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Natural Antibacterial Activity of Thai Red Curry Paste in Coconut Milk based Curry; Kang-Kati, Model on *Salmonella* sp. and *Listeria monocytogenes*

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Abstract

Since 2006, *Salmonella* sp. and *Listeria monocytogenes* outbreaks have occurred frequently in a variety food types all over the world. Thai red curry paste is composed of 7 herbs which have been investigated for their antimicrobial activity in different independent laboratories. The investigation aimed to study the antibacterial activity of Thai red curry paste in a coconut milk based curry; Kang-Kati, as a real food model against *S. enteric* Enteritidis (human) and *L. monocytogenes* 10403S. The standard plate count method as CFU/ml was used to evaluate the Thai red curry paste's *in vitro* antibacterial activity every hour for 6 h at room temperature. The Thai red curry paste was extracted according to the traditional Thai home cooking as Kang-Kati. The log CFU/ml of *S. enteric* Enteritidis (human) level was significantly lower ($P < 0.05$) in the Kang-Kati model than in nutrient broth (NB) as control at only 3rd and 4th h; 3rd h; 5.53±0.027 and 5.65±0.019, and at 4th h; 5.62±0.07 and 5.80±0.03 log CFU/ml, respectively. While the log CFU/ml of the *L. monocytogenes* level was also significantly lower ($P < 0.05$) in Kang-Kati than in NB at 3rd and 4th h; 3rd h; 5.49±0.01 and 5.61±0.02, and at 4th h 5.63±0.02 and 5.70±0.04 for log CFU/ml, respectively. The Thai red curry paste in Kang-Kati as a real food model showed promising natural antibacterial activity against the food borne pathogens, *enteric* Enteritidis (human) and *L. monocytogenes* 104003S.

Keywords: Food natural antibacterial, Thai curry paste, herbs, *Salmonella enterica* Enteritidis, *Listeria monocytogenes*

Introduction

Thai curry paste is a gorgeous aromatic mixture of freshly ground spices and herbs. Thai curry paste has a key role in Thai foods because it is the main component in the curry. The basic authentic Thai red curry paste's ingredients are *Capsicum annuum* (chili), *Citrus hystrix* (Kaffir lime), *Cuminum cyminum* L. (Cumin), *Allium ascalonicum* L. (Shallot), *Allium sativum* (Garlic), *Cymbopogon citratus* (Lemongrass), *Alpinia galangal* (Galangal). However, the exact formulation also depends on the area and the individual.

In 2011, there was an outbreak of both *Salmonella* sp. and *Listeria monocytogenes* [1]. It was reported that almost 400 illnesses were due to Salmonellosis, according to a 2011 report [1]. In 2012, the reported outbreaks of *Salmonella* Bareilly in Sushi were 141 in 20 states and the district of Columbia [2]. *Salmonella* sp. can result in symptoms of diarrhea, fever, and abdominal cramps within about 12 to 72 h after infection. In addition, the symptoms will last for about a week; mostly the symptoms will fade without treatment. However, in some cases, *Salmonella* sp. infection can spread from the intestines to the bloodstream, and then to other organs and can cause death unless the person is treated punctually with antibiotics [3].

For *L. monocytogenes,* about 300 deaths occur each year [4]. Symptoms of Listeriosis are similar to mild-flu, fever, muscle aches, nausea, and diarrhea. These symptoms normally last up to a week and recovery occurs without treatment. However, in some cases *L. monocytogenes* can infect the brain of the patients and cause the symptoms of meningitis which leads to a stiff neck, headache, and fever, or altered mental status which leads to confusion, reduced mental activity and balance problems. In addition, pregnant women who are usually healthy will develop only minor symptoms, but in some pregnant females, the infection can cause miscarriage, stillbirth, premature birth, or cause infection, and death of the newborn [5,6].

The individual antibacterial activity of each Thai red curry paste's ingredients has been studied under different extraction conditions [14-30]. The individual antibacterial activity of each Thai red curry paste's ingredients namely Kang-Pa (water), Kang-Kati (fresh coconut milk), and Kang-Kati (UHT coconut milk) extraction conditions against *S. enterica* Typhimurium DT104b has been studied [37]. All previous studies [14-30,37] showed the potential antibacterial activity in the food itself. Hence food is an ideal medium for the spread of harmful agents due to the ability of food to mask the harmful agents by strong flavors, strong odors, various textures or intense colors. Food and food ingredients are widely distributed over great distances; there is increased potential for widespread impact from food and food ingredients [8]. Therefore the objective of this experiment is to investigate the combination antibacterial activity of Thai curry paste in Thai coconut milk based curry (Kang-Kati) model against *S. enteric* Enteritidis (human) and *L. monocytogenes* 10403S.

