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A study of the antioxidant and anti-inflammatory properties of Thai yellow curry (Keang-hleung) paste with finger chili and bird chili and its consumer acceptability

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Abstract

Southern sour curry or 'Keang-Som' or 'Keang-hleung' is a popular dish for people wishing to lose weight and is consumed throughout Thailand. The original recipe of sour curry paste was obtained from small and medium enterprises in Chumphon province and consisted of finger chili, shallot, garlic and turmeric rhizome. The designed formula used bird chili to substitute the finger chili in order to provide a more hot sensation to meet the southern Thai curry dish characteristic. The total phenolic content, antioxidant activities, anti-inflammatory property and consumer acceptability were determined. The paste substituted with bird chili had total phenolic content and antioxidant as FRAP activity higher than the control paste. Moreover, the pastes with either finger chili or bird chili possessed more anti-inflammatory property compared with individual chili. The sour curry soup made from the paste with bird chili substitution had higher sensory scores in every attribute, with the overall liking score of 7.57 determined by 9-point hedonic scale in comparison to the control (5.96).

Introduction

The sour curry paste of southern Thailand or 'Keang-hleung' paste is popular not only in southern Thailand but also throughout a lot of Asia because of its good taste, spicy, unique flavour and health benefits. Herbs and spices used in the curry paste are typically: turmeric, garlic, shallot and chili which are sources of phytochemicals or bioactive compounds which are claimed to prevent non-contagious diseases such as high blood pressure, diabetes and cancer (Kaefer and Milner, 2008). Many ingredients used in the southern Thai sour curry paste have been found to contain antioxidant and anti-inflammatory substances and have medicinal value (Tuntipopipat *et al*., 2011).

Curcumin, the major bioactive component of turmeric rhizome may have a positive impact on arterial health (Ashaf *et al*., 2005). The use of turmeric has also been linked to a reduction in leukaemia, breast cancer, lung cancer and colon cancer. This polyphenol has antioxidant and anti-inflammatory properties (Jayaprakasha *et al.*, 2006; Mueller *et al.*, 2010). In addition, curcumin was capable of scavenging oxygen free radicals such as superoxide anions and hydroxyl radicals, which are the initiators of lipid per-oxidation (Pulla-Reddy and Lokesh, 1992). Additionally, garlic contains flavonoids and sulphur compounds such as diallylsulphate, alliin, ajoene and allicin. Antioxidant effects of garlic is primarily of interest in the treatment of arteriosclerosis. Garlic constituents are able to inhibit the formation of free radicals, promote the radical scavenger process, and protect low-density lipoprotein cholesterol against oxidation by free radicals (Ide and Lau, 1997). Both allicin and allyl sulfides are metabolized in blood and liver to the same metabolite, allylmercaptan, which is a strong antioxidant (Koch, 1996). Shallots have a higher phenolic content than many onions, making them particularly effective against liver cancer cells. An extensive phytochemical compound of the polar extracts from bulbs of shallot is furostanolsaponins, such as ascalonicoside (Fattorusso *et al.*, 2002). Shallot has been reported to exhibit anti-oxidative and free radical scavenging abilities. These properties appear to be related to the high contents of flavones, sulfur-containing compounds, and polyphenolic derivatives in the bulb of shallot. More importantly, it is shown that the antioxidant potential of shallot is superior to several onion varieties and some garlic preparations (Yang *et al*., 2004; Leelarungrayub *et al.*, 2006). Red chilies contain high amounts of vitamin C and carotene (pro-vitamin A). Capsicum fruit have long been recognized as an excellent source of

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ascorbic acid, which is a required nutrient for humans. However, ascorbic acid content of chili can depend on variety, mature stage, storage conditions and so on (Tilahun *et al*., 2013). Capsaicin can prevent heart attacks and strokes, reduce serum cholesterol levels, relieve backaches and psoriasis, as well as help cure strains and sprains (Zimmer *et al*., 2012).

