การศึกษาฤทธิ์ต้านจุลชีพของพริกแกงในแกงป่าในการยับยั้งเชื้อ *Listeria monocytogenes* 10403S

Natural Antibacterial Activity of Thai Red Curry Paste in Thai Red Curry (Kang-Pa) on *Listeria monocytogenes* 10403S

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บทคัดย่อ

สารสกัดธรรมชาติที่มีฤทธิ์ต้านจุลชีพนั้นได้รับความสนใจอย่างมากโดยเฉพาะในด้านความปลอดภัยของอาหาร การศึกษาอาหารที่มีฤทธิ์ต้านจุลชีพนั้นเป็นเรื่องที่น่าสนใจ พริกแกงแดงเป็นเครื่องปรุงหลักที่ใช้ในการทำแกงป่า โดยทั่วไปส่วนผสมของพริกแกงแดงประกอบไปด้วย พริกแห้ง กระเทียม หอมแดง ตะไคร้ ผิวมะกรูด ข่า และเมล็ดยี่หร่า งานวิจัยนี้มีจุดประสงค์ที่จะศึกษาฤทธิ์ความเป็นไปได้ที่พริกแกงในแกงป่าจะมีฤทธิ์ในการยับยั้งเชื้อ *Listeria monocytogenes* 10403S แกงป่าเตรียมโดยใช้วิธีการต้ม1 ชั่วโมง ในสัดส่วน น้ำ 500 มิลลิลิตรต่อพริกแกง 45 กรัมรอให้เย็น และใส่เชื้อลงไป 1 % v/v ศึกษาฤทธิ์ต้านจุลชีพของพริกแกงโดยใช้วิธี in-vitro by cell count serial dilution ลงบนอาหารเลี้ยงเชื้อ BHI ทุกๆ 1 ชั่วโมงเป็นเวลา 6 ชั่วโมง ที่อุณหภูมิห้อง และควบคุมการปนเปื้อนจากเชื้อชนิดอื่นโดยการเก็บตัวอย่างจากชั่วโมงที่ศูนย์ก่อนการถ่ายเชื้อ ผลการศึกษาแสดงให้เห็นว่าการเจริญเติบโตของ *L. monocytogenes* 10403S ในแกงป่านั้นต่ำกว่าอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับ positive control (BHI) ($P < 0.05$) ตั้งแต่ชั่วโมงที่ 1-6, พริกแกงในแกงป่านั้นมีฤทธิ์ต้านจุลชีพในการยับยั้งเชื้อ *L. monocytogenes* 10403S

คำสำคัญ: ฤทธิ์ต้านจุลชีพ *Listeria monocytogenes* 10403S พริกแกง แกงป่า

Abstract

The natural antimicrobial agent generates a lot of attentions especially on food safety. The investigation on the food having antibacterial activity itself becomes more interesting. Thai red curry paste is a traditional condiment used in making red curry (Kang-Pa). In general, the ingredients used in the red curry paste are *Capsicum annuum*, *Cymbopogon citrates*, *Alpinia galangal*, *Allium ascalonicum* L, *Allium sativum*, *Citrushystrix* and *Cuminumcyminum*. This study aimed to investigate the potential of Thai red curry paste in Kang-Pa model acting as natural antibacterial agent against *Listeria monocytogenes* 10403S. Kang-Pa was prepared by using Thai traditional cooking and was inoculated with 1% culture. Thai red curry paste antibacterial activity was investigated in-vitro by cell count serial dilution method on BHI media every hour for 6 hours at room temperature. The results 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. Thai curry paste in Thai red curry showed promising antibacterial activity against *L. monocytogenes* 10403S.

Keywords: Antibacterial, *Listeria monocytogenes* 10403S, Thai Curry Paste, Kang-Pa model

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Introduction

Thai food is one of the most popular foods consumed all around the world withthe signature spicy flavors. Thai red curry paste is a traditional condiment used in cooking 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), *Cuminumcyminum* (Cumin).

Those herbs have also been used since ancient time for flavoring foods and beverages, and formedicinal 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 (Negi, 2012). 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 (Negi, 2012). 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 (Negi, 2012).

