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Evaluation of functional foods properties on Thai curry soups without coconut milk by using water extraction *in Vitro* experiments

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Abstract
This study aimed to determine the functional foods properties of water extract on Thai curry soups namely Gaeng Pah, Gaeng Liang, Gaeng Som, Gaeng Leung and Tom Yam. The water extract of these curry soups were tested for 5 functional foods properties for example; Thrombosis, Fibrinolytic activity, Antioxidant activity, Anti-aging activity, and Anti-hypertension activity. The results showed that Gaeng Leung soup had highest Anti-thrombosis activity and all curry soups did not have Fibrinolytic activity. All curry soups also had very high antioxidant activity especially Tom Yum and Gaeng Som soups. Tom Yam and Gaeng Pah soups inhibited Tyrosinase activity and resulted to increase Anti-aging activity, respectively. Finally, Gaeng Liang soup showed the highest Anti-hypertension activity compared to the other curry soups.

Keywords: functional foods properties, Thai curry soups without coconut milk, thrombosis, fibrinolytic activity, antioxidant activity, anti-aging activity, anti-hypertension activity

1. Introduction
Non-communicable disease (e.g. Cardio vascular disease, Hypertension, Cancer) is one of the most concerning health problems in many countries around the world including Thailand. It is well documented that types of food consumed and the eating pattern are largely contributed to its cause. Recently, there is an idea of using food in prevention and perhaps control these diseases. The word "Functional Foods" therefore, was introduced. It means "the foods that provide a health benefit beyond the traditional nutrients it contains" the foods that provide a health benefit beyond the traditional nutrients it contains" (1). Thai food is not only well-known for the delicacy but also known to have physiologically health benefits due to its ingredients; local vegetables, herbs and spices. These ingredients are usually used in a mixed-form called 'Curry Paste'. However, there is still a lacking of scientific evidence supporting the health benefits of Thai foods or Thai food products as commonly consumed. Thus, they must reassure and convince the consumer for health benefits of Thai food. There is a need for the research to determine whether there is any functional property in these products. The information about health benefits of these Thai food products would be beneficial for opening the new market for Thai food business and industry.

2. Materials and Methods
2.1 Food samples
There are five curry pastes from one industry such as Gaeng Pah, Gaeng Liang, Gaeng Som, Gaeng Leung, and Tom Yum. Each curry pastes was prepared 300 g for each curry and make triplicate for each curry. Then each curry was kept at -20^{O}C.

2.2 The preparation of each curry soup
The soup was filtered to separate the solid waste by using centrifugation at 10,000 rpm, 4^{o}C for 10 minutes. The supernatant was kept at -20^{o}C until the analysis in the next step.

2.3 The extraction of each curry soup
The curry was weighted 2 g and put into centrifuge tube 50 ml. The ratio of food sample to water was 1:2 (w/v). The sample was homogenized at 13,500 rpm for 1 minute. The homogenate was separated by using centrifugation at 15,000 g (10,000 rpm) for 20 minutes at 4^{O}C. The supernatant was kept at 4^{o}C until the analysis was started.

2.4 Health benefits testing
2.4.1 Thrombosis
The technique was measured Thrombin Time (TT) assay for fibrin formation *in vitro* (2). This reaction between Thrombin with food sample was recorded in TT compare to time in the presence of water as control experiment by using Coagulometer (AMELUNG KCl Δ). The 2% fibrinogen 100 µl was pipetted into plastic cup with metal ball and incubated at 37^{o}C in rotating well on Coagulometer. The APTT program on Coagulometer will be counted backword from 60 second. After the time stopped and then pipette enzyme Thrombin with concentration 100 µl/ml in 0.18 M boric acid buffer pH7.5 and 0.15 $CaCl_2$ with 50µl filled in plastic cup above. Automatic pipette was connected to the machine after the solution was delivered until empty solution. The machine will be measured time automatically and stopped when metal ball was stopped motion because fibrin formation was connected. The time showed on the machine was time for fibrin formation by using Thrombin as the enzyme. This was called Thrombin Time expressed as unit in second.

2.4.2 Fibrinolytic activity
The technique was called Fibrin Plate Assay (3) by fibrin formation or blood coagulation in petri dish. The enzyme Thrombin 10 units/ml was pipetted 4 ml on petri dish and swirled as circle for equal distribution of enzyme. Then 0.6%

Fibrinogen was added 8 ml and left it until the solution showed white opaque after left it overnight at room temperature. The extract from food sample was pipetted 30 µl on Fibrin plate and shown clear zone diameter after left it 24 hours. The diameter of clear zone expressed in mm^2.

2.4.3 Antioxidant activity

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 (4). 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.

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

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

2.4.4 Anti-aging activity

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(4). 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 37ºc for 20 minutes. The absorbance was measured at 475 nm with UV-visible spectrophotometer. %Inhibition of Tyrosinase was calculated as the equation below.

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

2.4.5 Anti-hypersion activity

The hypertension was studied by using Angiotensin I-converting Enzyme (ACE) inhibition assay (3). The reaction of ACE was used substrate Hippuryl-His-Leu (HHL) to convert to Hippuric acid (HA) and His-Leu (HL). The measurement of Hippuric acid was detected by spectrophotometer at wavelength 228 nm. The inhibitor in food sample stopped the reaction of Angiotensin I-converting Enzyme and increased the anti-hypertension activity. The assay was showed in table as the following Table 1(5).