Materials and methods

Preparation of red curry paste

The red curry paste formula was 40 % w/w chili (*C. annuum*), 20 %w/w lemongrass (*C. citrates)*, 15 % w/w garlic (*A. sativum)*, 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 % of cumin powder (*C. cyminum* L), which were bought from Rama 4 local road market, Bangkok, Thailand. The raw materials were hand ground in a rock mortar. In grinding, the raw materials were added in order and time as follows; chili and salt for 5 min, garlic and shallot for 5 min, galangal and lemongrass for 4 min, kaffir lime peel and cumin powder for 2 min and shrimp paste for 4 min. The red curry paste was prepared according to an authentic homemade Thai recipe.

Preparation of Kang-Kati

The Kang-Kati was prepared according to an authentic homemade Thai recipe. The ready-to-use pasteurized coconut milk was boiled for 5 min using a hot plate (VELP SCIETIFICA, model Are2), and when the oil and water phase in the coconut milk separated then the 45 g prepared curry paste was added, and stirred for 5 min. Then, 500 ml coconut milk solution (1 coconut milk: 2 water) was added and boiling continued for 1 h. The curry was stirred every 5 min. The temperature was in the range of 90 - 92 °C.

Preparation of the culture

The stock culture was prepared by inoculating one loopful of *S. enteric* Enteritidis (human) into 50 ml fresh nutrient broth (NB) and 50 ml of Brain Heart Infusion (BHI) for *L. monocytogenes* 10403S and incubated overnight at 37 °C, 100 rpm (IKA LABORTECHNIK, model KS 501 Digital). Then 1 % v/v overnight culture was inoculated into 50 ml of fresh NB, incubated at 37 °C in a culture tube Rotator SCI (Stuart Scientific), until OD_{600} reach 0.1 as 10^6 CFU/ml (SPECTRONIC, model GENESYS 5) which is early log phase of both *S. enteric* Enteritidis (human) and *L. monocytogenes* 10403S.

Antibacterial assay

The 1 % v/v of 0.1 OD_{600} *S. enteric* Enteritidis (human) and *L. monocytogenes* 10403S were inoculated in 100 ml room temperature-Kang-Kati and NB as a positive control, then incubated at room temperature (28 - 30 °C) for 6 h with samples taken every 1 h for 6 h. The standard plate count method as CFU/ml was used to evaluate antibacterial activity by using the Salmonella- Shigella (SS) agar for *S. enteric* Enteritidis (human) and BHI agar for *L. monocytogenes* 10403S. The minus hour (–1 h = r) was done to ensure that there was no contamination before the inoculation. The colony forming unit was counted after 24 h incubation at room temperature.

Statistical analyses

The experiment was performed in duplicate and done 3 times independently. An independent 2-sample t-test to study the effect of antibacterial properties from the Kang-Kati on the growth of *S. enteric* Enteritidis (human) and *L. monocytogenes* 10403S at different times using the SAS program.

Results and discussion

The results in **Tables 1** and **2** showed that the curry paste had promising antibacterial activity.

Table 1 Log CFU/ml of *S. enteric* Enteritidis (human) growth in Kang-Kati and NB.

Time (h)	log CFU/ml	
	Kang-Kati	**NB**
–1	0	0
0	ND	ND
1	ND	ND
2	ND	ND
3	5.53±0.03*	5.65±0.02
4	5.62±0.07*	5.80±0.03
5	5.79±0.11	5.91±0.01
6	5.88±0.08	5.96±0.00

*: Significantly different ($P < 0.05$) from NB
ND: The colonies presented on the media is less than 30 colonies

Table 2 Log CFU/ml of *L. monocytogenes* 10403S growth in Kang-Kati and NB.

Time (h)	log CFU/ml	
	Kang-Kati	**NB**
-1	0	0
0	ND	ND
1	ND	ND
2	ND	ND
3	5.49±0.01*	5.61±0.02
4	5.63±0.02*	5.70±0.04
5	5.75±0.01	5.84±0.06
6	5.89±0.02	5.95±0.04

*: Significantly different ($P < 0.05$) from NB
ND: The colonies presented on the media is less than 30 colonies

The results from **Table 1** reveal that there is no significant difference in log CFU/ml of *S. enteric* Enteritidis (human) between in Kang-Kati and the nutrient broth during the 1st and the 2nd h. But, after the 3rd and 4th h the results showed that the log CFU/ml of *S. enteric* Enteritidis (human) was significantly lower ($P < 0.05$) in Kang-Kati than in nutrient broth at 5.53 ± 0.03 and 5.65 ± 0.02, and at 5.62±0.07 and 5.80 ± 0.03 log CFU/ml, respectively.

