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Comparison of antioxidant and anti-aging activities in selected Thai curry pastes and dishes using water extraction and simulation of gastrointestinal digestion *in vitro* experiments

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Abstract
Fresh herbs and spices pastes used in Thai cooking are one of the uniqueness of Thai cuisine. Some of them have been scientifically proved for health benefits when there is no evidence on health benefits of the ingradients for Thai foods. The objectives were determined antioxidant and anti-aging activities of Thai curry pastes and Jasmine rice/Thai spaghetti with those curries and chickens. Food samples were extracted with water or digested with simulation of gastrointestinal digestion. The results of water extraction showed differences in their activities. The distinctive activities were found in the water extract from green curry paste. Most of the water extracted from Jasmine rice/Thai spaghetti with curries and chickens had less potent than that of their curry pastes except for both activities. After simulation gastrointestinal digestion, some activities of foods were altered.

Keywords: Curry pastes, Jasmine rice/Thai spaghetti with curries, Antioxidant activity, Anti-ageing activity

1. Introduction
Thai food is not only well-known for the delicacy but also known to have physiologically health benefits due to its ingradients; local vegetables, herbs and spices. These ingradients are usually used in a mixed-form called 'Curry Paste'. Although, many health benefits of an individual herb and spice were studied and identified, there is a lacking of scientific evidence supporting the health benefits of Thai foods or Thai food products as commonly consumed. Thus, Thai food consumption was promoted domestically and internationally under the functional foods concept, there is a need for the research to determine antioxidant activity and anti-aging activity in these products. Antioxidant activity was determined radical scavenging activity of food sample by use of stable free radical, α,α-diphenyl-β-picryhydrazyl (DPPH). The method is widely acceptable for the antioxidant activity. The other property Anti-aging activity was determined Tyrosinase which played the major role in melanogenesis by converting L- DOPA to DOPA chrome. Tyrosinase inhibitory activity of food samples was therefore a good indicator for predicting the anti-aging property of the food. The expected outputs were provided an useful information on functional properties of selected Thai curry pastes and their curry dishes for manufacturer and general consumers. This information could be used for screening Thai foods/dishes with high potential to further study in human for more supportive evidences.

2. Materials and methods
2.1 Chemicals
All chemicals used in this study were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2 Sample preparation
There were 4 kinds of curries such as red curry, green curry, phanang curry and musaman curry. All curries were come from the only one factory. The weight of each curry was one kilogram from triplicating processes at difference time interval and was collected for each testing using nitrogen gas at temperature 4ºC until the experiment was done. Each curry was divided for cooking with chicken as the representative meat in the preparation such as red curry chicken, green curry chicken, phanang curry chicken and musaman curry chicken. Those formulations have been studied and accepted. The detail of composition was shown in recipes for each curry. After that each curry was prepared each curry sample for eating as one plate meal as the following such as rice with red curry chicken, rice with green curry chicken, Thai spaghetti with green curry chicken, rice with phanang curry chicken and rice with musaman curry chicken, respectively. Each plate meal had Jasmine rice variety 105 or Thai spaghetti with varies curries according to unit consumption. One unit consumption was average amount of food which was suitable for eating per person per meal as the following. One unit of consumption rice or spaghetti was 180 grams. One unit of consumption with red curry chicken or green curry chicken was 200 grams. One unit of consumption with phanang chicken curry was 120 grams. Finally, one unit of consumption with musaman chicken curry was 270 grams. Each one plate meal with each curry including rice or spaghetti was blended into fine pieces and collected each portion under nitrogen gas at -20 ºC until the experiment was started.

2.3 Preparation of samples for study functional health effects
There were two methods for food extraction such as water extraction and simulation gastrointestinal digestions similar to the function of human stomach and small intestine. The water extraction of food was started with the weighted each sample 4 grams was put into 50 ml of centrifuge tube and added water 8 grams (triplicate each sample). Then the sample solution was homogenized into homogeneous solution with Homogenizer (ultra turrax T25) at 13,500 rpm for 1 minute. The supernatant was centrifuged (HERMLE Z400 K) at 6,000 rpm for 10 minutes at temperature 4ºC. The filtrate was separated by Whatman no.

