International Journal of Food Science and Nutrition
ISSN: 2455-4898, Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 1; Issue 3; May 2016; Page No. 24-26

Evaluation of thrombosis in selected Thai curry pastes and dishes using *In Vitro* experiments

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Abstract
Fresh herbs and spices used in Thai cooking is one of the uniqueness of Thai cuisine. They are usually blended together to make a paste-like so called 'Curry Paste'. Some of them have been scientifically proved for health benefits when testing in the form of an individual extract. The objective was evaluated Thrombosis of Thai curry pastes and Jasmine rice/Thai Spaghetti with those curries. Food samples were extracted with water or digested with the simulation of gastrointestinal digestion. The solutions of treated solutions were tested for Thrombosis in *vitro* experiments. The results of water extracts from curry pastes showed the highest in anti-thrombosis. The water extract from green curry paste was different from the other curry pastes. Most of the water extracts from Jasmine rice/Thai spaghetti with curries were less potent than that of their curry pastes. After simulation of gastrointestinal digestion, most foods were reduced in anti-thrombosis.

Keywords: Curry pastes, Jasmine rice/Thai spagehetti with curry, Thrombosis, Anti-thrombosis

1. Introduction
Cardiac vascular disease is one of the most concerning health problems in many countries around the world including Thailand [1]. It is well documented that types of food consumed and the eating pattern are largely contributed 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 threat these diseases. The word 'Functional Foods' therefore, was introduced. It means "the foods that provide a health benefit beyond the traditional nutrients it contains". 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'. 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, they were to promote thai foods consumption domestically and internationally under the functional foods concept. There is a need for the research to determine Thrombosis was used Thrombin Time (TT) assay which was a measurement of the time elapsing until the fibrin formation with Coagulometer.
The expected outputs was provided an useful information on Thrombosis 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 intervals and was collected for each testing using nitrogen gas at temperature 4 ºC until the experiment was done. Each curry was divided for cooking with chicken as the representative meat in the preparation such as red chicken curry, green chicken curry, phanang chicken curry and musaman chicken curry. After that each curry was prepared each curry sample for eating as one plate meal as the following such as rice with red chicken curry, rice with green chicken curry, thai spaghetti with green chicken curry, rice with phanang chicken curry and rice with musaman chicken curry, 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 chicken curry or green chicken curry 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 Thai 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 simulated digestions 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 4 ºC. The filtrate was separated by Whatman no. 541 and collected each supernatant at 4 ºC for next experiment. The simulated digestion 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 N_aCl 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 N_aCl. 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 N_aHCO_3. The bile extract concentration 40 mg/ml in 100 mM N_aHCO_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 N_aOH and finally added 120 mM N_aCl to 100 ml. The solution was digested as in small intestine with shaking 85 rpm at 37 ºC 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 4 ºC for further experiment.

2.4 Methods for testing functional health effects

2.4.1 Thrombosis activity

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 ºC in rotating well on Coagulometer. The APTT program on Coagulometer will be counted backward 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.

3. Results and Discussion

3.1 Thrombosis

The result on food samples extraction with water showed that green curry had the highest TT with 191.9±12.3 second. The second high of red curry was 87.2±6.9 second, the third high was phanang curry and finally masaman curry was the last high 34.4±1.1 second. For Jasmine rice, Thai spaghetti alone was shown the similar TT with water. All of this result was not difference from the control at significant $p < 0.05$ (Table 1)

Table 1: The testing anti-thrombosis of the extraction with water from Jasmine rice, Thai spaghetti with various curries by Thrombin Time (TT) assay and reported as mean±SD, n=3.