To make curry paste, many ingredients used in the recipe need to be prepared, mixed then pounded or blended until it forms a fine paste; a process which is time consuming. However, busy life, individual or separate families into single family living together in skyscraper, it is difficult to cook starting from raw material preparation. Ready-to-cook and convenient foods such as instant curry powder products are becoming popular among these groups of consumers. However, there is a few scientific data mentioning about functional properties of the paste particularly for southern sour curry which is made from the hotter chili variety bird chili. Therefore, this experiment aimed to determine total phenolic content, antioxidant properties, anti-inflammatory activity and consumer acceptability, when bird chili was added into the paste compared to the paste with finger chili.

Materials and Methods

Raw materials

Both red chilies (*Capsicum* spp.); finger chili (*Capsicum annuum* Linn.) and bird chili (*Capsicum frutescens* Linn.), garlic bulb (*Allium sativum* Linn.), shallot bulb (*Allium ascalonicum* Linn.) and turmeric rhizome (*Curcuma longa* Linn.) were purchased from a local market in Hatyai, Songkhla province, Thailand.

Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride hexahydrate ($FeCl_3$•$6H_2O$), gallic acid were purchased from Sigma-Aldrich Corporation (St. Louis, Missouri, United States). Folin-Ciocalteu's phenol reagent, sodium acetate, hydrochloric acid, sodium carbornate (Na_2CO_3), 99.5% absolute ethanol were purchased from Merck Millipore (Darmstadt, Germany). Roswell Park Memorial Institute medium (RPMI medium), lipopolysaccharides (LPS), phosphate buffered saline (PBS), fetal bovine serum (FBS), penicillin, streptomycin and 0.25% trypsin-EDTA were purchased from Gibco (Carlsbad, California). All chemicals and reagents were of analytical grade.

Keang-hleung paste preparation

Red state of fresh finger chili and bird chili, garlic bulb, shallot bulb and turmeric rhizome were sorted, trimmed and washed to remove dust and dirt. The sample was soaked in chlorine solution 150 ppm (chlorine solution: sample, 3:1) for 1 min then removed to soak in chlorine solution 10 ppm (chlorine solution: sample, 3:1) for 1 min. Thereafter, the sample was rinsed with tap water to complete halal processing. The sample was drained on the sieve for 60 min and chopped 0.2-0.3 cm for size reduction Keang-hleung paste, a basic formula followed the small and medium enterprise in Chumphon province consisted of garlic clove, shallot, finger chili and turmeric rhizome as 12%, 38%, 38% and 12% (W/W), respectively (P1). Another paste consisted of the same proportion with the paste; P1 except the bird chili was used instead of finger chili (P2). The ingredients of each formula were mixed and blended until it became fine paste sized 20-40 mesh. Both pastes were also brought to determine pH value using pH meter (Duco-pH^+ meter, Sartorius).

Preparation of aqueous extract from Keang-hleung paste and chili

The pastes (P1 and P2), and both chilies (finger chili and bird chili), 100 g were taken to mix with 300 ml DI water, stirred for 12 h and subjected to filter with cheese cloth. The liquid part was centrifuged at 1,300 x g at 4°C for 20 min to obtain the supernatant before being subjected to freeze drying. The dry sample, 0.1 g was dissolved with 10 ml DI water and used as a stock solution for determination of phenolic content and antioxidant activity assay.

Total phenolic compound content

Total phenolic contents of the extracts were determined by using Folin-Ciocalteu assay modified from Kahkonen *et al*. (1999). Briefly, 20 µl of the extract at appropriated concentration was introduced into 96 well plates, then added 100 µl Folin-Ciocalteu's reagents and 80 µl of 7.5% (w/v) sodium carbonate and shaken slightly at ambient temperature (29°C) for 30 min in the dark. The absorbance was determined at 765 nm using the micro-plate reader (PowerWare X, Biotek, USA). Gallic acid was used as standard reagent, and reported as g gallic acid equivalent (GAE)/100 g sample of dry weight.

DPPH scavenging activities

DPPH scavenging activity was determined by the modified method of Wu *et al.* (2004). Briefly, 100 µl of sample was mixed with 100 µl of 0.2 mM DPPH dissolved in 75% ethanol. The mixture was shaken

slightly and stand at ambient temperature (29 °C) for 30 min in the dark. Then the absorbance was determined at 517 nm using the micro-plate reader (PowerWare X, Biotek, USA). Gallic acid was used as antioxidant standard, and reported as g gallic acid equivalent (GAE)/100 g sample of dry weight.

