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Antioxidant activity of some instant sour curry pastes

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Abstract:

Sour curry is a Thai cuisine that often consumes in all regions of Thailand. Currently, many instant curry pastes are provided on shelf in supermarkets; nevertheless, antioxidant activity of instant form of curry pastes is still lacking. With this reason, six instant sour curry pastes such as Lobo, Mae Ploy, Rosdee, Knor compared to Kanokwan and Lobo (yellow sour curry paste) were selected to determine their antioxidant capacity including DPPH scavenging activity, FRAP assay, total phenolic and total flavonoid contents (TPC and TFC, respectively). Additionally, the qualitative assays were also investigated to identify types of bioactive compounds. The results showed that Kanokwan strongly exhibited DPPH scavenging activity with IC_{50} of 2.18±0.19 mg/mL. This result resembles to quercetin with IC_{50} of 2.51±0.09 mg/mL. Furthermore, it also displayed the highest FRAP value of 45.25±0.13 mg $FeSO_4$/g sample. For TPC and TFC determination, Mae Ploy at 1 mg/mL contained the highest contents of 4.28±0.11 mg GAE/g sample and 39.64±4.27 mg QE/g sample, respectively. Moreover, the qualitative colorimetric assays found that flavone, chalcone, aurone and flavanonol were the principal active ingredients in most samples. In conclusion, this study provides direct comparative data on antioxidant capacity of six instant sour curry pastes particularly yellow pastes that show stronger antioxidant potential.

Introduction

Free radicals are unstable and highly reactive, and energized molecules have unpaired electron such as superoxide, hydroxyl, peroxyl and alkoxyl. Outside the living cell, these compounds are produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes; however, they are continuously produced in the human body and also controlled by endogenous enzymes (superoxide dismutase, glutathione peroxidase, and catalase etc.). An overproduction of these species, exposure to external oxidant substance or failure in the defense mechanisms, leads to damaging of valuable biomolecules (DNA, lipids, proteins) which associated with and increased risk of cardiovascular disease, cancer and other chronic disease[1]. In recent years, human health related to nutrition, fitness and beauty has exaggerated concerns over diet. Therefore, a new diet health paradigm is more interesting.

Sour curry or Keang-som soup is traditional popular spicy-sour curry consumed in not only in Thailand but also throughout a lot of Asia because of its good taste, spicy, unique flavor and health benefits. It is claimed as a healthy food because of low calories due to less fat but high proportion of vegetable. Sour curry normally contains many kinds of vegetables; therefore, it has high fiber, which is good for health. Moreover, the ingredients used in the paste are turmeric rhizome, garlic, shallot and chili, which have been reported as a source of antimicrobial and antioxidant compounds[2]. Herbs and spices used in the curry paste are sources of phytochemicals or bioactive compounds, which are claimed to prevent non-communicable

diseases (NCDs) such as high blood pressure, diabetes and cancer[3]. Many ingredients used in the southern Thai sour curry paste have been found to contain antioxidant and anti-inflammatory substances and have medicinal value[4]. Currently, many instant curry pastes are provided on shelf in supermarkets; nevertheless, antioxidant activity of instant form of curry pastes is still lacking. Therefore, six instant sour curry pastes in supermarkets were selected to determine their antioxidant potential and also evaluated bioactive compounds.

Methodology

Samples preparation

Six instant sour curry pastes including Lobo, Mae Ploy, Rosdee, Knor, Kanokwan and Lobo (yellow sour curry pastes) were selected and purchased from supermarkets in Chon Buri province, Thailand during January to February 2018. All purchased sour curry pastes were manufactured during July 24, 2017 to August 25, 2017 and will expire during June 4, 2018 to September 7, 2019. All samples were prepared at appropriated concentrations by dilution with distilled water 50 mL at 95^0C for 5 min prior to different analyses.

Total phenolic content (TPC)

Total phenolic contents of sour curry paste samples were determined using Folin-Ciocalteu assay modified from Suvajasuwan et al.[5] Briefly, 100 µL of the sample at 1 mg/mL was introduced into 96 well plates, then added 100 µL Folin-Ciocalteu's reagents, 500 µL distilled water and 1 mL of 7% (w/v) sodium carbonate and shaken slightly at ambient temperature (29^0C) for 45 min in the dark. Then the mixture was pipetted about 150 µL into the microplate and the absorbance was determined at 765 nm using the microplate reader (VERSA max, USA). Gallic acid was used as standard reagent, and reported as mg gallic acid equivalent (GAE)/g sample.

