KKU Res. J. 2013; 18(4): 559-566
http : //resjournal.kku.ac.th

Natural Antibacterial Activity of Thai Curry Paste in Thai Red Curry-Water Base (Kang-Pa) Model *on Salmonella enterica* 4, 5, 12: i: - (human) and *Salmonella enteric* Enteritidis (human)

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Abstract

Natural antibacterial is now a very interesting food safety trend. The investigation on the food having antibacterial activity itself, as functional food, becomes more dynamic. *Salmonella* sp. is a food pathogen which has been reported its outbreaks frequently in variety of foods. Thai red curry (Kang-Pa) is a Thai cultural dish and become worldwide well-known menu. The Thai curry paste, Thai red curry main ingredients, compose of seven 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). This study aimed to investigate the potential of Thai curry paste in Thai red curry-water base (Kang-Pa) model as natural antibacterial agent against S. *enterica* 4, 5, 12: i - (human) US clone and *S. enteric* Enteritidis (Human). Thai curry paste *in-vitro* antibacterial activity was evaluated by standard plate count method on SS media every hour for 6 hrs at room temperature. Thai red curry was prepared according to Thai homemade authentic cooking method as it has been served in Thai cuisine. The result showed that the log CFU/ml of *S. enterica* 4, 5, 12: i - (human) US clone in Kang-Pa was significantly lower than in nutrient broth, as positive control, ($P < 0.05$) since 2^{nd} - 6^{th} hour: 2^{nd} hr; 5.14±0.06 and 5.44±0.17, 3^{rd}hr; 5.86±0.19 and 6.76±0.28, 4^{th}; 5.85±0.16 and 6.97±0.6, 5^{th} hr; 5.92±0.22 and 6.26±0.27 and 6^{th} hr; 6.88±0.04 and 7.51±0.20 log CFU/ml, respectively. While the log CFU/ml of *S. enteric* Enteritidis in Kang-Pa was significant lower than those of positive control (NB) ($P<0.05$) only at 2^{nd} and 3^{rd} hour: 2^{nd} hr: 5.705±0.199 and 6.370±0.085 log CFU/ml, at 3^{rd} hr: 5.872±0.255 and 6.878±0.177 log CFU/ml, respectively. The t-test has been done by using SAS on log CFU/ml with $P < 0.05$. Thai curry paste in Kang-Pa model showed promising antibacterial activity against food-borne pathogenic bacteria, *S. enterica* 4, 5, 12: i - (human) and *S. enteric* Enteritidis (human).

Keywords: Natural Antibacterial, Thai Red Curry Paste, Kang-Pa, *Salmonella enterica* 4, 5, 12: i, *Salmonella enteric* Enteritidis

1. Introduction

Thai food is one of the most popular foods consumed all around the world due to the signature spicy flavors. Thai curry paste or red curry paste is a traditional condiment used in making red curry (Kang-Pa). Kang-Pa 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). Those herbs have also been used since ancient time for flavoring foods and beverages, and for medicinal purposes with varying success to cure and prevent diseases. Herbs contain innumerable constituents and are valuable sources of new and biological active molecules possessing antimicrobial properties (1). The extracts from plants either as standardized extracts or as a source of pure compounds provide unlimited opportunities for control of microbial growth owing to their chemical diversity (1). Many of them possess antimicrobial activity against a range of bacteria, yeast and mold however the variation in quality and quantity of their bioactive constituents is the major detriments in their food usage (1).

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 (2), resulting in the outbreak found in various types of food. One of the microorganisms that frequently cause the outbreak is *Salmonella*. Thus from Centers for Diseases Control and Prevention Reports of Selected *Salmonella* Outbreak Investigations in year 2012, showed wide sources of *Salmonella* e.g. peanut butter, hedgehogs, mangoes, cantaloupe, ground beef, live poultry, dry dog food, raw scraped ground Tuna product, small turtles and restaurant chain A (3).

From that wide sources of Salmonella resulted in the outbreak, some of them are food ingredients that have been used in many dishes. For the purpose of this experiment is to investigate the potential of Thai curry paste in Thai red curry-water base (Kang-Pa) model acting as functional food and natural antibacterial agent against foodborne pathogen. Thus that *Salmonella* is an interesting food-borne pathogenic bacteria for this investigation.