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 (Sobel and Watson, 2009). Result in the outbreak found in various types of food. Though *L. monocytogenes* isn't one of the most commonly found foodborne pathogens, the mortality rate associate with *L. monocytogenes* is very high (Rocourt and Cossart, 1997). The elderly, people with compromised immune systems, pregnant women, children and infants are most at risk of serious illness from foodborne (CDC, 2011).

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. The food was still not spoiled. This comes to this project's objective is to investigate the potential of Thai red curry paste in Thai red curry-water base (Kang-Pa) model acting as functional food and natural antibacterial agent against food-borne pathogens.

Methodology

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)I*, 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 *(Cuminumcyminum)* . 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. 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 $^{\circ}$C. Then red curry (Kang-Pa) was cool down to room temperature before culture inoculation.

3. Culture preparation. Stock culture of *Listeria monocytogenes* 10403S was prepared by inoculating one loopful of culture into 10 mL fresh Brain Heart Infusion broth (BHI) and shaken overnight by Culture tube Rotator SCI (Stuart Scientific). Then 1% v/v of overnight culture was inoculated into 50 mL of fresh BHI and shaken for 100 rpm, until optical density at 600 nm reach 0.1(SPECTRONIC, model GENESYS 5).

4. Antibacterial Assay. The 1% v/v of *L. monocytogenes* 10403S at 600 nm optical density equaled to 0.1 was inoculated into 100 mL of fresh BHI as control and 100 mL of red curry soup (Kang-Pa) in comparison at room temperature. The cell count serial dilution method was used to evaluate antibacterial activity, using Brain Heart Infusion (BHI) 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.

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 of *L. monocytogenes* 10403S, at different time by SAS program

Results&Discussion

The results from statistical program showed that the levels of *L. monocytogenes* 10403Sin Kang-Pa was significant lower than those of positive control (BHI), since 1^{st}– 6^{th} hour. The t-test has been done by using SAS on log CFU/ml with $P < 0.05$. The mean and standard deviation of Log 10^6 CFU/ml of *L. monocytogenes* 10403Sin Kang-Pa and in control (BHI) showed in Table 1.

Table 1 Mean and SD of *L. monocytogenes* 10403S growth in Kang-Pa and Control (BHI) up to six hour

Hour	Log 10^6 CFU/ml in Kang-Pa	Log 10^6 CFU/ml in Control (BHI)
0	5.95±0.056[a]	6.02±0.015[a]
1	6.17±0.040[a]	6.34±0.100[b]
2	6.29±0.030[a]	7.03±0.040[b]
3	6.67±0.020[a]	7.36±0.010[b]
4	7.09±0.110[a]	8.22±0.004[b]
5	7.17±0.120[a]	8.26±0.004[b]
6	7.31±0.003[a]	8.91±0.010[b]

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

Growth of *L. monocytogenes* 10403S was monitored at room temperature for 6 hr in Kang-Pa food modeland in brain heart infusion broth (control). The 1% v/v of *L. monocytogenes* 10403S was inoculated into both Kang-Paand BHI. Results that were presented in Figure 1.