Total flavonoid content (TFC)

Total flavonoid content was evaluated based on the formation of flavonoid-aluminium as described by Djeridane et al.[6] 0.5 mL of sample was mixed with 0.1 ml of 5% sodium nitrite. After incubation at room temperature for 6 min, 0.2 mL of 10% aluminium trichloride solution was added and allowed to stand for 5 min. 0.5 mL of 1 M sodium hydroxide was then mixed with the mixture. Adjusted the final volume to 1.5 mL with distilled water. Then the mixture was pipetted about 150 µL into the microplate and the absorbance of the reaction mixture was measured using the microplate reader (VERSA max, USA) at 510 nm. Quercetin was used as a standard to plot the calibration curve. The amount of flavonoid was expressed as mg quercetin equivalent (QE)/g sample.

DPPH scavenging activity

DPPH scavenging activity was determined by the modified method of Kantangkul et al.[7] Briefly, 100 µl of appropriated concentrations of sample was mixed with 200 µl of 0.2 mM DPPH dissolved in methanol. The mixture was shaken slightly and stand at ambient temperature (29^0C) for 30 min in the dark. Then the absorbance was determined at 517 nm using the microplate reader against ethanol blank and distilled water as negative control. Ascorbic acid and quercetin were used as comparative standards. Antioxidant activity (AA) was expressed as the percentage of DPPH scavenging activity as the equation (1) and IC_{50}.

$$\% \text{ DPPH scavenging activity} = (A_c - A_s - A_b)/A_c \times 100 \qquad (1)$$

Where, $A_c = A_{517}$ of DPPH in methanol, $A_s = A_{517}$ of sample mixed with DPPH in methanol and $A_b = A_{517}$ of blank sample

Ferric reducing antioxidant power (FRAP assay)

FRAP assay was determined by the modified method of Benzie and Strain[8]. Briefly, the stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ 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 $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml $FeCl_3 \cdot 6H_2O$ solution then heated at 37°C for 30 min before use. The sample (100 µl) was allowed to react with 200 µl of the FRAP solution for 30 min in the dark condition. The absorbance was determined at 596 nm using the microplate reader. Trolox was used as antioxidant standard, and reported as mg ferrous sulfate/g sample.

Qualitative determination by colorimetric assays

The samples were prepared at 10 mg/mL prior to qualitatively determine the kinds of flavonoids by different colorimetric assays including ferric chloride test, Shinoda test, Pew test, alkaline test, anthocyanin test (acid-base test), leucoanthocyanin test and gelatin test[9]. 1. The ferric chloride test was carried out by mixing between 1% $FeCl_3$ about 2-3 drops and sample 2 mL. 2. Shinoda test, 0.1 g of magnesium fillings (ribbon) were added to 1 mL of sample followed by a few drops of concentrated hydrochloric acid. A reddish color was observed. 1 mL of water was then added with 1 mL of octanol. Shaked and allowed to separate the layer and observed the present color. 3. Pew test, 0.5 g of zinc powder was mixed with 1 mL of sample followed by 2 drops of 2N HCl. After mixed well about 1 min, 10 drops of concentrated HCl were administered. 4. The reaction with base, 1 mL of sample was mixed with a few drops of 10% ammonia solution. The color was observed. 5. Anthocyanin test, 1 mL of sample was mixed with a drop of 2N HCl, and then followed by a few drops of 10% ammonia solution. 6. Leucoanthocyanin test, 2 mL of 2N HCl was added to 1 mL of sample, the mixture was boiled in water bath, and then the color was observed. 7. Gelatin test, 0.5-1% of gelatin solution was added to 1 mL of sample, and then the precipitate was observed.

Statistical analysis

All results were presented as mean ± SEM and were done in triplicate independent analyses. Data were subjected to analysis of variance, and mean comparison were made using One-way ANOVA. Statistical analyses were carried out using the Minitab software version 17.

Results and Discussion

TPCs of sour curry paste sample were in the order of Mae Ploy > Lobo (yellow sour curry paste) > Kanokwan (yellow sour curry paste) > Lobo > Rosdee > Knor (**Table 1**). For sour curry paste, Mae Ploy demonstrated the highest TPC and was significantly different ($p < 0.05$) with all the others. However, TPC of all samples of sour curry paste was rather much indifferent ($p < 0.05$) than yellow sour curry paste. The ingredients mixed in sour curry paste may affect to their phenolic contents particularly shallot, chili and turmeric rhizome. Lu et al.[10] presented the total phenolic content of shallot (*Allium oschaninii*) of 17.18±1.88 mg GAE/g extract. Moreover, Kantangkul et al.[7] showed the total phenolic content of different chili ranged from 0.965±0.007 to 1.031±0.011 g GAE/100 g dry weight.

