OC-2

Antibacterial Activity of Thai Red Curry Paste in Kang-Kati (Thai Red Curry) Model on *Salmonella enterica* Typhimurium DT104b

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Abstract

Nowadays food safety becomes an international concern. Natural antibacterial is now become very interesting food safety trend. The investigation on the food itself having antibacterial activity becomes more dynamic. *Salmonella* sp. has been reported its outbreaks recently in different variety of food. Kang-Kati (Thai red curry) is a Thai cultural dish and also worldwide well-known menu. The Thai red curry paste, Thai red curry's main ingredients, compose of many herbs including *Capsicum annuum* (Red chili), *Cymbopogon citrates* (Lemongrass), *Alpinia galangal* (Galangal), *Allium ascalonicum* L (Shallot), *Allium sativum* (Garlic), *Citrus hystrix* (kaffir lime), *Cuminum cyminum* (Cumin). Therefore, this study aimed to investigate the potential of Thai red curry paste in Kang-Kati using fresh coconut milk model as natural antibacterial agent against *S. enterica* Typhimurium DT104b. Kang-Kati was prepared according to Thai homemade authentic cooking method as it has been served in Thai cuisine and was inoculated with 1% culture. Thai curry paste in-vitro antibacterial activity was evaluated by cell count serial dilution method on SS media every hour for 6 hrs at room temperature. The result showed that the level of *S. enterica* Typhimurium DT104b in Kang-Kati was significant lower than of positive control (NB) ($P<0.05$) in 6th hour; 8.32±0.04, 8.02±0.08 log CFU/ml, respectively. The t-test has been done by using SPSS on log CFU/ml with $P < 0.05$. It is indicated that Thai red curry paste in Kang-Kati model showed promising combinated antibacterial activity against food-borne pathogenic bacteria, *S. enterica* Typhimurium DT104b.

Keywords: Antibacterial, Salmonella Typhimurium DT104b, Thai Red Curry Paste, Kang-Kati

Introduction

Food is one of many things that affected by globalization era. Therefore, many traditional food of one country can be accessed in many places. One of the traditional foods that popular to consume all around the world is Thai food which is have signature spicy flavor. Thai curry paste is a traditional ingredient used in cooking Thai red curry (Kang-Kati). Kang-Kati is a Thai traditional food that can be found in almost every parts of Thailand. The ingredients to making Thai curry paste are of *Capsium annuum* (red chili), *Cymbopogon citrates* (lemongrass), *Alpinia galangal* (galangal), *Allium ascalonicum* L (shallot), *Allium* *sativum* (garlic), *Citrus hystrix* (kaffir lime), and *Cuminum cyminum* (cumin).

Those herbs have been used since ancient time for flavoring food and beverages, and also for medicinal purposes to cure and prevent diseases. Many herbs are containing antimicrobial properties to prevent microbial growth in their own body. So, the extract herbs either as standardized extracts or as a source of pure compounds can be used for controlling of microbial growth and their chemical diversity (Negi 2012).

In the modern era where food safety has begun to get more attention, a food that free from human pathogenic microorganism become the first criteria to choose food. Food and food ingredients are easily in distribution over great distances, there is increased potential for widespread impact from food and food ingredients (Sobel and Watson 2009). Therefore, food borne illness has become a major concern worldwide, since it can cause several adverse health effects, even death. Through the global incidence, foodborne diseases are difficult to estimate. WHO reports that in the year 2005, 1.8 million people died from diarrheal diseases, those were mainly caused by contaminated food and drinking water (WHO 2006).

Nowadays, food safety concerns have been focused on pathogenic microorganisms, such as *Salmonella.sp* which is recognized as one of the major causes of foodborne bacterial diseases especially diarrheal diseases. The problems of human salmonellosis following consumption of contaminated foods and drinking water have increased worldwide. *S. enterica* is a common cause of human gastroenteritis and bacteremia worldwide. The number of people that are suffering from food-borne diseases in developed countries is up to 30%. In United States of America, it is estimated that 76 million cases of food-borne diseases with 25,000 peoples hospitalized and 5000 peoples died, occur each year (WHO 2006).