2. Materials and Methods

2.1 Preparation of red curry paste

The formula of red curry paste composes of 40% w/w dried red chili (*Capsicum annuum*), 20% w/w lemon grass (*Cymbopogon citrates*), 15% w/w garlic (*Allium sativum*), 10% w/w galangal (*Alpinia galangal*), 10% w/w shallot (*Allium ascalonicum* L), 3% w/w shrimp paste, 1% w/w kaffir lime peel (*Citrus hystrix*), 0.5% w/w salt and 0.5% cumin seed (*Cuminum cyminum*) . The ingredients were bought from the market near Pattanakarn, Bangkok, Thailand. All ingredients have been trimmed, cut and washed before putting into mechanic mortar. Firstly, chili 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 were ground for 2 minutes. Finally shrimp paste was ground for 2 minutes.

2.2 Preparation of red curry (Kang-Pa)

The 45g red curry paste were weighed, mixed with 500 mL of water and heated using hot plate (VELP SCIETIFICA, model Are2). Then, it was stirred every 5 minutes for 1 hour. Cooking temperature was in the range of 90-92 ˚C. Then red curry (Kang-Pa) was cool down to room temperature before culture inoculation.

2.3 Culture preparation

Biotechnology Faculty, Assumption University's Stock culture of *S. enterica* 4, 5, 12: i: - (human) US clone and *S. enteric* Enteritidis (Human) were prepared by inoculating one loopful of culture into 10 mL fresh Nutrient Broth (NB) and shake overnight by Culture tube Rotator SCI (Stuart Scientific). Then 1% v/v of overnight culture was inoculated into 50 mL of fresh NB and shake for 100 rpm, until optical density at 600 nm reach 0.1 (SPECTRONIC, model GENESYS 5), approximately equal to 2×10^8 CFU/ml.

2.4 Antibacterial Assay

The 1% v/v of *S. enterica* 4, 5, 12: i: - (human) US clone and *S. enteric* Enteritidis (Human) at 600 nm optical density equaled to 0.1, approximately equal to 2×10^8 CFU/ml, was inoculated into 100 mL of fresh NB as positive control and 100 mL of red curry soup (Kang-Pa) in comparison at room temperature. The cell count by using standard plate count method was used to monitoring on the number of cells (CFU/ml) as the evaluation of antibacterial activity, using Salmonella shigella (SS) agar as selective media. Both control and Kang-Pa 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.

2.5 Statistical analysis

The experiment was performed in duplicate and done in three replications independently. Using independent two-sample t-test to study the effect of antibacterial properties from the red curry (Kang-Pa) on the growth *S. enterica* 4, 5, 12: i: - (human) US clone and *S. enteric* Enteritidis (Human), at different time by SAS program.

3. Results and Discussion

Growth of *S. enterica* 4, 5, 12: i and *S. enteric* Enteritidis (Human) were monitored at room temperature for 6 hours in Kang-Pa as real food model and in nutrient broth (NB) as positive control. The 1% v/v of *S. enterica* 4, 5, 12: i and *S. enteric* Enteritidis (Human) were inoculated into both Kang-Pa and NB and incubated at room temperature for 6 hours.

The results from table 1 showed that the log CFU/ml of *S. enterica* 4, 5, 12: i in Kang-Pa was significantly lower than in nutrient broth, as positive control, ($P < 0.05$) since 2nd - 6th hour. The log CFU/ml of *S. enterica* 4, 5, 12: i in Kang-Pa and NB as positive control at 3rd, 4th, and 6th hour are different approximately 1 log. The results from table 2 showed that the log CFU/ml of *S. enteric* Enteritidis (Human) in Kang-Pa was significantly lower than in nutrient broth, positive control, ($P < 0.05$) at only 2nd and 3rd hour. The log CFU/ml of *S. enteric* Enteritidis (Human) in Kang-Pa and NB at 3rd is different approximately 1 log. Kang-Pa showed potentiality antimicrobial effects on from 2nd hour to 6th hour *S. enterica* 4, 5, 12: I and 2nd to 3rd hour on *S. enteric* Enteritidis (Human). This indicted that the red curry paste, which its ingredients are different herbs, is a source of natural antibacterial substance.