FRAP activity

FRAP assay was determined by the modified method of Benzie and Strain (1996). Briefly, the stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2$•$3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-Tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM $FeCl_3$•$6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml $FeCl_3$•$6H_2O$ solution then heated at 37°C for 30 min before use. The extracted sample (15 µl) was allowed to react with 285 µl of the FRAP solution for 30 min in the dark condition. The absorbance was determined at 593 nm using the micro-plate reader (PowerWare X, Biotek, USA). Gallic acid was used as antioxidant standard, and reported as g gallic acid equivalent (GAE)/100 g sample of dry weight.

Cell culture

Growth and activation of cells by using murine macrophage RAW264.7 cells were grown in cell culture medium (RPMI-1640 medium) supplemented with 10% fetal bovine serum, 100 units/ml penicillin and 100 µg/ml streptomycin and incubated at 37°C in humidified atmosphere of 5% CO_2 and 95% air in a cabinet incubator. Cells were used at 80% confluence between passages 7. Cells (1×10^6 cells/ml) were seeded for 24 h to allow attachment (Matsuda *et al*., 2003). The attached cells were incubated with 0.01-3 µg/ml of extract for 30 min before co-incubation with 0.5 µg/ml of LPS.

Measurement of nitric oxide

Nitrite concentration was used as an assessment of nitric oxide (NO) production. Cells were plated and treated with test extract in the presence or absence of LPS. After incubation for 24 h, the spent medium was collected to measure nitrite concentrations using Griess's reaction by adding 100 µl of Griess's reagent to 100 µl sample. Then the reaction was stopped by adding 100 µl of 0.04 M HCl in isopropanol and the optical density (OD) was detected at 570 nm. Inhibition (%) of NO production was calculated using the following equation (1).

$$\text{NO Inhibition (\%)} = \left[\frac{(OD_C - OD_{Bc}) - (OD_S - OD_{Bs})}{(OD_C - OD_{Bc})}\right] \times 100 \quad \text{Equation (1)}$$

Where:
Bc = blank control – RPMI,
C = control -- RPMI+ LPS,
S = Sample -- Sample + LPS,
Bs = Blank sample – RPMI + Sample

Determination of cytotoxic effects

Cytotoxicity was evaluated by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Briefly, after 24 h of incubation with test compounds, MTT (10 µl, 5 mg/ml in PBS) solution was added to the wells. After 4 h incubation at 37°C with 5% CO2, the medium was removed, and isopropanol containing 100 µl 0.04 M HCl was added to dissolve the formazan produced in the cells. The optical density (OD) of the formazan solution was measured with a micro-plate reader at 570 nm. If the OD of the sample-treated group dropped below 80% of that in the vechicle-treated group, the test compound was considered cytotoxic (Matsuda *et al.*, 2003). The cell survival (%) was calculated as shown below.

$$\text{Cell survival (\%)} = OD_s/OD_c \times 100 \quad \text{Equation (2)}$$

Where C = control -- RPMI+ LPS
S = Sample -- Sample + LPS

Sensory quality

Each paste (50 g) was added in boiling water (500 ml) and heated for more 4 min then followed by shrimp paste (20 g), salt (6 g), sugar (7 g), garcinia powder (8 g) and fresh white shrimp (Litopenaeus vannamei) meat (200 g) then boiled for more 2 min. Only the soup at 45-50°C was served to 30 panelists who regularly consumed the southern sour curry and evaluated using 9-point hedonic scale for appearance, color, odor, taste, hot sensation and overall liking.

Statistical analysis

Completely Randomized Design (CRD) was used for total phenolic content, antioxidant activities and anti-inflammatory property. Randomized Completely Block Design (RCBD) was used for sensory quality determination. Data was subjected to Analysis of variance (ANOVA) and mean comparisons were performed using the Duncan's new multiple range test (DMRT). Statistical analyses were carried out using the SPSS statistical software (SPSS, Inc., Chicago. IL).

