

Evaluation of Fibrinolytic activity 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'. These herbs and spices have been used in Thai folk remedy for centuries. Some of them have been scientifically proved for health benefits when testing in the form of an individual extract. The objective was determined Fibrinolytic activity 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 Fibrinolytic activity *in vitro* using biochemical assay. The results of water extracts from curry paste showed differences in Fibrinolytic activity. After simulation of gastrointestinal digestion, some Fibrinolytic activity of food was altered, either higher or less than their water extract.

Keywords: Curry pastes, Jasmine rice/Thai spaghetti with curry, Fibrinolytic activity

1. Introduction

Cardiac vascular disease is one 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 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 the disease. This research on Fibrinolytic activity was used Fibrin Plate Technique to observe if food sample can break the fibrin gel formed in the Petri-dish by measuring the diameter of clear zone formation. The expected output was provided an useful information on Fibrinolytic activity of selected Thai curry pastes and their curry dishes for manufacturer and general consumers.

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's interval and was collected for each testing using nitrogen gas at temperature 4 °C 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. Those formulations have been studied. 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 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 spaghetti was blended into fine pieces and collected each portion under nitrogen gas at -20 °C 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 °C. The filtrate was separated by Whatman no. 541 and collected each supernatant at 4 °C 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 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 shaken 85 rpm at temperature 37°C for one hour. Then the solution was adjusted to pH 6.0±0.1 with 1M NaHCO₃. The bile extract concentration 40 mg/ml in 100 mM NaHCO₃ was added 6 ml and the pancreatine-lipase 10 mg/ml, lipase 5 mg/ml in 100 mM NaHCO₃ 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 37 °C for 2 hours. The supernatant was centrifuged at 6,000 rpm, temperature 4 °C. 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 °C for further experiment.

2.4 Methods for testing functional health effects

2.4.1. Fibrinolytic activity

The technique was called Fibrin Plate Assay (1) 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 mm².

4. Result and Discussion

4.1 Fibrinolytic activity

The Fibrinolytic activity was performed with fibrin plate assay. The fibrin fiber was simulated on glass plate. The water extracted of sample was dropped on fibrin plate compared to control water. The control water had no effect on fibrin fiber and the water extracted curry pastes showed the three kind of curries had clear zone on the fibrin plate. (Table 1).

Table 1: Fibrinolytic activity of water extracted curry by measuring the clear zone around the sample dropped on fibrin plate.

Food sample	Clear zone(mm ²) [n]	Remark
Red Curry	49.9±11.2 [6]	Clear zone
Green Curry	95.9±12.2 [6]	Clear zone
Phanang Curry	46.8±6.6 [6]	Clear zone
Masaman Curry	-	No clear zone
Jasmine Rice	48.8±7.0 [3]	Clear zone
Thai Spaghetti	-	No clear zone
Jasmine Rice with Chicken and Red Curry	-	No clear zone
Jasmine Rice with Chicken and Green Curry	-	No clear zone
Thai Spaghetti with Chicken and Green Curry	-	No clear zone
Jasmine Rice with Chicken and Phanang Curry	-	No clear zone
Jasmine Rice with Chicken and Masaman Curry	-	No clear zone

[n] = number of clear zone on fibrin plate from the dropped sample.

The simulation of stomach and small intestine digestion was performed *in vitro* by using the mixing of enzymes. After the digestion was completed and the enzymes were stopped by heating, the clear zone still had on fibrin plate. The Jasmine rice after digestion also had clear zone and Thai spaghetti after digestion had clear zone. The curry with Jasmine rice after digestion had no clear zone except Jasmine rice with green curry and Thai spaghetti with green curry digestion. Finally, Jasmine rice with green curry and Thai spaghetti with green curry showed the bigger clear zone. (Table 2)

The report on food for Fibrinolytic activity found in fermented with salt and had a specific characteristic in Asia continent (2). This food had microorganism with enzyme production which

has property in Fibrinolytic activity such as fermented soy bean or Natto, Tofuyo, fermented fish Skipjack shikaras, soybean sauce, Kimchi, Chinese Kapi (3, 4). The main composition of Thai Kapi was salt taste and a specific flavor. The amount was between 5-8% for each curry. Therefore it was very high possibility that Thai Kapi may have microorganism producing enzyme in the curry. The most enzyme had not tolerated to heat and would destroy these properties. The masaman curry was stirred fried and lost the enzyme activity which had no Fibrinolytic activity. The five curries with Jasmine rice had no Fibrinolytic activity because there were heated for a long time. The enzyme activity was denatured and had no Fibrinolytic activity.

Table 2: Fibrinolytic activity of curry samples after simulation of stomach and small intestine digestion

Food sample	Clear zone (mm ²) [n]	Remark
Enzyme Mixtures	47.6±8.2 [9]	Clear zone
Jasmine Rice	38.6±9.3 [9]	Clear zone
Thai Spaghetti	45.5±4.9 [4]	Clear zone
Jasmine Rice with Chicken and Red Curry	-	No clear zone
Jasmine Rice with Chicken and Green Curry	55.4 [2]	Clear zone
Thai Spaghetti with Chicken and Green Curry	58.21±6.24 [4]	Clear zone
Jasmine Rice with Chicken and Phanang Curry	-	No clear zone
Jasmine Rice with Chicken and Masaman Curry	-	No clear zone

[n] = Number of clear zone on fibrin plate with the tested curry from the dropped sample.

The food digestion was used of the mixture of enzymes, pepsin and pancreatin-lipase, in simulation the same digestion in the stomach and small intestine. The enzymes was denatured with heat at 100 °C for 20 minutes and resulted to a little Fibrinolytic activity. The Jasmine rice with green curry and Thai spaghetti with green curry were extracted with water still had Fibrinolytic

activity. Whereas the other curries with Jasmine rice or Thai spaghetti had no Fibrinolytic activity. The possible explanation was the relation interaction between curry and enzyme mixtures showed the promotion and inhibition of Fibrinolytic activity as shown in Table 2.

5. Conclusion

There were three curries, green curry, red curry and phanang curry which extracted with water were shown Fibrinolytic activity from highest to lowest, respectively. In addition, Jasmine rice after water extraction was the only surprising one that had Fibrinolytic activity. The other Jasmine rice or Thai spaghetti with Thai curries and chicken after water extraction did not have Fibrinolytic activity.

The simulation of gastrointestinal digestion consisted of the mixture of enzymes which had Fibrinolytic activity. Jasmine rice was digested with the mixture of enzymes and reduced the Fibrinolytic activity from 48.8 ± 7.0 in Table 1 to 38.6 ± 9.3 mm² in Table 2. Whereas Thai spaghetti had no Fibrinolytic activity after water extraction and after simulation of gastrointestinal digestion had shown Fibrinolytic activity 45.5 ± 4.9 mm². Finally, Jasmine rice or Thai spaghetti with green curry and chicken had both high Fibrinolytic activity but lower than green curry extracted with water significantly.

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