In **Table 2**, the results show that there is no significant difference in log CFU/ml of *L. monocytogenes* between Kang-Kati and in nutrient broth during the 1st and 2nd h. After the 3rd and 4th h, the results showed that there was a significant difference in log CFU/ml of *L. monocytogenes*. The *L. monocytogenes* level was significantly lower ($P < 0.05$) in Kang-Kati than in nutrient broth at the 3rd h; 5.49±0.01 and 5.61±0.02, and at the 4th h 5.63±0.02 and 5.70±0.04 log CFU/ml, respectively. The minus hour (−1 h), which is the time point before the culture inoculation, the log CFU/ml result is shown as 0, this indicated that the prepared Kang-Kati does not have any contamination.

The results showed a significant difference in log CFU/ml in only the third and the fourth hour in both *S. enterica* Enteritidis (human) and *L. monocytogenes* 10403S. This phenomenon might be due to both *S. enterica* Enteritidis (human) and *L. monocytogenes* 10403S having a longer lag phase when grown in Kang-Kati. The herbs in red curry paste might contribute in this phenomenon. However, *S. enterica* Enteritidis (human) was significantly different in log CFU/ml from the 3rd to the 6th h between Kang-Kati and nutrient broth [36]. The shifting of both bacteria growth during the second and third hour might due to the mechanism of starvation survival. The bacteria still remains in a high metabolic state while the nutrient conditions in the surrounding environment is low [31]. The change in the environment may lead the bacteria to adapt themselves and preparing for the new environment and cause the low growth [31]. Moreover, the mechanism that controls the growth and non-growth of the microorganisms is mostly encountered in the aquatic habitat [31]. It has also been shown that the changing of the growth and non-growth mode depends on the effect of transient phase and the cellular makeup from the starvation condition [31]. At the 5th and 6th h, the growth of both bacteria entered into the log phase after adapting themselves in new environment, as in the 3rd and 4th h.

Table 3 Specific growth rate of *S. enterica* Enteritidis (human) and *L. monocytogenes* 10403S growth in Kang-Kati and NB.

Microorganism	Specific Growth Rate (h^{-1})	
	Kang-Kati	NB
S. enterica Enteritidis (human)	0.46	0.55
L. monocytogenes 10403S	0.51	0.54

In **Table 3**, the specific growth rate was defined by the division of *ln*2 which is the twice increase of biomass and time of microorganisms [6]. Moreover, the *S. enterica* Enteritidis (human) specific growth rate in Kang-Kati was lower than in the nutrient broth, which is 0.46 h^{-1} and 0.55 h^{-1}, respectively. The specific growth rate of *L. monocytogenes* 10403S in Kang-Kati was lower than the control, which were 0.51 h^{-1} and 0.54 h^{-1}, respectively. The lower specific growth rate indicates that the herbs of red curry paste in Kang-Kati show a combination inhibitory activity against both *S. enterica* Enteritidis (human) and *L. monocytogenes* 10403S. The results indicate that the Thai curry paste in Kang-Kati can inhibit both gram positive bacteria, *S. enterica* Enteritidis (human) and gram negative bacteria, *L. monocytogenes* 10403S.

Coconut milk plays a very important role in making Kang-Kati and also acts as an extractant that can extract both water soluble and oil soluble compounds from the herbs in Thai curry paste. Previous studies showed that the main fatty acid found in the coconut milk is lauric acid, which is a medium chain fatty acid that can act as antiviral, antibacterial, and some yeast against *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* [9-12]. In this experiment, the coconut milk was boiled until the water phase and

the oil phase separated so the chemicals obtained from the extraction process would be both polar and non-polar substances. However, the active compounds in the extract should be identified and tested in a further experiment of both of oil and water phases.

Kaffir lime or *C. hystrix* was reported in previous experiments that the major constituents in the essential oil of kaffir lime peels are β-pinene (30.6 %), limonene (29.2 %), and sabinene (22.6 %) [13]. The former study also reported that the essential oil from *C. hystrix* can inhibit the growth of many microorganisms such as *S.* Enteritidis [14], *Staphylococcus aureus*, *B. cereus*, *L. monocytogenes*, *Saccharomyces cerevisiae* var. sake, *Aspergillus fumigatus* TISTR 3180 [15], *E. coli* TISTR 292, *Pseudomonas aeruginosa*, *Campylobacter jejuni*, and *C. perfringens* DMST 1591 with the inhibition zone ranging from 6 - 90 mm using hydrodistillation [16]. Another study also indicated that the oil extraction method provides a better antibacterial activity than the fresh extraction by manual extraction and squeezing [17] so that the chemical compounds in the extract are possibly active compounds from kaffir lime in the oil phase. However, the chemical analysis is required in order to understand the mechanism of the active compound from kaffir lime and should be done as a further investigation.