Results and Discussion

The pH(s) value of finger chili, bird chili, and the pastes; P1 and P2 were 4.06, 4.34, 5.14 and 5.22, respectively. This result pointed out that the paste made from finger chili had lower pH value compared with the paste used bird chili may due to higher ascorbic acid content (Tilahun *et al*., 2013) and or some organic acids as citric acid (Kumar and Tata, 2009) containing in the finger chili. These organic acids are mainly responsible for pH and acidity in fruits and vegetables. The more organic contents the less pH value and more acidity in the sample is addressed. Tilahun *et al.* (2013) reported that Indian pepper, Capsicum varieties as C. annuum (5 varieties) contained ascorbic acid in the range 76-189 mg/100 g while C. frutescens contained this acid about 61 mg/100 g. Kumar and Tata (2009) also concluded that there was variation in ascorbic acid content among eighteen genotypes and repining stages of chili peppers (*Capsicum* L.).

Total phenolic content and antioxidant activity

Total phenolic content of aqueous extracts from the paste used finger chili and bird chili was presented in Table 1. The result showed that the paste with bird chili was higher in total phenolic content, 1.031± 0.011 g GAE/100g dried sample. Zimmer *et al.* (2012) reported that total phenolic content of Capsicum baccutum ranged from 149.28 to 187.51 mg of GAE/g dried weight. It pointed out that difference of total phenolic content in each sample may due to many factors such as location of planting, raw material, preparation procedure, freshness and extraction method as well season and so on.

Table 1. Total phenolics contents and antioxidant activities of Keang-hleung pastes and chilies.

Sample	TPC	DPPH	FRAP
	(g GAE/100g dry sample)		
Paste with finger chili (P1)	0.959^{b}±0.007	0.130^{c}±0.000	1.176^{b}±0.009
Paste with bird chili (P2)	1.031^{a}±0.011	0.203^{a}±0.006	1.913^{a}±0.020
Finger chili	0.107^{d}±0.001	0.208^{a}±0.007	0.411^{d}±0.005
Bird chili	0.178^{c}±0.000	0.183^{b}±0.012	0.643^{c}±0.002

Each value is expressed as a mean ± SD (n=3)
$^{-d}$ means that with different letters within a column are significantly different ($p<0.05$)

Antioxidant activities

DPPH and FRAP activities of the sour curry pastes were increased when substituted with bird chili. It was noticed that the smell of the paste with finger chili was differed from the paste with bird chili. Therefore, it was hypothesized that many compounds were generated during blending the paste leading to difference in antioxidant activity and organoleptic quality. Moreover, it was found that FRAP activity was higher compared with DPPH may due to high polarity of active compounds such as ascorbic acid mainly containing in chili (Tilahun *et al*., 2013), soluble organosulfur containing in shallot and garlic (Banerjee *et al*., 2003) used in the paste were easily extracted by aqueous. And the most important antioxidant found in chili is ascorbic acid that is water soluble compound.

Anti-inflammatory property of Keang-hleung paste extracts

As generally known that NO is an inflammatory mediator then an increase of NO means the cell is inflamed. However, a decrease of NO could be explained in 2 possibilities; (1) no injured or inflamed cell and (2) cell is already death. Therefore, toxicity of test sample or cell survival needed to be evaluated before anti-inflammatory testing. In this experiment, the RAW264.7 cell line treated with the samples, the extracts of pastes and chilies were evaluated and the concentrations made the cell survival higher than 80% were selected. The results showed that the cell treated with both extracts could inhibit NO production as concentration dependent as showed in Table 2.

Table 2. Inhibition on NO production (%) in RAW264.7 cells and IC_{50} of 2 Keang-hleung pastes and chili extracts

Sample	% Inhibition (µg/ml)				IC_{50}
	0.01	1	3		(µg/ml)
Paste with finger chili (P1)	0.52	29.36	74.69		1.967^{e}
Paste with bird chili (P2)	9.82	47.02	61.15		2.021^{d}
Finger chili	2.87	12.29	79.40		2.021^{d}
Bird chili	1.69	21.71	64.09		2.334^{c}
Medical standard	**10**	**50**	**100**	**200**	**(µg/ml)**
Indomethacin	26.70	31.31	39.97	82.68	106.126^{b}
Ibuprofen	2.92	8.80	23.81	67.10	160.006^{a}

Each value is expressed as a mean ± SD (n=3)

Moreover, it was found that finger chili had higher NO inhibition than bird chili. Then the sour curry paste made from finger chili was higher in NO inhibition compared with the paste made from bird chili. However, when IC_{50} of each sample were determined, it was found that the pastes possessed higher inhibition than the bird or finger chili. It pointed out that there were more active compounds inhibiting NO production producing in the pastes.