Table 1: Log CFU/ml of *S. enterica* 4, 5, 12: i growth in Kang-Pa and Control (NB) up to six hour

Hour	Kang-Pa	Control (NB)
0	ND	ND
1	ND	ND
2	5.14±0.06 [a]	5.44±0.17 [b]
3	5.86±0.19 [a]	6.76±0.28 [b]
4	5.85±0.16 [a]	6.97±0.60 [b]
5	5.92±0.22 [a]	6.26±0.27 [b]
6	6.88±0.04 [a]	7.51±0.20 [b]

*Remark: Different superscript within a row show significant different ($P < 0.05$)

ND = Not Detectable

Table 2: Log CFU/ml of *S. enteric* Enteritidis growth in Kang-Pa and Control (NB) up to six hour

Hour	Kang-Pa	Control (NB)
0	ND	ND
1	5.652±0.034 [a]	5.922±0.036 [a]
2	5.705±0.199 [a]	6.370±0.085 [b]
3	5.872±0.255 [a]	6.878±0.177 [b]
4	7.168±0.047 [a]	7.239±0.144 [a]
5	7.714±0.059 [a]	7.839±0.291 [a]
6	7.669±0.415 [a]	8.075±0.100 [a]

*Remark: Different superscript within a row show significant different ($P < 0.05$)

ND = Not Detectable

In table 3, the specific growth rate of both *S. enteric* in Kang-Pa was lower than in NB as positive control. This indicate that the red curry paste in Kang-Pa have inhibitory effect on the growth of both *S. enteric.*

Table 3: The specific growth rate of *S. enterica* 4, 5, 12: I and *S. enteric* Enteritidis in Kang-Pa and Control

Microorganism	specific growth rate (hr^{-1})	
	Kang-Pa	Control
S. enterica 4, 5, 12: I	0.25	0.96
S. enteric Enteritidis	0.41	1.22

The red curry paste compose of seven herbs including *C. annuum* (Red chili), *C. citrates* (Lemongrass), *A. galangal* (Galangal), *A. ascalonicum* L (Shallot), *A. sativum* (Garlic), *C. hystrix* (kaffir lime), *C. cyminum* (Cumin). They are natural antibacterial substrates source. Garlic (*A. sativum*) has traditional dietary and medicinal applications as an antimicrobial agent, mostly due to the presence of allicin (allyl 2-propene thiosulphinate) that inhibits various thiol-dependent enzymatic systems of bacteria (4). It is one of the active ingredients found during crushing garlic. Allicin has variety of antimicrobial activities (5). Thus by making red curry paste, mechanic mortar will be able to extract allicin out. Also it had been reported the use of 5.5% v/v garlic oil and 12.5–25% v/v garlic powder to completely inhibit the growth of *S. enterica* at 37 °C (6). It was noticed 2-log reduction in *S. enteric*a concentration with 1% v/v garlic oil at 37 °C (7). In fresh garlic, found that raw garlic extract is a more effective antimicrobial agent than antibiotics currently in use; Ciprofloxacin and Ampicillin when testing on *Salmonella* sp (8). Also the effect of garlic extract is most pronounced on enteric bacterial pathogens (8). It was studied the effect of raising the temperature on the effectiveness of garlic (9). It found that the activity of garlic increased with increase in temperature up to 80 °C (9), beyond which the activity remained constant or decreased, similar to the reports presented (10). It is known that raising the temperature increases the solubility of chemical compounds (9). Thus from the founding, heating process during cooking wouldn't destroy the antimicrobial properties inside garlic. From the review of above works show possible perspective of garlic to be effective antimicrobial agent both during in chili paste making and process of making Kang-Pa.

It reported that Lemongrass essential oil inhibit the growth of *E. coli* O157:H7 and *S. enterica* ser. Enteritidis completely when the concentration of lemongrass was increased to 3µl/ml (11). It was studied crude extracts and essential oils of many herbs including crude ethanolic extract of lemongrass which was active against 17 strains of *Salmonella* sp. (7-11 mm) from total 25 strains including *S. Enteritidis* (12). Shallot as part of red curry paste ingredient also has been studied about its antimicrobial properties. It has been reported to have a heat stable antimicrobial activity against bacteria and