From **Table 1**, total flavonoid content (TFC) of instant sour curry paste samples were in the order of Mae Ploy > Kanokwan (yellow sour curry paste) > Lobo (yellow sour curry paste) > Rosdee > Knor > Lobo. At 1 mg/mL, Mae Ploy showed the highest TFC while Lobo showed the lowest TFC (differed 9 times). This finding was consistent with the findings by Dangubon[11] showing that the flavonoid contents of all herbs commonly used in Thai dish varied from 0.07 to 48.81 mg per 100g wet weight. From qualitative colorimetric assays, the results showed that instant sour curry paste contained polyphenols including flavone, chalcone, aurone and flavanonol. Furthermore, Rosdee and Knor also found leucoanthocyanin (data not shown).

Table 1. Total phenolic and total flavonoid contents at 1 mg/mL of sour curry paste samples

Sour curry paste sample	Total phenolic content (mg GAE[1]/g sample)	Total flavonoid content (mg QE[2]/g sample)
Kanokwan (yellow sour curry paste)	3.64±0.10[b]	36.19±2.46[a]
Lobo (yellow sour curry paste)	3.82±0.10[b]	23.96±2.89[b]
Mae Ploy	4.28±0.11[a]	39.64±4.27[a]
Lobo	2.90±0.13[c]	4.08±2.21[d]
Rosdee	2.13±0.18[d]	13.76±2.02[c]
Knor	1.74±0.14[e]	11.72±1.17[c]

[1]GAE = gallic acid equivalent
[2]QE = quercetin equivalent
[a-e] significant difference in the same column at *P* value < 0.05

DPPH free radical scavenging assay was based on the reduction of DPPH radicals, which causes an absorbance decrease at 517 nm where the purple color changed into yellow color. Antioxidants, on interactions with DPPH, transferring an electron from a hydrogen atom to DPPH free radical, hence neutralizing its free radical activity[12, 13]. Instant yellow sour curry paste both Kanokwan and Lobo exhibited stronger DPPH radical scavenging activity than other sour curry paste samples as presented in **Figure 1**. This may be the role of curcumin, the major bioactive component of turmeric rhizome that may have a positive impact on H atom donation to DPPH radical. The DPPH radical scavenging activity when considered with the IC_{50} value was in the order of ascorbic acid > quercetin > Kanokwan > Lobo (yellow sour curry paste) > Mae Ploy > Lobo > Rosdee > Knor (**Table 2**). Interestingly, Kanokwan that was a yellow sour curry paste displayed a strong radical scavenging activity (2.18±0.19 mg/mL) that lower than of quercetin, a standard used in the experiment (2.51±0.09 mg/mL). This potential of instant sour curry paste may due to high quantity of active compounds such as ascorbic acid mainly containing in chili[14], soluble organosulfur containing in shallot and garlic[15] used in the paste.

FRAP assay, a popular method used to determine the antioxidant activity that based on the electron donating ability of an antioxidant compound with ferric-TPTZ to form ferrous-TPTZ[8]. This activity of instant sour curry pastes was increased when added with turmeric rhizomes. It was noticed that the smell of the paste with this ingredient was differed from the other pastes. Therefore, it was hypothesized that many compounds were generated during blending the paste leading to difference in antioxidant activity. The highest value of FRAP was found in Kanokwan; yellow sour curry paste (45.25±0.13 mg $FeSO_4$/g sample) (**Figure 2**) whereas the lowest value was in Lobo, Knor and Rosdee sour curry pastes (17.33±0.03, 16.86±0.20 and 16.22±0.11 mg $FeSO_4$/g sample). The antioxidant activities of phenolic compounds are mainly of redox properties, including free radical scavenging, hydrogen donating and singlet oxygen quenching[16]. Pulido et al.[17] reported that the reducing capacity of polyphenols, as determined by the FRAP assay seemed to depend on the degree of hydroxylation and extent of conjugation of the phenolic compounds.

