Natural Antibacterial Activity of Thai Curry Paste in Thai Red Curry (Kang-Kati) Model against *Listeria monocytogenes* 10403S

Patchanee Yasurin[1]* and Thidarat Udchachonand[2]

[1]Assumption University, Faculty of Biotechnology, Food Biotechnology Program, Bangkok, Thailand
[2]Assumption University, Faculty of Biotechnology, Agro Industry Department, Bangkok, Thailand

Abstract : In 2012, outbreak-associated strains of *Listeria monocytogenes* have been reported to CDC. Foods as natural antibiotics itself might be considered as an alternative for food safety. Thai red curry is a Thai cultural dish which has become well-known worldwide for having fresh coconut milk and red curry paste as main ingredients. The main ingredients of red curry paste include various types of herbs: *Capsicum annuum* (chili), *Citrus hystrix* (Kaffir lime), *Cuminum cyminum* L. (Cumin), *Allium ascalonicum* L. (Shallot), *Allium sativum* (Garlic), *Cymbopogon citratus* (Lemongrass), and *Alpinia galangal* (Galangal). All of the mentioned herbs have been investigated for their antibiotics activity. This project aimed to investigate the antibacterial activity of red curry paste in fresh-coconut-milk-based Thai red curry (Kang-Kati) model on *L. monocytogenes* 10403S. Thai curry paste in-vitro antibacterial activity was evaluated by standard plate count method on BHI media every hour for 6 hours at room temperature. Thai red curry was prepared by Thai homemade authentic cooking method as served in Thai cuisine. Results showed that the *L. monocytogenes* 10403S log CFU/ml level in Thai red curry (Kang-Kati) was significantly lower than in nutrient broth, as positive control, ($P < 0.05$) since 2nd - 6th hour; 2nd hr; 6.28±0.01 and 6.38±0.02, 3rd hr; 6.39±0.04 and 6.91±0.14, 4th; 6.96±0.05 and 7.24±0.10, 5th hr; 7.02±0.05 and 7.38±0.10 and 6th hr; 7.04±0.04 and 7.43±0.03 log CFU/ml, respectively. The t-test has been done by using SAS on log CFU/ml with $P < 0.05$. The Thai red curry paste in Kang-Kati model showed promising antibacterial activity against food-borne pathogenic bacteria, *L. monocytogenes* 10403S.

Keywords : *Listeria monocytogenes* 10403S, Natural Antibacterial, Thai Curry Paste, Kang-Kati

*Corresponding Author: patchaneeYsr@au.edu Tel. +662-300-4553 Ext.3797

1. Introduction

Thai food is one of the most popular foods consumed all around the world due to the signature spicy flavors. Thai red curry paste is a traditional condiment used in making red curry coconut base (Kang-Kati). Kang-Kati can be found commonly in almost every parts of Thailand. In general, the ingredients used in the paste are *Capsicum annum* (Red chili), *Cymbopogon citrates* (Lemongrass), *Alpinia galangal* (Galangal), *Allium ascalonicum* L (Shallot), *Allium sativum* (Garlic), *Citrus hystrix* (kaffir lime), *Cuminum cyminum* (Cumin). Most of herbs and spices in red curry paste consist of antimicrobial agents that inhibit bacterial growth and act as natural preservative agents due to their secondary metabolism.

Thus Food is the 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 easily in distribution over great distances, there is increased potential for widespread impact from food and food ingredients [1]. Result in the outbreak found in various types of food. Though *Listeria monocytogenes* isn't one of the most commonly found foodborne pathogens, the mortality rate associate with *L. monocytogenes* is very high [2]. The elderly, people with compromised immune systems, pregnant women, children and infants are most at risk of serious illness from foodborne [2].

However, it can be seen from the Thai culture that we tend to keep our foods overnight, reheat them and consume again in the next day. Also from the old times, we didn't have refrigerator. We kept our foods in storage cabinet and the food still not spoiled. This comes to this research objectives is to investigate the potential of Thai curry paste in Thai red curry-coconut base (Kang-Kati) model acting as functional food and natural antibacterial agent against *L. monocytogenes* 10403S, foodborne pathogen.