fungi (13). Thus by cooking, Kang-Pa didn't reduce the potential of antimicrobial agents inside shallot. The oil of shallot also has been reported to have bacteriostatic effect against *S. enterica* (14). Chili is main ingredient used to make red curry paste. Thus red chili is in Capsicum spp. and it contains capsaicin which is reported as antimicrobial agents (15, 16). There also have been studies about different levels of dietary capsaicin, either natural or synthetic on broilers and leghorn; has demonstrated reductions in *S. enteritidis* (17, 18). A rhizome part of galangal has been used in making red curry paste. The essential oils from both fresh and dried rhizomes of galangal have antimicrobial activities against bacteria, fungi, yeast and parasite (19). Kaffir lime peels also been used in red curry paste. There was the study in 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 (20). The use of Kang-Pa cooking model in heating red curry paste might extract the phenolic compound content in kaffir lime peel out. The last ingredient is cumin seed. It has been used in the treatment of mild digestive disorders as a carminative and eupeptic, as an astringent in bronco pulmonary disorders, and as a cough remedy, as well as an analgesic (21).

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 (22, 23). The mechanism of action for the antimicrobial activity of natural preservatives is not fully understood, however, membrane disruption by terpenoids and phenolics; metal chelation by phenols and flavonoids; and effect on genetic material by coumarin and alkaloids are thought to inhibit growth of microorganisms (24). 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 (25) thus that the subsequently of each action result in microbial death or injured.

The effectiveness of antimicrobial compound depends on pH of the food, type and number of contaminating microorganisms, and type and concentration of antimicrobial (1). Storage temperature may also influence the effectiveness of antimicrobial as diffusibility of compounds is related to the temperature (26). So the ability of antimicrobial properties of plants varies according to those influences. The study of real food model from that particular reason could give more information on the antimicrobial properties of plants in food environments. From above mentioned properties, the major targets for those antimicrobials could be food poisoning microorganism and spoilage microorganism. From previous mentioned, the ingredients use in making red curry paste using Kang-Pa food model show promising antimicrobial activity.

There're few studied about the results of the combined effect of plants. It was explained the ability of combined effects that the combined antimicrobials are preferred as microbial tolerance is less likely to develop against substances having more than one type of modes of action (27). It was thus necessary to check the antimicrobial activities of these spices in combinations as used in conventional cooking or salad dressing or as in Kang-Pa. Combinations like aqueous extract of cumin and fenugreek showed synergistic activity against *Proteus vulgaris* and additive effects against *Staphylococcus aureus, Bacillus cereus* and *Aspergillus niger* (28). Thus from that results the synergistic or additive effects of those plants extracts give an alternative way of using plants and giving higher effectiveness. It was studied antibacterial activity of red curry paste in Kang-Pa showed that the levels of *L. monocytogenes*

10403S in Kang-Pa was significant lower than of positive control (BHI) ($P < 0.05$), since 1st-6th hour (29). Although the combination of above spices and herbs that have been used as food not yet been investigated From the results in this project, the herbs in curry paste formula might have synergistic or additive effects against foodborne pathogen because the amount of active compounds in each herbs varying from the formula couldn't come from either one of the herbs but could be the combining of them. The effective spice-combinations may be engaged in food preservation and may lead to new choices for antimicrobial agents. However the combined effects of herbs inside curry paste should have further investigation.

Although the combination of above spices and herbs that have been used as food not yet been investigated. The results 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. enterica* 4, 5, 12: i and *S. enterica* Enteritidis between Kang-Pa and NB showed significantly different in growth level .However as can be seen from above mentioned previous results, even in the same species of *Salmonella* sp. different serotype showed different antimicrobial activity effect. Thus those, the function of the combination of herbs and spices in food-model need further investigate on the active molecular level of how antimicrobial agents react to subjected microorganism.

4. Conclusion

Thai red curry paste in Thai red curry (Kang-Pa) model showed promising antibacterial activity against food-borne pathogenic bacteria *S. enterica* 4, 5, 12: i level in Thai red curry was significantly lower than in Nutrient broth, as positive control, ($P < 0.05$) from 2nd hour to 6th hour. While in *S. enterica* Enteritidis was significantly lower than in NB, as positive control, ($P < 0.05$) in 2nd hour and 3rd hour only. Due to the different in the growth of *Salmonella* in Kang-pa showed significant number of cells in reduction of *Salmonella* sp. This might be another explanation as food safety aspect that why Kang-Pa was kept in food cabinet at room temperature without spoilage.

5. Acknowledgement

The authors would like to thank you Dr. Aussama Sontrunnarudrungsri for the statistical analysis

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