However, it can be seen from Thai culture that usually tend to keep foods overnight and reheat those foods to consume again in the next day without keeping in the refrigerator. And the foods are not spoiled by microorganism. This come to this project objective is to investigate the potential of Thai red curry paste in Thai curry-oil base (Kang-Kati) model acting as functional food and natural antibacterial agent against food-borne pathogenic microorganism.

Materials and Methods

Materials

Materials used in this research are the ingredients for making red curry (Kang-Kati), which are *C. annuum* (Red chili), *C. citratus* (Lemongrass), *A. sativum* (Garlic), *A.galangal* (Galangal), *A.ascalonicum* L. (Shallot), shrimp paste, *C.hystrix* (Kaffir lime), salt, and *C. cyminum* L. (Cumin); bacteria culture of *S. Typhimurium* DT104b, coconut milk, *Nutrient Broth* (NB), *Salmonella- Shigella* (SS) agar, and water.

Equipments

Equipments used in this research are knife, mortar, hot plate, micropipette, petri dish, weight balance, vortex, waterbath, microwave, incubator, rotator, spoon, autoclave, tubes, beaker glass, loop, and petri dish.

Methods

Preparation of red curry paste

The formula of red curry paste composed of 40% w/w dried red chili (*C.annuum*), 20% w/w lemon grass (*C. citrates*), 15% w/w garlic (*A. savitum*), 10% w/w galangal (*A. galangal*), 10% w/w shallot (*A. ascalonicum* L), 3% w/w shrimp paste, 1% w/w kaffir lime peel (*C. hystrix*), 0,5% w/w salt and 0,5% cumin seed (*C. cyminum*). All ingredients have been trimmed, cut and washed before putting into mechanic mortar. Firstly, chilli and salt were added and ground for 4 minutes, then, followed by garlic and shallot for 3 minutes. Galangal and lemongrass were sequentially ground for 3 minutes. Then, kaffir lime peel and cumin seed ground for 2 minutes. Finally shrimp paste was ground for 2 minutes.

Preparation of red curry (Kang-Kati)

The 45g red curry paste was weighed, and mixed with 200 ml coconut milk and heated using hot plate and stirred 5 minutes. After that, 200 ml water and 100 ml coconut milk were added into the soup. Then, it was stirred every 5 minutes for 1 hour. Cooking temperature was in the range of 90^{o}C-92^{o}C. After heated and stirred until 1 hour, red curry (Kang-Kati) was cool down to room temperature before culture inoculation.

Culture preparation

Stock culture of *S. Typhimurium* DT104b was prepared by inoculating one loopful of culture into 10 mL fresh Nutrient Broth (NB) and shaken overnight by Culture Tube Rotator. Then, 1% v/v of overnight culture was inoculated into 50 mL of fresh NB and shaken for 100 rpm, until optical density at 600 nm reach 0,02 (early log phase).

Antibacterial Assay

The 1% v/v of *S. Typhimurium* DT104b at 600 nm optical density equal to 0,02 was inoculated into 100 mL of *Nutrient Broth* (NB) as a control and 100 mL of red curry soup (Kang-Kati) in comparison at room temperature. The cell count serial dilution method was used to evaluate antibacterial activity, using *Salmonella-Sighella* (SS) agar as selective media. Both control and Kang-Kati samples were taken before inoculation as zero hour and every 1 hour after inoculation up to 6 hour at room temperature. The colony forming unit was counted after 24 hours.

Statistical analysis

The experiment was performed in duplicate and repeated in three replications independently. The experiment was performed in duplicate and three times. The independent two-sample t-test was used to study the effect of the antibacterial from the curry paste on the growth of *S. enterica 4,5,12:i:*-(human), at different time by using SPSS program.

Results

The results from statistical program showed that the levels of *S. Typhimurium* DT104b in Kang-Kati was significant lower than those of positive control (NB) at 6th hour. The t-test has been done by using SPSS on log CFU/mL with $P < 0,05$. The mean and standard deviation of Log 10^6 CFU/mL of *S.Typhimurium* DT104b in Kang-Kati and in control (NB) showed in Table 1.