This may be explained by possible ways; (1) some active compounds such as curcuminoids derived from turmeric rhizome (Jurenka, 2009), ajoene from garlic (Dirsch and Vollmar, 2001) and quercitin from shallot (Cho *et al.*, 2003) and/or (2) new active compounds generating during blending the paste and/ or (3) each active compound synergized each other (Tuntipopipat *et al*., 2011). Interesting, it was found that both pastes and chilies extracts possessed much higher NO inhibition compared with the drug indomethacin and ibuprofen. This may due to the pastes and chilies containing several bioactive compounds which possessed synergized anti-inflammatory effect via different mechanisms and targets (Cho *et al.*, 2003). Yeh *et al.* (2009) reported that beta-carotene plus quercetin had higher ability to synergistically inhibit inflammatory response and DNA damage compared with using an individual compound. Some researchers discovered that using alpha- and beta- carotene helps increase the antinociceptive activity of morphine (Penn, 1995). The increase of anti-inflammatory properties of the paste in this experiment also supported the folk medicine idea which states that using washed water from the mortar after making curry paste in the household could cure normal stomachache problems.

However, there was no relationship between antioxidant and anti-inflammatory activities of the pastes. Since the paste substituted with bird chili had higher total phenolic content and antioxidant activities determined by DPPH and FRAP assay but this paste was lower in anti-inflammatory activity compared with the paste used finger chili. It meant that the active compounds responded to antioxidant activity differed from the active compounds responded to anti-inflammatory activity. Tewtrakul *et al.* (2005) reported that volatile oil of dried rhizome of *Kaempferia galanga* obtained by water distillation did not show antioxidant activity while Ridtitid *et al.* (2008) reported that methanol extract of *Kaempferia galanga* markedly exhibited the anti-inflammatory activity.

Sensory evaluation

The result of sensory evaluation confirmed that the sour curry soup prepared from the paste with bird chili was higher in overall liking score, 7.57/9 (Table 3) mainly due to high spiciness which correlated to other sensations. The spiciness or hot sensation depends on amount of hot chili variety used (Tilahun *et al*., 2013). In Thailand bird chili was claimed as one of the hottest chili types and normally used for southern food. Then using bird chili could improve sensory acceptability though finger chili also used in some provinces of southern Thailand. It meant that consumer preference of Chumporn province differed from Songkhla province even in the same dish. Therefore, making food could not only use scientific reason but it is an art which needs to adapt to client preferences.

Table 3. Sensory score of Keang-hleung soups with finger chili and bird chili

Formula	Appearance	Color	Flavor	Taste	Spiciness	Over all liking
Paste with finger chili (P1)	6.86^{b}±0.85	7.00^{b}±0.86	6.36^{b}±0.99	6.07^{b}±0.77	5.14^{b}±0.93	5.96^{b}±0.88
Paste with bird chili (P2)	7.64^{a}±0.73	7.61^{a}±0.88	7.43^{a}±0.96	7.46^{a}±1.04	7.32^{a}±1.33	7.57^{a}±0.84

Each value is expressed as a mean ± SD (n=3)
a-b means that with different letters within a column are significantly different ($p<0.05$)

Conclusions

The result showed that the paste with bird chili was higher in total phenolic content than the paste with finger chili. DPPH and FRAP activities of the paste were increased when substituted with bird chili. Moreover, it was found that the paste with finger chili had higher NO inhibition than the paste with bird chili. To sum up both pastes and chilies had NO inhibition higher than standard drug using indomethacin and ibuprofen. Panelists prefer the soup made from the paste bird chili compared with the soup made from finger chili. Sour curry or 'Keang-hleung' paste showed both antioxidant and anti-inflammatory activity, which could be claimed as functional or healthy ingredient.

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