541and collected each supernatant at 4ºC for next experiment. The simulation gastrointestinal digestion similar to the function of human stomach and small intestine was started with each weighted sample 4 grams into flask (triplicate each sample) and added 120 mM NaCl 60 ml. then homogenized into homogeneous solution with Homogenizer (ultra turrax T25) at 13,500 rpm for 30 seconds. The solution was adjusted pH into acidic condition as in human stomach pH 2.1±0.1 with 1N HCl. The enzyme pepsin solution concentration 40 mg/ml in 100 mM HCl was added 4 ml and adjusted the total volume 80 ml with 120 mM NaCl. The stomach digestion was shaked 85 rpm at temperature 37 ºC for one hour. Then the solution was adjusted to pH 6.0±0.1 with 1M $NaHCO_3$. The bile extract concentration 40 mg/ml in 100 mM $NaHCO_3$ was added 6 ml and the pancreatine-lipase 10 mg/ml, lipase 5 mg/ml in 100 mM $NaHCO_3$ was added 4 ml. The pH was adjusted to pH 6.9±0.1 with 1 M NaOH and finally added 120 mM NaCl to 100 ml. The solution was digested as in small intestine with shaking 85 rpm at 37ºC for 2 hours. The supernatant was centrifuged at 6,000 rpm, temperature 4 ºC. for 30 minutes. The supernatant was filtered on Whatman no.541 and heated to stop the enzyme function in the digestion for 20 minutes then put in ice box immediately. The supernatant solution was cooled at 4ºC for further experiment.

2.4 Methods for testing functional health effects

2.4.1. Antioxidant activity (6)

The radical scavenging activity was used α,α-diphenyl-β-picrylhydrazyl (DPPH) as the stable free radical in solution and shown violet-blue color. The assay was measured the color change from violet-blue color to yellow color of hydrazine by antioxidant such as Butyryl hydroxyanisole (BHA). The changing in color was detected by microplate spectrophotometer. The control was water 22 µl in case of water extraction from food sample or enzymes solution in digestion 22 µl of the food sample digestion with enzymes. The 96-well plate was used to fill up with the solution above and 150 µM of DPPH in 80% methanol with the volume 200 µl into the control and the sample. The incubation at room temperature in the dark was measured absorbance at 520 nm with the Microplate Reader (Tecan: Sunrise). The % radical scavenging activity (%RSA) was calculated from the equation as the following.

$\%RSA = (OD_{t\ 30}\ control\text{-}OD_{t30}\ sample) \times 100 / OD_{t30}\ control$

OD_{t30} was absorbance at 520 nm for 30 minutes

2.4.2. Anti-aging activity (5, 6)

The inhibition of Tyrosinase activity was measured by the food sample solution. Tyrosinase enzyme had an important in Melanogenesis which is one mechanism for aging. The activity of Tyrosinase was used spectrophotometer to convert L-DOPA to detect absorbance of DOPA chrome at wavelength 475 nm *in vitro* with or without the food sample. The Tyrosinase 100Units/ml was pipetted 0.3 ml into food sample solution 0.3 ml and mixed very well. The solution was incubated 37ºC for 10 minutes Then 0.15% L-DOPA was added 0.3 ml in the ice box and incubated 37 ºc for 20 minutes. The absorbance was measured at 475 nm with UV-visible spectrophotometer. %Inhibition of Tyrosinase was calculated as the equation below.

$\%\ Tyrosinase\ Inhibition = OD_{475}\ control\text{-}OD_{475}\ sample \times 100 / OD_{475}\ control$

3. Results & Discussion

3.1 Antioxidant activity

The water extraction of all food samples were tested antioxidant activity by comparison with the standard BHA at concentration 1 mg/ml. The results showed that all food samples had antioxidant activity (Table1). The extraction with water of Jasmine rice and Thai spaghetti had equal antioxidant activity, respectively but lower than the standard BHA with significant figure (p<0.05). The Jasmine rice with red curry and chicken, Jasmine rice with green curry and chicken / Thai spaghetti with green curry and chicken were shown from the increase in antioxidant activity, respectively. For Jasmine rice with phanang curry and chicken had highest antioxidant activity than Jasmine rice with musaman curry and chicken. However, the antioxidant activity was increased less than the standard BHA with significance figure (p<0.05). For the water extraction of all curries paste, green curry had the highest antioxidant activity among musaman curry, red curry and phanang curry, respectively.