2. Materials and Methods

2.1 Preparation of red curry paste

The curry paste formula were 40% w/w chilli (*C. annuum*), 20% w/w lemon grass (*C. citrates*), 15% w/w garlic (*A. sativum*), 10% w/w galangal (*A. galangal*), 10% w/w shallot (*A. ascalonicum* L), 2% w/w shrimp paste, 1% w/w kaffir lime peel (*C. hystrix*), 0.5% w/w salt, and 1.5% of cumin powder (*C. cyminum* L). The raw materials were hand grinded by the mortar, approximately 100 rpm. In grinding, the raw materials were added in order and time as following; chili and salt for 10 min, garlic and shallot 5 min, galangal and lemongrass for 5 min, kaffir lime peel and cumin powder for 3 min, shrimp paste 2 min, and continue grinding for 5 minutes. Table 1 List of Thai herbs used in this experiment

Table 1: List of Thai herbs used in this experiment

Scientific name	Common name	Used part
Capsicum annuum	Chili	Fruit
Citrus hystrix	Kaffir lime	Peel
Cuminum cyminum L.	Cumin	Seed
Allium ascalonicum L.	Shallot	Tuber
Allium sativum	Garlic	Tuber
Cymbopogon citratus	Lemongrass	Stem
Alpinia galangal	Galangal	Tuber

2.2 Preparation of red curry (Kang-Kati)

The fresh coconut milk was prepared by weight 1 coconut: 1 water; mixed it together, soaked for 5 minutes and separated coconut part out of coconut milk by squeezing. The prepared fresh coconut milk was boiled for 5 minutes using hot plate (VELP SCIETIFICA, model Are2) as heater. At the step, oil and water phase in coconut milk was separated then the 45 grams prepared curry paste was added, and stirred for 5 minutes. Then, coconut milk solution (1 coconut milk: 2 water) was added and continue boiling until 1 hr. The curry was stirred every 5 minutes. The temperature is in the range of 90-92 °C.

2.3 Preparation of the culture

The stock culture was prepared by inoculating one loopful of *L. monocytogenes* 10403S into 50 ml fresh NB and shake on the shaker (IKA LABORTECHNIK, model KS 501 Digital) with 100 rpm at room temperature overnight. Then 1 % v/v overnight culture was inoculated into 50 ml of fresh NB, at room temperature by Culture tube Rotator SCI (Stuart Scientific), until OD600 reach 0.1 (SPECTRONIC, model GENESYS 5) which is early log phase.

2.4 Antibacterial Assay

1% v/v of early log phase *L. monocytogenes* 10403S was inoculated in 100 ml curry inoculated curry then incubated at room temperature. The cell count serial dilution method was used to evaluate antibacterial activity by using the BHI agar. The curry was taken every hour for 6 hrs. The colony forming unit was observed after 24 hours incubation at room temperature. The control was done in the same way in NB, inoculated at room temperature, 100 rpm, and to show the real growth pattern *L. monocytogenes* 10403S.

2.5 Statistical analyses

The experiment was performed in duplicate and repeated three times. The independent two-sample t-test was used to study the effect of the antibiotic from the curry paste on the growth of *L. monocytogenes* 10403S, at different time by using SAS program.

3. Results and Discussion

From Table 2, it showed that curry paste showed promising in antibacterial activity.

Table 2: CFU count of *L. monocytogenes* 10403S growth in curry and control (Nutrient Broth)

Time (Hours)	log CFU/ml	
	Kang-Kati	Control
0	ND	ND
1	ND	ND
2*	6.28±0.01	6.38±0.02
3*	6.39±0.04	6.91±0.14
4*	6.96±0.05	7.24±0.10
5*	7.02±0.05	7.38±0.10
6*	7.04±0.04	7.43±0.03

*: there is significantly different ($P < 0.05$) ND: less than 30 colonies

It showed that there is no significantly different of *L. monocytogenes* 10403S level ($P>0.05$) between curry and control (NB) only at first hours. However, since the third hour to the sixth hour, the result showed that there is significantly different of *L. monocytogenes* 10403S level ($P>0.05$) between curry comparing with control (NB) since 2nd – 6th hour; 3rdhr; 2nd hr; 6.28±0.01 and 6.38±0.02, 3rd hr; 6.39±0.04 and 6.91±0.14, 4th; 6.96±0.05 and 7.24±0.10, 5th hr; 7.02±0.05 and 7.38±0.10 and 6th hr; 7.04±0.04 and 7.43±0.03 log CFU/ml, respectively.

In Table 3, the specific growth rate in curry was less than control, which is 1.06 $hour^{-1}$ and 1.33 $hour^{-1}$ respectively. The reducing of specific growth rate indicated that curry paste inhibited *L. monocytogenes* 10403S.