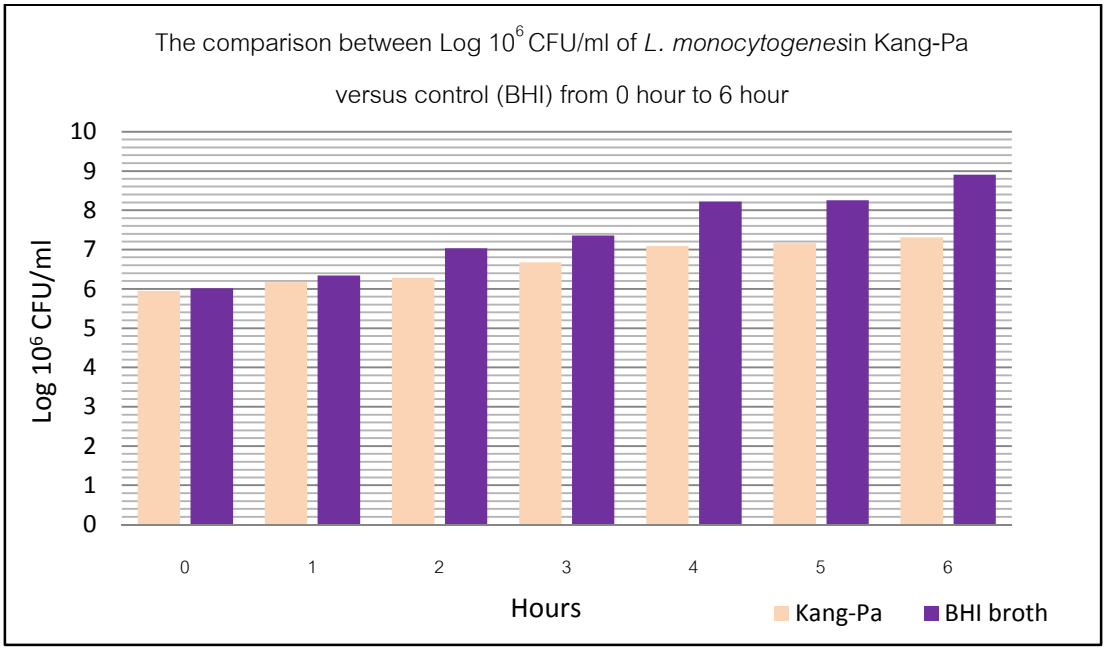

Figure 1 The histogram chart of *L. monocytogenes* 10403S (log CFU/ml) between in Thai red curry and in control (BHI) from 0 to 6 hours.

Kang-Pa statistically reduced the cell number of *L. monocytogenes* 10403Scompared to brain heart infusion broth (BHI) in approximately 1 log cycle. Kang-Pa showed promising bacteriostatic effects up to 6 h might come from the antimicrobial activities inside the red curry paste ingredients. Garlic which is one of the condiments in red curry paste contains allicin. It is one of the active ingredients found during crushing garlic. Allicin has variety of antimicrobial activities (Hughes and Lawson, 1991).Thus by making red currypaste, mechanic mortar will be able to extract allicin out. Also from the work of Kumar andBerwal (1998) and Singh *et al*, (2001) reported that garlic was found to be effective against *L. monocytogenes*. Lis-Balchin and Deans (1997) studied 93 commercial essential oils against 20 *L. monocytogenes* strains. Lemongrass was among the oils that exhibited antibacterial activity against all the Listeria strains tested. 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 by Amin and Kapadnis (2005). 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 bactericidal effect against *L. monocytogenes* (Rattanachaikunsopon and Phumkhachorn, 2009). 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 (Cichewicz *et al*, 1996; Molina-Torres *et al*, 1999). Also from the work of Leuschnerand Lelsch(2003) showed that by adding 1% w/v of dried chili in BHI can slightly inhibited the growth of *L. monocytogenes*. Hence by increasing the amount of chili might increase the inhibition activity. 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 (Farnsworth and Bunyapraphatsara, 1992). Kaffir lime peels also been used in red curry paste. It contains antimicrobial compounds. The work of Khuwijitjaru*et al* (2008) studied 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. 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 (De *et al*, 2003). The report of cumin seed extracts or essential oil on *L. monocytogenes* inhibition has not yet been found.

From the works of Ikigai *et al*, (1993) and Otake *et al*, (1991), they suggest 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 may preserve the foods by reducing lipid oxidation as they are reported to have significant antioxidant activity (Scwarz *et al*, 2001;

Shahidi *et al*, 1997; Shan *et al*, 2009; Tanabe *et al*, 2002; Yanishlieva *et al*, 2006).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. Although the combination of above spices and herbs that have been used as food not yet been investigated. The results from this experimentshowed that when cooked herbs and spices using food model, the spices and herbs still have antimicrobial properties. The different in the growth of *L. monocytogenes* 10403Sbetween Kang-Pa and BHI showed significantly different in growth level. However 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.

Conclusion

Thai red currypaste in Thai red curry (Kang-Pa)model showed promising antibacterial activity against food-borne pathogenic bacteria, *Listeria monocytogenes* 10403S. *L. monocytogenes* 10403S level in Thai red curry was significantly lower than in Brain heart infusion broth, as positive control, ($P < 0.05$) from first hour up to sixth hour. This might be another explanation as food safety aspect that why Kang-Pa was kept in food cabinet at room temperature without spoilage.

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