Table 1: Antioxidant activity of water extracted food sample by using DPPH technique

Food samples	%RSA[a]
Standard BHA (1 mg/ml)	89.1
Jasmine Rice	44.6±13.9
Thai Spaghetti	25.1±5.9
Red Curry	63.7±12.5
Green Curry	102.6±9.0
Phanang Curry	61.6±9.0
Musaman Curry	92.3±8.5
Jasmine Rice with Red Curry and Chicken	29.8±7.6
Jasmine Rice with Green Curry and Chicken	37.4±7.8
Thai Spaghetti with Green Curry and Chicken	47.3±8.0
Jasmine Rice with Phanang Curry and Chicken	82.9±6.7
Jasmine Rice with Musaman Curry and Chicken	79.7±10.6

[a]%Radical Scavenging Activity (%RSA) : mean±SD, n=3

The simulation of gastrointestinal digestion of food samples with mixture of enzymes. The results showed that all food samples had decreased the antioxidant activity when compared to the standard BHA 1 mg/ml with significance figure at p<0.05. The digestion of Jasmine rice had not difference from the heated mixture of enzymes (Table2).

Table 2: Antioxidant activity of all digested food samples were determined by DPPH techniques

Food Samples	%RSA[a]
Standard BHA (1 mg/ml)	89.9±0.4
The Mixture of Digestion Enzymes (Heat)	2.2±1.3
Jasmine Rice	5.0±1.1
Thai Spaghetti	6.1±1.7
Jasmine Rice with Red Curry and Chicken	20.0±1.7
Jasmine Rice with Green Curry and Chicken	23.2±1.7
Thai Spaghetti with Green Curry and Chicken	17.7±2.2
Jasmine Rice with Phanang Curry and Chicken	21.0±1.7
Jasmine Rice with Musaman Curry and Chicken	17.2±2.2

[a]% Radical Scavenging Activity (%RSA) : mean±SD, n = 3

Each herb was mixed to make curries and composed of vitamin that had antioxidant activity such as vitamin C, vitamin E, β-carotene and phytochemicals. Chili was composed of compounds that affected antioxidant activity such as

capsaicinoid, flavonoids and phenolic compounds [1, 2]. The plant family Allium such as onion, garlic had antioxidant activity by important chemicals which were allicin, diallyl disulphide, diallyl trisulphide [3]. and flavonoid compounds such as quercetin [4]. Curries were composed of herbs with high antioxidant activity and showed the property of antioxidant activity equal to the standard BHA. In this experiment the green curry had the highest antioxidant activity and there was used fresh chili instead of dried chili. The freshness of raw materials and the type of chili was affected the antioxidant activity in the curries. Whereas the musaman curry was used heat in cooking and resulted in destroy some of antioxidant activity. From the research of Gazzani *et al*., 1998 [5] showed the heat of garlic and onion at 102 ºC for 30 minutes did not destroy all of the antioxidant activity. In addition, musaman curry was composed of various spices which has the important composition and unique for musaman curry. Therefore the antioxidant activity of these spices may replace or increase the higher efficiency of antioxidant activity than the other curries.

The curry pastes were cooked and made Jasmine rice with curries which caused difference antioxidant activity (Table 1). Some samples was decreased on antioxidant activity such as Jasmine rice with red curry and chicken and Jasmine rice/Thai spaghetti with green curry and chicken. In addition, some samples was increased the antioxidant activity such as Jasmine rice with phanang curry and chicken. There was no changed in Jasmine rice with musaman curry and chicken. Finally, the change of antioxidant activity was not decreased the ratio of curry paste. Because the effect was come from Jasmine rice or Thai spaghetti. Both Jasmine rice and Thai spaghetti had increased antioxidant activity to replace. Moreover the composition of food was to be ready to eat. There were added the compostion such as various vegetables including in curries, peanut, potato and onion in musaman curry which helped to increased antioxidant activity or the increased in value would replace the looseness of some antioxidant activities from the decreased in ratio of curries. The heat would also reduce the antioxidant activities.

The results of the simulation of gastrointestinal digestion were found the reduction a lot of antioxidant activity (Table 2). But the residues of antioxidant activity was appeared a little when compared to the only mixture of enzymes solution. This was the example of *in vitro* simulation of gastrointestinal digestion which may alter the antioxidant activity *in vivo*.

3.2 Anti-aging activity

Tyrosinase was the enzyme involved in skin Melanogenesis which resulted in skin melanin pigments and then resulted in aging. The inhibition of Tyrosinase would help for anti-aging activity. The results of water extraction of Jasmine rice, Thai spaghetti, curry pastes, and Jasmine rice/Thai spaghetti with various curries and chickens were studies. The negative anti-aging control was water and the positive anti-aging control was sodium benzoate 5 mM. Jasmine rice and Thai spaghetti did not inhibit Tyrosinase activity. The water extraction of various curries and various Jasmine rice with curries and chickens were changed with significance figure (p<0.05) on Tyrosinase inhibition. Most of all had higher than the standard sodium benzoate 5 mM except Thai spaghetti with green curry and chicken and Jasmine rice with phanang curry and chicken had lower anti-aging activity than the standard sodium benzoate, respectively (Table 3).

