International Journal of GEOMATE, Dec., 2016, Vol. 11, Issue 28, pp.2906-2909
Special Issue on Science, Engineering and Environment, ISSN: 2186-2990, Japan

# TOTAL PHENOLIC CONTENTS AND ANTIOXIDANT ACTIVITY OF SOME FRESH THAI CURRY

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**ABSTRACT**: The objectives of this work were to study the total phenolic contents (TPC) and antioxidant activity of methanolic extracts of six fresh Thai curry. The TPC of the fresh Thai curry were found in the range of 0.989-1.1132 mg GAE/mg of extract. Among the curry, red curry present the highest amount of TPC. Antioxidant activity of the extracts was measured by DPPH-radical-scavenging assay, lipid peroxidation inhibition and metal ion chelating assay. The Thai red curry extract exhibited the highest inhibition of lipid peroxidation of linoleic acid emulsion (81.41±0.55%), radical-scavenging activity ($IC_{50}$ = 0.0476 mg/mL). In addition, the fresh of Thai red curry had the highest of metal chelating activity (76.58±0.72%). However, all of fresh Thai curry showed metal chelating similar potential activity and not significant statistical differences. These results suggest that the fresh Thai curry had interesting antioxidant activities and should be propose as potential sources of safe natural antioxidants and preservatives for curry industry.

*Keywords: Phenolic, Antioxidant, Chelating, DPPH, Fresh Thai curry*

## 1. INTRODUCTION

Thai food has gained popularity, and Thai curry such as Tom-Yum, Red curry and Green curry are important ethnic food consumed world wide due to as tastes, colors and health effect. The major ingredients of Thai curry include garlic, chili and shallot (red onion). Garlic and onion have generally found to be a grate antibacterial [1], antidiabetic, hypocholesterolemic and cancer preventive agent [2]. Allicin, one of the active components of freshly crushed garlic homogenated, has a variety of antimicrobial, antifungal, antiparasitic and antiviral activities [3]. Spices has a lot of carotenoids which have antimutagenic or anticarcinogenic properties [4]. Essential oil and flavonoid from shallot are used as flavoring or seasoninf agents. Therapeutic properties include the antioxidant activity, the alleviation of discomfort stomach, and as an antiheimintic, antidiarrhoeal, expectorant, antitussive, diuretic and antiflu agents. In addition, other ingredients of Thai curry include galagal root, lemon grass, kaffir lime leaves, shrimp paste, and kaempferia pandurata, which are effective in inhibiting tumors in the digestive tract [5]-[8]. The essential oil of lemon grass is mainly comprised of citral which exhibited a broad antifungal spectrum [9], [10] while galangal root can be purchased fresh or dried. It resembles ginger root and is known as the "defisher" as it reduces the fishy smell of foods and kaffir lime leaves have antioxidant properties and contain volatile oil. A digestive aid, Kaffir leaves cleanse the blood while helping maintain healthy teeth and gums. Kaffir can be applied to the hair and scalp, and even prevents hair loss. It is used as a deodorant. The fresh lime fruits can also be used if available. Therefore, the objectives of this work were to study the total phenolic contents (TPC) and antioxidant activity of methanolic extracts of six fresh Thai curry.

## 2. METHODOLOGY

Thai curry, 6 cultivars of fresh Thai curry; Green curry, Tom-Yum, Kaeng-Sum, Tom-kha, Pa-naeng and Red curry were from the 2012 of the department store in Mahasarakham province, Thailand. All chemical reagents used were analytical grade.

### 2.1 Extraction of Crude Antioxidants

The Thai curry cultivars (25.0g) were extracted with 80% ethanol (3×100 mL) and 0.5% TFA-80% ethanol (3×80 mL) for 30 min with intermittent shaking at room temperature. The extracts were combined and filtered through a 0.45 µm Nylon membrane filter. After which, the extracts were then slowly concentrated under reduced pressure, below 40 ℃, on a rotary evaporator to yield the crude antioxidants. The crude samples were used for the determination of antioxidant activity.

### 2.2 Antioxidant Activities

*2.2.1Total phenolic compounds*

The total phenolic contents of crude Thai curry extracts were determined by spectrophotometric method using Folin–Ciocalteu's phenol reagent (24). The crude extract in ethanol (0.5 mL) was placed in a test tube and was diluted to 5.0 mL with a glass of

distilled water. Folin–Ciocalteu's phenol reagent (5.0 mL) was added, and the contents of the test tube were mixed thoroughly. After 3.0 min, 5 mL of 10% sodium carbonate solution was added, and the mixture was allowed to stand for 1 h with intermittent shaking. The absorbance of the blue color was measured in a DU-7500 Spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA) at 750 nm. The concentration of total phenolic compounds was determined using the gallic acid equation (mg/g extract) obtained from the standard gallic acid calibration curve. This experiment was carried out three times, and the results were averaged for the different fractions in the Thai curry varieties.