Table 1: Composition of Chemicals and /or Food sample and methods for Anti-hypertension assay

Sample or Chemicals	Volume (µl)	
	Control tube (BI)	Reaction tube (Rx)
Captopril 1µM as inhibitor/ Food extract	50	50
0.3% Hip-His-Leu peptide	200	200
1.0 M HCl	300	-
Incubate 37ºC for 5 minutes		
0.33 Unit/ml ACE	50	50
Incubate 37ºC for 15 minutes		
1.0 M HCl	-	300
Ethyl acetate for separation product	3000	3000
Vortex vigorously for 60 seconds		
Centrifuge to separate phase at 5,000 rpm for 5 minutes		
Pipette Ethyl acetate (Top phase)	2000	2000
Evaporate until dryness in waterbath 100ºC		
Dissolve with water	3000	3000
Measure Absorbance at wavelength 228 nanometer		

The calculation of ACE inhibition was used this equation.

% ACE inhibition = (Rx Control-BI Control) - (Rx Sample-BI Sample) x 100 / (Rx control – BI Control)

2.4.6 Statistic analysis

For all the experiments all assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The difference between the different extracts were analyzed using one-way analysis of variance (ANOVA) followed by $p<0.05$.

3. Results and Discussion

3.1 Thrombosis

Thrombosis was shown Thrombin Time in second which each curry soup was tested the time for Thrombin to make Fibrin in Table 2. Gaeng Leung soup was the highest Thrombin Time (TT) at 72.2±19.9 second compare to water at 28.0±2.4 second. The TT was highest time which explained "Anti-thrombosis".

Table 2: Thrombosis of Thai curry soup without coconut milk

Food sample	TT (second)
Water	28.0±2.4
Gaeng Pah soup	44.6±9.5
Gaeng Liang soup	37.2±2.2
Gaeng Som soup	51.5±9.5
Gaeng Leung	72.2±19.9
Tom Yam	44.2±1.5

mean±SD, n=3, $p<0.05$

3.2 Fibrinolytic activity

All curry soups did not have Fibrinolytic activity. That explained all curry soups without coconut milk did not have clear zone on Fibrin plate.

3.3 Antioxidant activity

Antioxidant activity of all curry soups were shown on Table 3. Tom Yam soup was the highest Antioxidant activity.

Table 3: Antioxidant activity of Thai curry soup without coconut milk

Food sample	Radical Scavenging Activity (%)
BHA 1 mg/ml (Reference standard antioxidant)	94.2±0.5
Gaeng Pah soup	73.4±10.0
Gaeng Liang soup	86.7±1.3
Gaeng Som soup	89.5±2.7
Gaeng Leung soup	83.0±5.9
Tom Yam soup	91.6±1.9

mean±SD, n=3, $p<0.05$

3.4 Anti-aging activity

Anti-aging activity was inhibited Tyrosinase activity. The higher %Tyrosinase inhibition activity was the higher Anti-aging activity in Table 4. Tom yum soup was the highest % Tyrosinase inhibition activity which was the best Anti-aging activity.

Table 4: Anti-aging activity of Thai curry soup without coconut milk

Food sample	Inhibition of Tyrosinase Activity (%)
Sodium benzoate 1 mM (Reference standard Anti-aging)	26.1±2.4
Gaeng Pah soup	54.8±4.1
Gaeng Liang soup	29.8±4.1
Gaeng Som soup	3.8±2.4
Gaeng Leung soup	33.7±6.9
Tom Yam soup	61.2±17.0

mean±SD, n=3, $p<0.05$

3.4 Anti-hypertension

Angiotensin-I converting enzyme (ACE) was the main enzyme that had effect to hypertension (Table 5). Therefore Gaeng Liang and Gaeng Leung were similar and with significant figure on %ACE inhibition activity 84.3±6.1 and 82.7±3.5, respectively,

Table 5: Anti-hypertension of Thai curry soup without coconut milk

Food sample	Angiotensin-I Converting enzyme (AEC) Inhibition (%)
Captopril 1 µM (Anti-hypertension drug as standard)	88.5±0.3
Gaeng Pah soup	52.6±3.6
Gaeng Liang soup	84.3±6.1
Gaeng Som soup	72.3±1.3
Gaeng Leung soup	82.7±3.5
Tom Yam soup	56.1±1.3

mean±SD, n=3, $p<0.05$

4. Conclusion

The experiment was significant proof that Thai curry soups without coconut milk were Functional foods for good health benefits. Anti-thrombosis, Fibrinolytic activity, Antioxidant activity, Anti-aging and Anti-hypertension were found in Thai curry soups. These results were the first scientific evaluated Functional properties of Thai curry soups without coconut milk. In 2006, our research team conducted the study on Functional properties of Thai curry pastes with coconut milk showed high potency in a particular Functional properties (2, 3, 4). Therefore these experiments were extended the study to another type of commonly consumed Thai curry without coconut milk. The information about health benefits of these Thai food products would be beneficial for opening the new market for Thai foods business and industry. The experiment had the typical results for further study bioactive compounds in all curry soups. The results showed that Gaeng Leung soup had highest Anti-thrombosis activity and all curry soups did not have Fibrinolytic activity. All curry soups also had very high antioxidant activity especially Tom Yum and Gaeng Som soups. Tom Yam and Gaeng Pah soups inhibited Tyrosinase activity and resulted to increase Anti-aging activity, respectively. Finally, Gaeng Liang soup showed the highest Anti-hypertension activity compared to the other curry soups.

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