The water extraction of each curry was shown the highest anti-aging activity in order from green curry (42.8±1.3%) and phanang curry (38.3±6.8%). Both curries were higher than the standard sodium benzoate (27.08±0.97%) with significant figure (p<0.05). The red curry and musaman curry was shown 30.5±6.5% and 21.2±1.4%, respectively. In addition, Jasmine rice with various curry and chicken were shown in order from Jasmine rice with musaman curry and chicken(47.7±2.6%), Jasmine rice with green curry and chicken (44.6±3.1%), Jasmine rice with red curry and chicken(40.8±2.6%) had very high efficient of anti-aging activity, respectively. The sodium benzoate 5 mM had lower than the curries above with significance figure (p<0.05). Finally, the water extraction of Jasmine rice with phanang curry and chicken (17.4±4.9%), Thai spaghetti with green curry and chicken (8.9±1.4%) had lower anti-aging activity compare to the standard sodium benzoate 5 mM. (Table 3).

Table 3: Anti-aging activity after water extraction of Jasmine rice, Thai spaghetti, curries, and Jasmine rice /Thai spaghetti with curries and chicken were examined Tyrosinase activity.

Food Samples	%Tyrosinase Inhibition[a]
Water	0±0.01
Sodium Benzoate 5 mM	27.08±0.97
Jusmine Rice	-4.33±1.72
Thai Spaghetti	0.67±1.23
Red Curry	30.48±6.50
Green Curry	42.79±1.34
Phanang Curry	38.26±6.82
Musaman Curry	21.23±1.39
Jasmine Rice with Red Curry and Chicken	40.75±2.60
Jasmine Rice with Green Curry and Chicken	44.65±3.09
Thai Spaghetti with Green Curry and Chicken	8.89±1.37
Jasmine Rice with Phanang Curry and Chicken	17.37±4.94
Jasmine Rice with Musaman Curry and Chicken	47.73±2.58

[a] % Tyrosinase Inhibition : mean±SD, n=3

The simulation of gastrointestinal digestion would increase % Tyrosinase inhibition on Jasmine rice (19.2±2.9%) and Thai spaghetti (8.8±0.7%), respectively. Jasmine rice had higher than the standard sodium benzoate (12.51±1.55%) with significant figure (p<0.05). After simulation of gastrointestinal digestion, most of Jasmine rice with curries and chickens was shown in order from high to low inhibition such as Jasmine rice with red curry and chicken (19.0±1.2%), Jasmine rice with green curry and chicken (17.9±1.8%), Jasmine rice with musaman curry and chicken (13.9±1.1%), Jasmine rice with phanang curry and chicken (11.8±0.1%), respectively. Finally, Thai spaghetti with green curry and chicken (9.1±0.1%) was shown the lowest % Tyrosinase inhibition (Table 4).

Table 4: Anti-aging activity of the solution after simulation of gastrointestinal digestion of Jasmine rice, Thai spaghetti, Jasmine rice/Thai spaghetti with curries and chicken by using %Tyrosinase inhibition

Food Samples	% Tyrosinase Inhibition[a]
Water	0±0.01
Sodium Benzoate 5 mM	12.51±1.55
Jasmine Rice	19.21±2.90
Thai Spaghetti	8.79±0.73
Jasmine Rice with Red Curry and Chicken	19.04±1.24
Jasmine Rice with Green Curry and Chicken	17.89±1.85
Thai Spaghetti with Green Curry and Chicken	9.10±1.25
Jasmine Rice with Phanang Curry and Chicken	11.81±0.10
Jasmine Rice with Musaman Curry and Chicken	13.86±1.10

[a] % Tyrosinase Inhibition : mean±SD, n=3

4. Conclusion

The highest antioxidant activity was found in green curry paste and musaman curry after water extraction. The other Jasmine rice/ Thai spaghetti with various curries and chickens did not showed the high % radical scavenging activity. The simulation of gastrointestinal digestion was shown the reduction of % radical scavenging activity with all samples.

The curries were shown that green curry, phanang curry and red curry had the high to low % Tyrosinase inhibition, respectively. Jasmine rice with musaman curry and chicken, Jasmine rice with green curry and chicken, Jasmine rice with red curry and chicken were shown the high to low %Tyrosinase inhibition in order with significance figure ($p<0.05$). The simulation of gastrointestinal digestion of most all curries did not have increasing in Tyrosinase inhibition except Jasmine rice with red curry and chicken, Jasmine rice with green curry and chicken, respectively.

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