*2.2.2 Thiocyanate method*

Antioxidative activity was carried out by using the linoleic acid system (24). In a well-stopped Erlenmeyer flask containing linoleic acid (0.13 mL) in a 0.2 M NaOH-phosphate buffer (10 mL, pH 7), the crude antioxidants in ethanol (1 mg) from the different fractions of Thai curry were added, and the volume increased to 25 mL with a glass of distilled water. The flasks were incubated at 40 °C for a two-week period, and the degree of oxidative was measured according to the thiocyanate method (25). The incubation mixture (0.2 mL) was reacted with $NH_4SCN$ (30%, 0.2 mL), 9.4 mL of 75% EtOH, and 0.2 mL of $FeCl_2$ ($2.53\times10^{-2}$ g/ 10 mL 3.5 % HCl) solution. The absorbance of the blue color (peroxide value) was measured in a DU-7500 Spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA) at 500 nm. The control solution was prepared in a similar manner without the addition of any antioxidant, while α-tocopherol and butylated hydroxyanisole (BHA) at 200 µg per flask was used as a standard for comparison. This experiment was performed three times, and the results were averaged for the different fractions in the Thai curry varieties. The percentage of inhibition of lipid peroxidation was calculated using the following equation:

$$\text{Inhibition (\%)} = [(A_c - A_s)/A_c] \times 100,$$

where $A_c$ is the absorbance of the control solution and $A_s$ is the absorbance in the presence of the black rice bran extracts

*2.2.3 DPPH free radical-scavenging activity*

The radical scavenging activity of crude extracts was measured using the method of Yamaguchi et al. [11]. The crude extract and α-tocopherol (5-40 mg/mL) were added to 1.5 mL of 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) in ethanol. The mixture was shaken vigorously and was left to stand for 20 min at room temperature in the dark. The absorbance was measured in a DU 7400 spectrophotometer (Beckman Instruments Inc., CA, USA) at 517 nm. The control reaction contained all reagents except for the crude samples.
The radical scavenging effect was calculated by the following equation:

$$\text{Scavenging effect (\%)} = [(A_c - A_s)/A_c] \times 100,$$

where $A_c$ is the absorbance of the control at 517 nm, and $A_s$ is the absorbance of the extract/standard at 517nm. This experiment was repeated thrice, and the results were averaged for the different fractions in the Thai curry varieties.

*2.2.4 Metal ion chelating activity*

The chelating of ferrous ion was measured using the method of Dinis et al. [12]. The extracts (5-25 mg/mL) were reacted with 0.05 mL 0f 2.0 mM $FeCl_2$. The mixture was then added with 0.2 mL of 5.0 mM ferrozine. The control did not contain $FeCl_2$ and ferrozine. After which, the reaction was shaken and incubated at room temperature for 10 min. The absorbance of the red color was measured in a DU-7500 Spectrophotometer (Beckman Instruments Inc., CA, USA) at 562 nm. This experiment was carried out three times, and the results were averaged for the different fractions in the Thai curry varieties. The percentage of metal chelating activity was calculated by the following equation:

$$\text{\% Metal chelating activity} = [(A_c - A_s)/A_c] \times 100,$$

where $A_c$ is the absorbance of the control at 562 nm, and $A_s$ is the absorbance of the extract/standard at 562nm. This method was performed three times, and the results were averaged for the different fractions in Thai curry cultivars. $Na_2EDTA$ was used as a positive control.

## 3. RESULTS AND DISCUSSION

### 3.1 Total Phenolic Compounds

Total phenolic content was measured by the Folin-Ciocalteu reagent method using gallic acid as the standard. A linear calibration curve of gallic acid resulted with a correlation coefficient of $R^2 = 0.9990$ over the concentration range 20-120 µg/mL. This linear equation was used to determine the total phenolic compounds in the Thai curry extracts. The average quantity of the total phenolic compounds found in the Thai curry extracts is shown in Table 1. The amount of total phenolics content of fresh Thai curry were in the range of 0.989 – 1.1132 mg GAE/mg of extract, respectively. Among the curry, red curry present the highest amount of TPC.
Antioxidant Activities

Antioxidant activity of the extracts was measured by lipid peroxidation inhibition, DPPH-radical-scavenging assay, and metal ion chelating assay. The Thai red curry extract exhibited the highest inhibition of lipid peroxidation of linoleic acid emulsion (81.41±0.55%) and the Tom-kha extract showed the lowest lipid peroxidation It is suggested that the antioxidant activity in Thai curry comes from free radicals which promote chain reactions during the linoleic acid peroxidation system.