Sample	TT(second)
Water	27.3±0.4
Jasmine Rice	22.6±3.4[a]
Thai Spaghetti	21.8±0.5[a]
Red Curry	87.2±6.9[*b]
Green Curry	191.9±12.3[*c]
Phanang Curry	69.4±1.7[*d]
Masaman Curry	34.4±1.1[c]
Jasmin Rice and Chicken Red Curry	21.2±2.9[f]
Jasmin Rice and Chicken Green Curry	24.0±1.5[f]
Thai Spaghetti and Chicken Green Curry	25.4±0.7[f]
Jasmin Rice and Chicken Phanang Curry	23.4±1.3[f]
Jasmin Rice and Chicken Musaman Curry	27.6±0.8[f]

[*]different from the water control at significant figure at $p<0.05$ [a,b,c,d,e,f]different alphabets showed the different significant figure in group p<0.05 Anova

Chili, garlic and onion were composed of the curry paste. Those herbs were active in Thrombosis. When the herbs were combined with the other herbs and were not the complex processes. The only one process was crunched into the homogeneous composition and the curry paste was still shown the original Thrombin time of the fresh herbs except masaman curry. The masaman curry was fried with oil under heat. Therefore the heat was destroyed or changed the active gradient of herbs and they were lost the partial usefulness. But there was consisted of the important gradient which resisted to heat [3]. The result still had Thrombosis left in masaman curry.

Although most of the curry pastes had the same main compositions such as garlic, shallot bulbs, galangal tuber, lemon grass, leech lime leaves and chili, the compositions of some gradients were difference in each unique curry and resulted in the efficient in the highest Thrombin time. Because of green curry paste composed of fresh green chili instead of red dried chili. Then the green chili in green curry paste was the main factor to make the difference in anti-thrombosis above.

Garlic had many activities involved the blood circulation of human body which included the slow of Thrombin time too [4]. The important active chemical from garlic was another factor resulted in slow Thrombin time. The garlic for making curry was the same and the proportion of garlic was closed relation in each curry. So the different of efficiency in Thrombosis was the other composition such as fresh chili.

Although the water extraction of curry showed clearly no Thrombosis, the rice with each curry was tested and there was not shown the Thrombosis. The reason was the curry with each formula and mixed with rice may dilute the amount of curry between 3-8% and showed no Thrombosis. The simulation of gastrointestinal digestion of each rice and curry was done the same as the food intake to human body and did not show Thrombosis again (Table 2).

Table 2: The test of Thrombosis on the food sample was digested with enzymes from Jasmine rice, Thai spaghetti with various curries by Thrombin Time (TT) assay and reported as mean±SD, n=3.

Sample	TT(second)
Water	23.2±1.4
Enzymes (heat)	23.5±2.2
Jasmin Rice	28.8±1.0^{*a}
Thai spaghetti	30.0±3.5^{*a}
Jasmin Rice and Chicken Red Curry	25.8±2.6^{b}
Jasmin Rice and Chicken Green Curry	24.9±1.6^{b}
Thai Spaghetti and Chicken Green Curry	28.0±1.7^{bc}
Jasmin Rice and Chicken Phanang Curry	21.8±2.6^{bc}
Jasmin Rice and Chicken Musaman Curry	22.1±2.8^{c}

Different from the water control at significant figure at $p<0.05$
^{a,b,c} different alphabets showed the different significant figure in group $p<0.05$ ANOVA

4. Conclusion

The green curry paste showed the highest thrombosis time during *in vitro* assay. The green curry has green chili to increase thrombin time. If some patients had the problem of blood cloting after meal, then the green curry was the best choice to reduce Thrombosis. The red curry was the second choice thrombin time to reduce Thrombosis. The phanang curry paste had tendency to reduce Thrombosis at the third choice. Finally, the masaman curry was the last choice to reduce Thrombosis. The simulation of gastrointestinal digestion was shown that all curry pastes dishes did not have Thrombin time changed from the water as the negative control. Therefore Thrombosis was similar to Thai curry pastes with dishes after simulation gastrointestinal digestion.

5. Acknowledgement

I wish to thank Dr. Somsri Charoenkiatkun for her kindly using Coagulometer. In addition, the financial supports from Thailand Research Fund and Numprik Nittaya Company Limitted were given the information of Thrombosis from various Thai curry pastes and dishes.

6. References

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