance with its total phenolic and flavonoid contents. Therefore, it was indicated that the Fe-chelating ability of the paste may occur as a result of mixing and blending during the production of the fine paste as mentioned above.

Effect of pH on total phenolic and flavonoid contents and antioxidant activities

It is well known that many factors such as processing treatment, temperature and pH of the media, antioxidant concentration and storage condition have strongly influenced the antioxidant activities (Gazzani *et al.*, 1998; Arabshahi-D *et al.*,

Table 3. Inhibition of NO synthesis in the LPS-induced macrophages cell of curry paste and ingredients extracts

Sample	IC_{50} (µg/ml)
Dried finger chilies	0.132
Garlic	400.911
Turmeric	0.045
Shallot	633.510
Keang-hleung paste	32.680
L-NA	54.680

IC_{50} values were calculated on the basis of nitrite concentration in the medium of activated macrophages cultured in the presence of various concentrations of test samples.

2007). The influence of pH on the total phenolic, flavonoid contents and antioxidant activities of the paste are shown in Table 2. Total phenolic and flavonoids content of the paste in pH 2-8 were in the range of 3.58±0.80 to 15.95±6.44 mg GAE/g sample and 0.36±0.01 to 2.55±0.16 mg CE/g sample, respectively. The results showed that total phenolic content of the paste was highest at pH 2 (15.95±0.64 mg GAE/g sample) while the highest of total flavonoid content was at pH 8 (2.55±0.16 mg CE/g sample). It pointed out that the pH variation could have a positive or a negative effect on extraction, depending on the interaction of the polyphenols in raw material. This indicated that the extraction of phenolic and flavonoid compounds were pH dependent (Chen *et al*., 2008).

The effect of pH on DPPH radical scavenging activity of the paste is reported in Table 2. The result showed the highest DPPH radical scavenging activity of the paste at neutral condition, pH 7 (1.49±0.12mg GAE/g sample). Settharaksa *et al*. (2012) reported the decreasing of DPPH activity of the hot curry paste extract at pH lower and higher than pH 6. It implied that the active compounds of the paste were easily changed or deformed by high acidic and basic condition. Chen *et al*. (2008) asserted that the DPPH scavenging ability in yam varieties under acidic pH (4-5) environments were greater compared to those at other pH 5. These differences may be due to different samples used and various compounds being extracted in each sample (Arabshahi-D *et al.*, 2007).

The FRAP value of the paste was increased significantly and was highest at pH 7, 2.46±0.02 mg GAE/g sample (Table 2), which is also similar to the DPPH radical scavenging activity. However, the paste extract showed a higher FRAP value than DPPH activity. This may be due to the type of solvent used in the paste and as well as FRAP preparation done with water while ethanol was used as solvent