The radical-scavenging activity of each crude Thai curry extract was measured by using the DPPH assay. When the DPPH radical was scavenged by an antioxidant through the donation of H˙ to form the reduced DPPH-H, the color changed from purple to yellow. In this study, the scavenging activity was amplified with the increased concentration of all the rice antioxidants in the range of 0-40 mg/mL, and it was constant at concentration above 15 mg/mL. In the DPPH radical-scavenging assay, the Red curry extract of fresh Thai curry showed the highest activity ($IC_{50}$ = 0.0476 mg/mL). The average quantity of the DPPH assay in the Thai curry extracts is shown in Table 1. In addition, the fresh of Thai red curry had the highest of metal chelating activity (76.58±0.72%). and the Tom-kha extract of pack Thai curry showed the lowest ($IC_{50}$ = 0.5553 mg/mL). the concentrations of α-tocopherol and the Thai curry extracts, at which the DPPH radicals were scavenged by 50 % ($IC_{50}$). The lower the $IC_{50}$, the higher the antioxidant activity. In this analysis, the possible mechanisms suggest that the radical-scavenging effects of black rice might be due to the hydroxyl groups in the antioxidants of the extracts.

**Table 1** The total phenolic compounds and The radical-scavenging activity by using the DPPH assay found in the Thai curry extracts

| Thai curry paste | Total phenolic content (mg GAE/mg) | $IC_{50}$ (mg/mL) |
|---|---|---|
| Green curry | 0.989 | 0.104 |
| Tom-yum | 1.092 | 0.067 |
| Kaeng-sum | 1.024 | 0.068 |
| Tom-kha | 0.936 | 1.749 |
| Pa-naeng | 1.111 | 0.053 |
| Red-curry | 1.132 | 0.048 |

The metal chelating activity of the Thai curry extracts was estimated by the ferrozine assay [12]. Ferrozine is a quantitative formation of a complex with Fe2+ ions. The results indicate that the chelating ability increased with the increased concentration of all Thai curry antioxidants in the range of 0-15 mg/mL. [Fig. 2 ]. EDTA was used as a reference chelating agent. At a concentration 5 µg/mL, the fresh Red curry variety showed the highest percentage of metal chelating, which was much lower than that of EDTA at the same concentration. The metal chelating activity was significant since it reduced the concentration of the catalyzing transition metal in lipid peroxidation. The chelating agents, which form α-bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential.

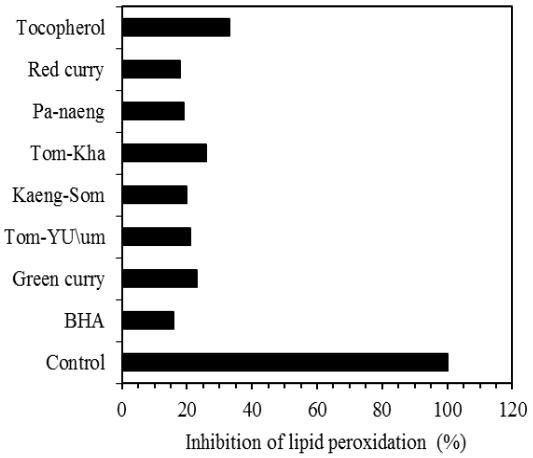


Fig 1. The thiocyanate method compared with α-tocopherol and BHA

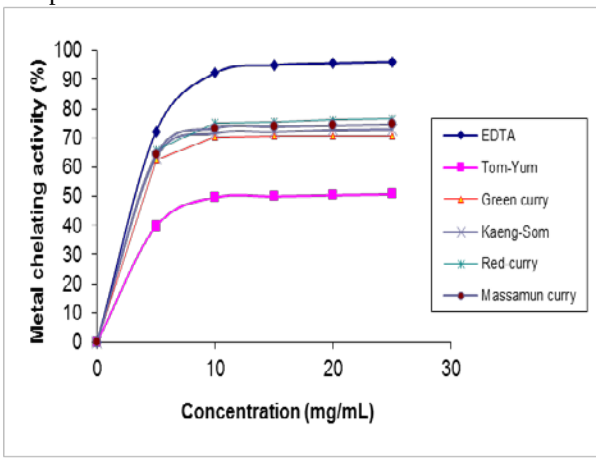


Fig 2. The metal chelating activity of the Thai curry extracts was estimated by the ferrozine assay

## 4. CONCLUSION

The results presented in this study showed that Thai curry cultivars possess a relatively strong antioxidant activity and that there is no significant difference between the antioxidant activity fresh Thai curry and pack Thai curry as assessed by all the methods used. There is also a correlation between the capacities of total and specific antioxidation and total phenolic contents of the Thai curry samples, which indicates that antioxidant activities in the fresh Thai curry are largely owed to the phenolic group of compounds. Therefore, the Thai curry cultivars is a potential source of antioxidative phytochemicals and is a useful ingredient for nutraceutical or functional food products.

## 5. ACKNOWLEDGEMENTS

The authors wish to thanks to Department of Chemistry, Faculty of Science, Mahasarakham University for a partial support of some chemicals. Financial support from Faculty of Science, Mahasarakham University is acknowledged.

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*International Journal of GEOMATE, Dec., 2016, Vol. 11, Issue 28, pp. 2906-2909.*
MS No. 1378 received on July 22, 2015 and reviewed under GEOMATE publication policies.

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