The main active compound of *C. annuum* or chili was previously reported that the most profuse component was capsaicin [18]. Capsaicin is a hydrophobic molecule, which has a wide range of antimicrobial activities covering *Fusarium semitectum*, *Helicobacter pylori*, *Botrytis cinerea*, and *A. niger* [19-22]. The boiling point of capsaicin is about 210 - 220 °C, so it is possible that capsaicin was extracted in the oil phase in the coconut milk. However, the amount and quality of capsaicin should be investigated in further experiments because using too much capsaicin may lead to adverse effects.

The major active compounds in *C. cyminum* L. or cumin oil were identified and reported as cuminaldehyde (20 - 72 %) and monoterpene hydrocarbons (e.g. β-pinene, γ-terpinene, p-cymene), which show inhibition of about 20 serotypes of *Salmonella* sp. with inhibition zones ranging from 8 - 10 mm [14]. In this experiment, the oil phase extraction in coconut milk might be a source of cuminaldehyde, however, further investigation is required to improve the understanding about extraction from cumin.

The main active compound of *A. ascalonicum* L. or shallot was investigated and reported as flavanols and phenolic compounds [23]. Moreover, they also have wide range of antimicrobial activities over bacterial and fungal such as *Syncephalastrum* sp., *A. niger*, *Penicillium* sp., *Paecilomyces* sp., *Scopulariopsis* sp., *B. cereus*, *E. coli* O157:H7, and *S. enterica* [24,25]. The group of sulfide compounds in shallots such as diallyl monosulfide, diallyl disulfide, diallyl trisulfide, and diallyl tetrasulfide, showed promise as an antimicrobial agent [24].

Allicin is a chemical component from *A. sativum* or garlic. It was previously identified and reported as an antibacterial and antifungal agent [26]. Allicin was proved to inhibit the growth of *E. coli*, *Shigella* sp., *Salmonella* sp. and *Proteus mirabilis* [27] by partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target [26]. However, allicin is not active unless the barriers of the enzyme alliin alkyl-sulfenate-lyase (EC 4.4.1.4) and the non-protein amino acid S-allylcysteine S-oxide (alliin) are broken-down [26]. The effects of temperature on garlic crude aqueous extract activity showed that the optimum temperature was 80 °C and the antimicrobial activity might decrease or be the same when the temperature is increased [26]. The temperature used for cooking Kang-Kati in this experiment is about 90 °C so it might decrease the antibacterial activity of garlic under aqueous extraction.

For *C. citratus* or lemongrass, it was reported that it can inhibit the growth of *Salmonella* sp. 17 serotypes with an inhibition zone ranging from 7 - 11 mm using ethanolic extraction [14]. The previous study also reported that the oil extraction method provided better antibacterial activity than the fresh extraction by manual extraction and squeezing [14]. So this confirmed the result in this experiment that the oil extraction in coconut milk might extract antibacterial compounds of lemongrass.

The major compound of *A. galangal* or galangal is terpinen-4-ol [27]. The active compound in *A. galangal* is more effective when it is extracted with a non-polar extractant, compared between hexane and water, which means that most active compounds are non-polar [29]. In addition it can inhibit the growth of many fungi and bacteria such as *S. typhimurium*, *S. aureus*, *Fusarium solani* KACC 40384, and

Botrytis. Cinerea KCTC 6973 [30]. The oil phase in coconut milk might give higher antibacterial compounds than aqueous extraction of food-borne pathogens.

The antimicrobial activity of herbs might come from the combined effects of adsorption of polyphenols to bacterial membranes with membrane disruption and subsequent leakage of cellular contents [32,33]. The mechanism of natural preservatives is not fully understood, however, membrane disruption by terpenoids and phenolics; metal chelation by phenols and flavonoids; and effects on genetic material by coumarin and alkaloids are thought to inhibit growth of microorganisms [34]. It was observed that membrane-disrupting compounds can also cause leakage of cellular content, interference with active transport or metabolic enzymes, or dissipate cellular energy in ATP form [35] thus subsequently resulting in microbial death or injury.

Conclusions

Thai red curry paste in Kang-Kati, which was prepared by traditional cooking, showed promising natural antibacterial activity against food borne pathogens; *S. enteric* Enteritidis and *L. monocytogenes* 10403S. However, the natural antibacterial combination mechanisms of the Thai red curry need further investigation.

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