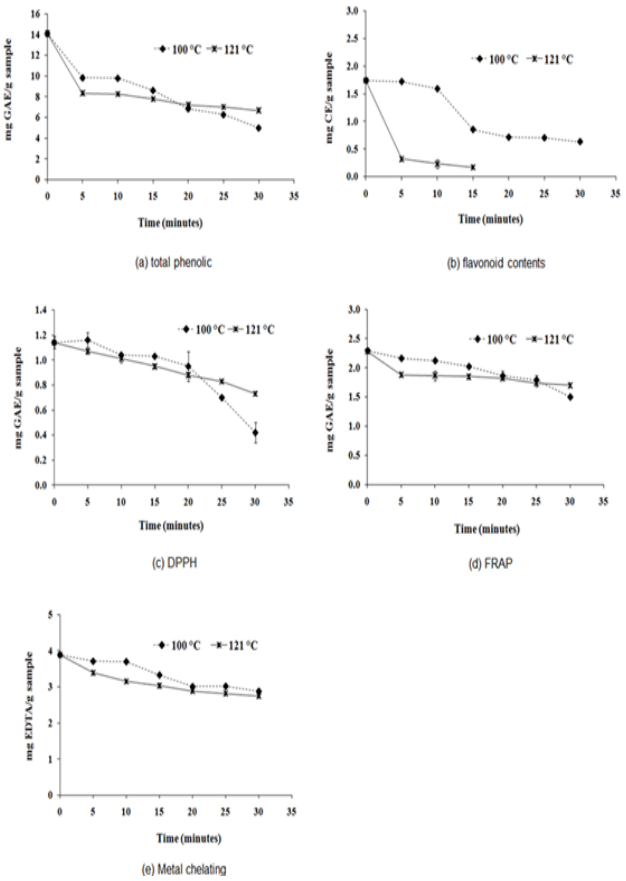

Figure 1. Effect of heating temperature and time on total phenolic (a), flavonoid contents (b), DPPH radical scavengaing (c), FRAP (d) and Metal chelating activity (e) of the paste.

in DPPH assay. It meant that the active compounds of spices used in the paste might have been dissolved or lost. In addition, for the metal chelating activity, the paste showed the highest chelating activity at slightly acidic pH 6 as 3.67±0.15 mg EDTA/g sample while the lowest was at pH 8 2.92±0.04 mg EDTA/g sample (Table 2). However, Chen *et al.* (2008) addressed the highest metal chelating activity of the different Chinese yams species was expressed at pH 8. The different results found in many researches may be due to difference in material used, extraction process, evaluation method etc. Therefore, it will be presumptuous to draw a similar conclusion.

Effect of temperature

The effect of heating temperature and time on total phenolic and flavonoid contents of the paste are represented in Figure 1a-b. Total phenolic and flavonoid content of the paste decreased at both heating temperature when heating time increased. It was implied that both heating temperature plus with increased time caused a significant change in total phenolic and flavonoid contents. This result was in agreement with the report of Xu *et al.* (2007) who

described that the heating could destroy the phenolic compounds in plant foods, which may be linked to various plant components through ester, ether, or acetal bonds (Robbins, 2003), by the cleaving of esterified bond and glycosylated bond, etc. When compared between the heating temperature at 100and 121°C , total phenolic content of the paste at 100°C declined continuously, whereas it was quite constant at 121°C . This may be due to sterilization method (121°C), a high temperature process may destruct bound phenolic compound (Arabshahi-D *et al.*, 2007; Chen *et al*., 2007; Antonia Murcia *et al.*, 2009) then converted insoluble phenolics compounds to soluble phenolics (Ayusuk *et al.*, 2009) which is easy to detect. In addition, the results showed that the decrease in total flavonoid contents (Figure 1b) of the paste was higher when compared with total phenolic contents. This could be implied that flavonoid compounds, with higher molecular weight compared with phenolic acid in the paste, were more sensitive to heating because of higher heat.

The effect of heating on antioxidant activities determined as DPPH radical scavenging, FRAP and metal chelating activity in the paste are presented in Figure 1c-e. The results showed that the DPPH radical scavenging activity of the paste extract slightly decreased during heating at 100°C and 121°C for 20 mins and significantly decreased at 100°C for 30 mins. This was similar to total phenolic changed (Figure 1a). The paste heated at 100°C showed the higher depletion of FRAP value when the heating time increased particularly for 30 mins when compared with heating at 121°C . Both heating at 100°C and 121°C caused a decrease in metal chelating activity of the paste during the first 20 mins, after which it remained constant. These observations confirmed that thermal processing were responsible for a depletion of natural antioxidants in plant due to weak bonds found in plant materials (Tomaino *et al.*, 2005) as synthetic product. However, it is generally accepted that thermal processing may have negative-positive effect for decrease, increase or even maintaining constant levels of activities (Yen and Duh, 1993; Nicoli *et al*., 1999; Tomaino *et al*., 2005; Seah *et al,* 2010). Therefore, it is important to consider the optimum technological condition and processing factors influencing activity and bioavailability of plant antioxidants for utilization in food and biological systems (Arabshahi-D *et al*., 2007).