Table 1. Mean and SD of *Salmonella Typhimurium* DT104b growth in Kang-Kati and Control (NB) up to six hour

Hour(s)	Log 10^6 CFU/mL in Control (NB)	Log 10^6 CFU/ml in Kang-Kati
0	5,64±0,170[a]	5,35±0,219[a]
1	6,04±0,191[a]	5,93±0,156[a]
2	6,16±0,141[a]	6,04±0,163[a]
3	6,61±0,502[a]	6,21±0,085[a]
4	6,91±0,643[a]	6,75±0,672[a]
5	7,82±0,587[a]	7,58±0,410[a]
6	8,32±0,042[a]	8,02±0,085[b]

* Remark: Different superscript within in a row show significant different (P < 0,05)

Growth of *Salmonella Typhimurium* DT104b was monitored at room temperature for 6 hours in Kang-Kati and in *Nutrient Broth* (NB) for control. The 1% v/v of *Salmonella Typhimurium* DT104b was inoculated into Kang-Kati and NB. The results showed in Figure 1.

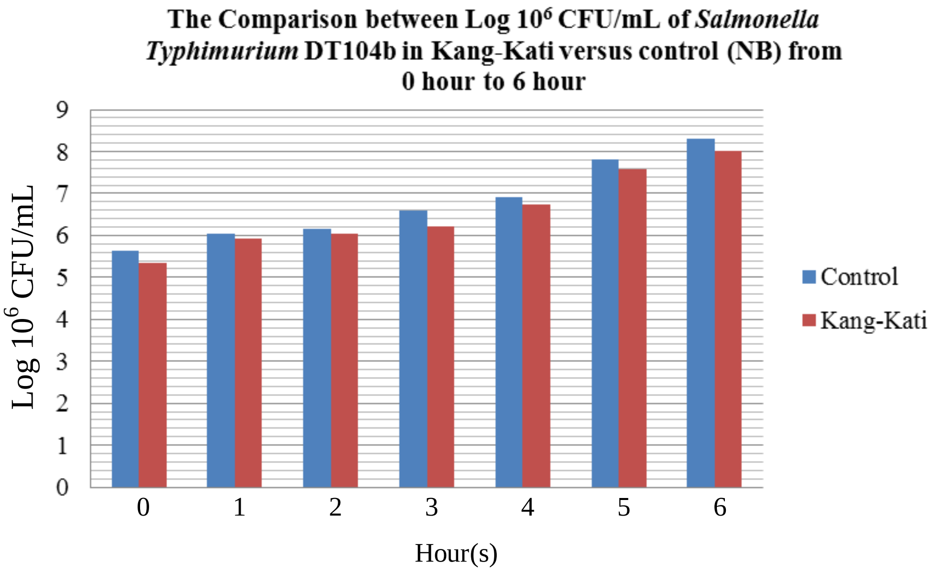

Figure 1. The histogram chart of *Salmonella Typhimurium* DT104b (log CFU/mL) between in Kang-Kati and in control (NB) from 0 to 6 hours.

Discussion

From Table 1. and Figure 1, those are shown that Kang-Kati have the potential antimicrobial activity to prevent growth level of *S. Typhimurium* DT104b in every hour. The significantly different between control (NB) and Kang-Kati is shown only in 6th hour;

8,32±0,04, 8,02±0,08 log CFU/mL, respectively. The red curry paste itself consist of *C. annuum* (Red chili), *C. citratus* (Lemongrass), *A. sativum* (Garlic), *A. galangal* (Galangal), *A.ascalonicum* L. (Shallot), *C. hystrix* (Kaffir lime), and *C. cyminum* L. (Cumin) which are those ingredients have antibacterial compounds. (Arora & Kaur 1999; Shelef 1983).

Red chilli contains capsaicin which is homovanillic acid derivative (8-methyl-N-vanillyl-6-moneamide) (McElroy and others 1994). Capsaicin is an irritant and vasoactive component from chili pepper (*C. annuum*). Although neither correlative nor causative investigations of enteric pathogen resistance and these capsaicin induced changes have been reported (McElroy and others 1994), capsaicin clearly has a number of known actions on enteric innervation and function in mammals. Previous studies with different levels of dietary capsaicin, either natural or synthetic, has demonstrated reductions in *S. enteritidis* organ invasion with no adverse effects on body weight and feed consumption on 11, 16 or 19 day old broilers and leghorn chickens (McElroy and others 1994; Tellez and others 1993).