Table 3: Specific growth rate of *L. monocytogenes* 10403S growth in curry and control (Nutrient Broth)

Specific Growth Rate ($hour^{-1}$)	
Kang-Kati	Control
1.06	1.33

This results showed that Thai curry paste in Kang-Kati model, which was kept at room temperature (Thai traditional style, approximately 30 ℃), inhibited the growth of *L. monocytogenes* 10403S. This indicated that the curry paste showed the synergistic effect of natural antibacterial activity against *L. monocytogenes* 10403S. And the curry paste is source of natural antibacterial compounds against *L. monocytogenes* 10403S growth.

The study of red curry paste's antibacterial activity in Kang-Pa as real food model showed that the levels of *L. monocytogenes* 10403S in Kang-Pa was significant lower than of positive control (BHI) ($P < 0.05$), since 1^{st}-6^{th} hour. This indicated that Thai curry paste in Thai red curry showed promising antibacterial activity [3].

Coconut milk contain a half the medium-chain fatty acids in coconut milk compose of lauric acid, which is anti-viral, anti-bacterial, anti-microbial and anti-fungal [4]. The higher fat content means the solvent become more non-polar. These polarity properties may affect on the extraction process and cause different extraction rate for the compound on herbs, and the amount of antimicrobial compound extracted may be different among these herbs [4]. Coconut milk plays a very important role for making Kang-Kati and also acts as an extractant that extract both water soluble and oil soluble compound from the Thai curry paste. The previous studies showed that the main fatty acid that 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 E. coli, B. subtilis* and *C. albicans* [5]. In this experiment, the coconut milk was boiled until the water phase and the oil phase is separate 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 the further experiment for both of oil and water phases.

A. ascalonicum L. (Shallot) was flavanols and phenolic compounds [6]. Shallot also has antimicrobial activity; it has been reported to have a heat stable antimicrobial activity against bacteria and fungi [7]. Moreover, they also have broad spectrum against both fungal and bacterial such as *B. cereus, Escherichia coli* O157:H7, and *S. enterica* [7]. *A. sativum* (Garlic) is one of the condiments in chili paste contains allicin. It is one of the active ingredients found during crushing garlic. Allicin has variety of antimicrobial activities [8].Thus by making chili paste, mechanic mortar will be able to extract allicin out. Also it was reported that garlic was found to be effective against *L. monocytogenes* [9]. The study of 93 commercial essential oils against 20 *L. monocytogenes* strains [10]. The *C. cyminum* L. (Cumin), mostly compost of oil was cuminaldehyde (20-72%) and monoterpene hydrocarbons (e.g. β-pinene, γ-terpinene, p-cymene), which showed that they can inhibit the growth of about 20 serotypes of *Salmonella* sp. by the ethanolic extracts[11].. The cumim using fresh coconut milk extracted, 1.6 ± 0.22 cm, against *S. enterica* Typhimurium DT104b [12]. In vitro antibacterial screening results, individual cumin in Kang-Kati model using fresh coconut milk showed highest antibacterial; 0.90±0.14cm, against *S. Enterica* 4,5,12:i:- (human) US clone [13]. Kaffir lime peels (*C.hystrix*) also been used in chili paste. The use of pressurized hot water extraction on kaffir lime fruit peel and found out that when increase temperature in extraction the

phenolic compound content increasing [14]. The use of Kang-Pa cooking model in heating chili paste might extract the phenolic compound content in kaffir lime peel out. For *C. citratus* or lemongrass, it was reported that it can inhibit the growth of about 17 serotypes of Salmonella sp. with inhibition zone range of 7-11 mm. by the ethanolic extracts [11]. The previous study also reported that the oil extraction method provide a better antibacterial activity than the fresh extraction by manual extractor and squeezing [15] so that the oil phase of the extract might be a source of antibacterial compounds in lemongrass extracted. The rhizome part of galangal (*A. galangal*)l has been used in making chili paste. The essential oils from both fresh and dried rhizomes of galangal have antimicrobial activities against bacteria, fungi, yeast and parasite [16]. The *C. annuum* (chili) which was investigated that the main chemical component was capsaicin. Capsaicin is a hydrophobic molecule with boiling point of 210-220°C [17-18]. It has also been show that capsaicin species and also capsaicin have antimicrobial effects with broadly activities on both bacterial and fungal such as *Fusarium* [19], *Helicobacter pylori* [20]. The Capsaicin might be a possible antibacterial agent against *S. enterica* 4,5,12:i:-(human) in this experiment.

4. Conclusion

The curry paste in Kang-Kati model (as real food model) showed natural antimicrobial combination effect against *L. monocytogenes* 10403S. This might be another explanation as food safety aspect that why Kang-Kati was kept in food cabinet at room temperature without spoilage.

5. References

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