Effect on the NO production

Nitric oxide (NO) is one of the inflammatory mediators causing inflammation in many organs. It is produced from L-arginine by a chemical reaction catalyzed by the enzyme inducible nitric oxide synthase (iNOS) in living systems (Makchuchit *et al*., 2010). After stimulation with stimuli agent inducing bacterial lipopolysaccharide (LPS), many cells including macrophages express the iNOS which is responsible for the production of large amount of NO (Darley-Usmar *et al*., 1995). To assess the effects of sample extracts on the LPS-induced NO production in RAW264.7 cells, cell culture media were harvested and nitrite levels were measured (Yoo *et al.*, 2008). And in order to avoid a possible cytotoxic effect of samples upon NO production, cell viability of the suspension was initially determined using an MTT assay (Tsai *et al.*, 2007). In this present work, all samples extracts at the treated concentrations (0-1,000µg/ml) of all samples were selected based on cell viability which was higher 80% data not shown).

The IC_{50} value of the paste and its ingredients extracts compared with the standard L-NA are shown in Table 3. Interestingly, the IC_{50} value of turmeric (IC_{50}=0.045 µg/ml), dried finger chilies (IC_{50}=0.132 µg/ml) and the paste (IC_{50}=32.680 µg/ml) were lower than that of L-NA (IC_{50}=54.680 µg/ml) which was used as standard drug. It pointed out that the NO synthase inhibition of these ingredients was more effective as multi mechanism compared with L-NA. Tuntipopipat *et al.* (2011) demonstrated that the ethanolic extract of Thai red curry decreased the production of inflammatory mediators by LPS-activated RAW 264.7 macrophages. The phytochemical in the red curry paste extract significantly inhibited the production of NO by suppressing the expressions of iNOS, COX-2, TNF-α, and IL-6 in a dose-dependent manner. As it has been established that the major compound in turmeric is curcumin, exhibiting anti-oxidant, antimicrobial, anticancer and anti-inflammatory effects (Surh, 2002; Yadav *et al*., 2013), its anti-inflammatory effect expresses by inhibition of cyclo-oxygenase-2 (COX-2, a key enzyme catalyzing the production of prostaglandins in response to inflammatory stimuli) and lipoxygenase activities in TPA-treated mouse epidermis (Huang *et al*., 1991). The active component in chili peppers is capsaicin which has a structure similar to that of curcumin and it is also known to have anti-inflammatory effect (Surh, 2002). Moreover, the anti-inflammatory action of capsaicin was illustrated by reducing of COX2 activity and expression of pro-inflammatory gene (Kim *et al.,* 2003). Capsaicin is claimed to inhibit adipogenesis and reduce the amount of intracellular triglycerides in mouse (Leiherer *et al.*, 2013). Kawada et al. (1986) reported that capsaicin can retard obesity-induced

inflammation which decreases fasting glucose, insulin, and triglyceride levels. Furthermore, it also counteracts the pro-inflammatory effect of saturated fatty acids induced by palmitic acid (Choi *et al*., 2011). Thus, capsaicin may be regarded as a useful phytochemical for attenuating obesity-induced inflammation and obesity-related pathologies (Leiherer *et al.*, 2013). This indicated that the curry or food that consists of turmeric and chili might have a great potential for health benefit, especially obese people.

Conclusion

In summary, the data showed that all ingredients used in the Keang-hleung paste contained total phenolic and flavonoid contents, with varying amounts of antioxidant activities. The paste showed very high levels of total phenolic, flavonoid contents, DPPH scavenging and metal chelating activity but no FRAP activity. Acidic and basic processing may facilitate or destruct some active compounds; however, those active compounds and their antioxidant activity were retained. After heating, total phenolic, total flavonoid contents, DPPH radical scavenging, FRAP and metal chelating activities of the paste decreased when heating temperature and time increased. Moreover, the paste and some of its ingredients showed higher anti-inflammatory activity when compared with L-NA. This investigation confirms that Southern curry consumption is good for health.

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