Lemongrass (*C. citrates*), a perennial herb, is widely planted throughout the warm tropical climates of the world. An infusion of this plant is used to induce sleep, loosen and reduce mucus, and treat fevers, cramps, and stress. The essential oil from lemongrass is used as food flavoring, and an ingredient in cosmetics and perfumes. Inhibitory activities of lemongrass oil against *B.* subtilis, *S. aureus*, *E. coli*, *Colletotrichum gloeosporioides* S. *enteridis* KCCM 12021, *S. enteridis* CCARM 8010, *S. enteridis* CCARM 8011, *S. typhimurium* KCCM 11862, *S. typhimurium* CCARM 8007, and *S. typhimurium* CCARM 8009 and insects like mosquito have been reported (Shin 2005).

Galangal (*A. galangal*) is indigenous spices from Southeast Asia e.g. Indonesia. The essential oils and extracts from galangal rhizomes have been studied and have been proven to exhibit antifungal, antigiardial, antiamoebic, antimicrobial, and antioxidant activities. The essential oils from both fresh and dried rhizomes of galangal have antibacterial activities against bacteria, fungi, yeast and parasite (Farnsworth and Bunyapraphatsara 1992).

Garlic (*A. sativum*) and Shallot (*A. ascalonicum* L) contains many biological activities including antimicrobial, antioxidant, anticarcinogenic, immune-modulatory and prebiotic action (Corzo-Martinez and others 2007). The biological and medical effects of garlic and shallot are mainly due to their high content of organosulphur compounds, such as alliin and allicin and their breakdown products. Flavonoids, abundant in shallot but practically absent in garlic, are also responsible for a great part of the health benefits of both vegetables (Corzo-Martinez and others 2007). Garlic has been proven to inhibit the growth of gram positive and gram-negative bacteria including strains of *Pseudomonas*, *Proteus*, *Escherichia*, *Staphylococcus*, *Salmonella* (Iwalokun and others 2004). Whereas shallot, unlike garlic, was not effective against gram-negative bacteria (Corzo-Martinez and others 2007).

Kaffir lime or leech lime (*C. hystrix*) has been used extensively in traditional Thai medicine. Fruit peel of kaffir lime helps relieve stomach pain, and possess antifungal, antiparasitic, antiviral, anticarcinogenic *in vivo* (V. Srisukh and others 2012). Cumin (*C.cyminum*) has a large antibiotic spectrum against both gram-positive and gram negative bacteria. Cumin is an aromatic plant that included in the *Apiaceae* family. It is usually used for flavoring foods, medical preparations, food industries and added to fragrances. Cumin is also used as antispasmodic, carminative, and appetite stimulant agent. Furthermore, cumin oil shows a high antifungal activity against various pathogenic fungi. It is also used as a fumigant or additive in the storage of foodstuff (Sheikh 2009).

Besides from red curry paste, the antibacterial activity of Kang-Kati might come from the coconut milk. Generally, coconut milk has antimicrobial activity because of the compound content in the coconut milk itself. The compound contents of coconut milk are medium chain fatty acid, such as lauric acid, caproic acid, and caprylic acid, which are have powerful antimicrobial properties. For example, lauric acid has been proven to inhibit growth of *Chlamydia trachomatis, Helicobacter pylori, Neisseria gonorrhoeae* and *Candida albicans* effectively (Sia and others 2010).

From works of Ikigai and others (1993) and Otake and others (1991), they say that the antimicrobial activity of plant in form of extract is most likely due to the combined effects of adsorption of polyphenols to bacterial membranes with membrane disruption and subsequent leakage of cellular contents. Herbs and spices also rich in phenolic compounds and besides exerting antimicrobial effect they can preserve the foods by reducing lipid oxidation as they are reported to have antioxidant activity (Scwarz and others 2001; Shahidi and others 1997; Shan and others 2009; Tanabe and others 2002; Yanishileva and others 2006). From above mentioned properties, the major targets for those antimicrobials could be food poisoning microorganism and spoilage microorganism. From the previous mentioned, the ingredients use in making red curry paste using Kang-Kati food model show promising antimicrobial activity. The result from this experiment showed that when cooked herbs and spices using food model, the spices and herbs still have antimicrobial properties. The different in the growth of *S. Typhimurium* DT104b between Kang-Kati and control (NB) showed significantly different in growth level.

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