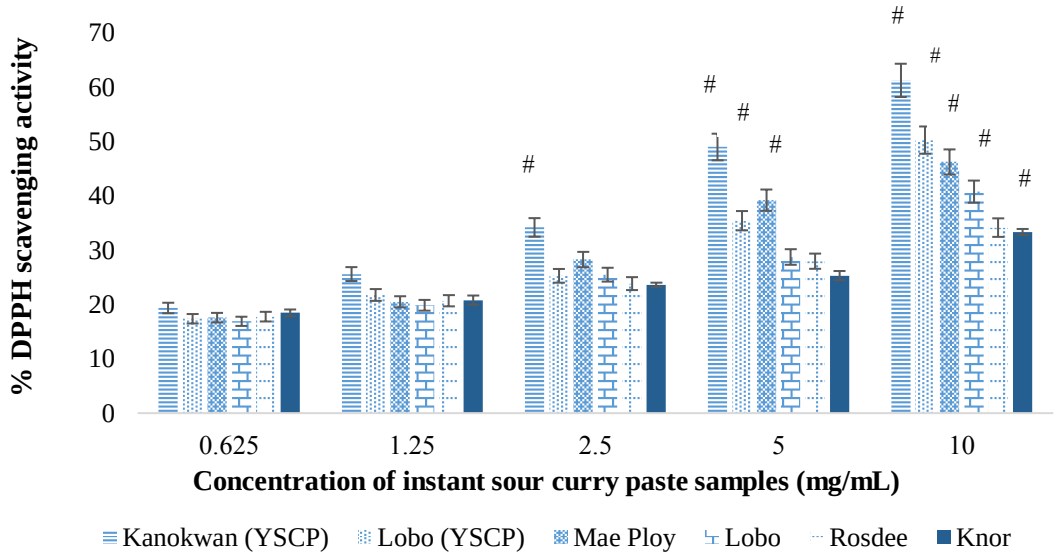

Figure 1. % DPPH scavenging property of instant sour curry paste samples including Lobo, Mae Ploy, Rosdee, Knor compared to Kanokwan and Lobo (yellow sour curry paste) # indicates the significantly difference at $P < 0.05$ of the same branding of instant sour curry pastes.
YSCP = yellow sour curry paste

Table 2. IC_{50} of DPPH scavenging activity of sour curry paste samples and standards

Sour curry paste sample	IC_{50} (mg/mL)
Ascorbic acid	0.014 ± 0.0002^{a}
Quercetin	2.51 ± 0.09^{b}
Kanokwan (yellow sour curry paste)	2.18 ± 0.19^{b}
Lobo (yellow sour curry paste)	9.71 ± 0.22^{c}
Mae Ploy	10.31 ± 0.18^{c}
Lobo	13.84 ± 0.28^{d}
Rosdee	19.12 ± 0.57^{e}
Knor	21.34 ± 0.78^{f}

[a-e] significant difference in the same column at P value < 0.05

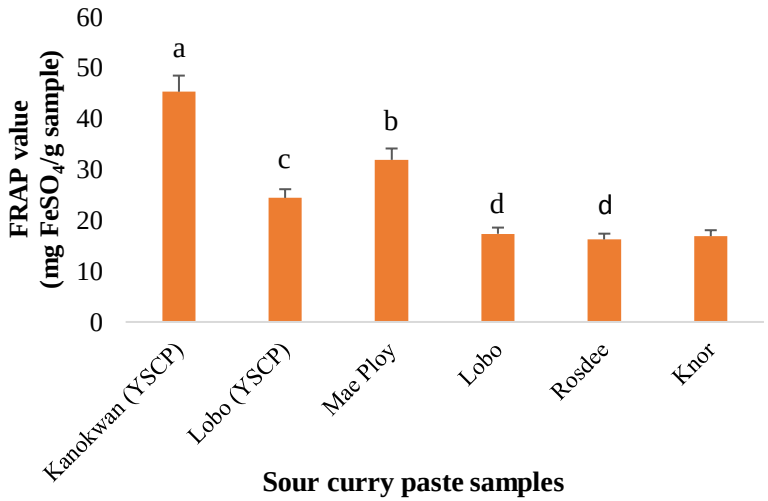

Figure 2. Ferric reducing power of instant sour curry paste samples
[a-d] significant difference when compared to the other bars at P value < 0.05
YSCP = yellow sour curry paste

Conclusion

Sour curry and yellow sour curry pastes showed strong antioxidant capacity with various flavonoids from their ingredients mixed together, which could be claimed as functional or healthy foods. Interestingly, both yellow sour curry pastes (Kanokwan and Lobo) seemed to exhibit more antioxidant activity than typical sour curry pastes. That may be due to their important ingredients such as turmeric (*Curcuma longa* L.) and finger root (*Boesecnergia pandurata* (Roxb.) Schltr.) rhizomes that cannot be found in general instant sour curry pastes. However, some researchers mentioned that in vitro antioxidant activity such as DPPH and FRAP assays may not responsible for antioxidant activity in vivo or even food system therefore using cellular antioxidant activity assay was more useful and close to body system.

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