Natural Antibacterial Activity of Thai Red Curry Paste in Coconut Milk based Curry model (Kang-Kati) on Salmonella sp. and Listeria monocytogenes

BY

Mr.Chuchad Sapabguy

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A special project submitted to the Biotechnology of Biotechnology, Assumption University in part of fulfillment of the requirement for the degree of Bachelor of Science in Biotechnology

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Title	:	**Natural Antibacterial Activity of Thai Red Curry Paste in Coconut Milk based Curry model (Kang-Kati) on *Salmonella* sp. and *Listeria monocytogenes***
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Mr. Chuchod Sapabguy
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ABSTRACT

Natural Antibacterial Activity of Thai Curry Paste in Thai Red Curry-Coconut Milk Base (Kang-Kati) model on *Salmonella* sp. and *Listeria monocytogenes*

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Past 5 years, *Salmonella* sp. and *Listeria monocytogenes* outbreaks occurred frequently in variety of food products. Foods, as natural antibiotics itself, might be alternative choice for food safety. Thai red curry is the cultural dish and become world popular dish. Thai curry paste, main ingredient in Thai red curry, composes of variety herbs including *Capsicum annuun* (chili), *Citrus hystrix* (Kaffir lime), *Cuminum cyminum* L. (Cumin), *Allium ascalonicum* L. (Shallot), *Allium sativum* (Garlic), *Cymbopogon citratus* (Lemongrass) , *Alpinia galangal* (Galangal). Therefore, the objective of this study was to investigate antibacterial activity of Thai curry paste in Thai Red Curry-Coconut Milk Base (Kang-Kati) model on three food-borne pathogenic bacteria: *S. enteric* Enteritidis, *S. enterica* 4,5,12:i:- (human) US clone and *L. monocytogenes* 10403S. The cell count serial dilution method was used to evaluate antibacterial activity by using the Salmonella-Shigella agar and BHI agar. The Kang-Kati was taken every hour for 6 hrs. Thai red curry-coconut milk base (Kang-Kati) was prepared by Thai homemade authentic cooking method using UHT coconut milk, as it has been served in Thai cuisine and was inoculated with 1 % culture. The result showed that *S. enterica* Enteritidis level in Thai red curry (Kang-Kati) was significantly lower than in NB, as positive control, ($P < 0.05$) at 3rd and 4th hour: 3rd hr; 5.53±0.027 and 5.65±0.019, and 4th hr; 5.62±0.07 and 5.80±0.03 log CFU/ml, respectively. While *S. enterica* 4,5,12:i:- (human) level in Thai red curry (Kang-Kati) was significantly lower than in NB, as positive control, ($P < 0.05$) since 4th - 6th hour: 4th hr; 5.72±0.06 and 5.84±0.01, 5th hr; 5.80±0.04 and 5.91±0.03, and 6th hr; 5.85±0.04 and 5.96±0.01 log CFU/ml, respectively. *L. monocytogenes* level in Thai red curry (Kang-Kati) was significantly lower than in BHI, as positive control, ($P < 0.05$) at 3rd and 4th hour: 3rd hr; 5.49±0.01and 5.61±0.02, and 4th hr 5.63±0.02 and 5.70±0.04 for 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 Thai red curry-coconut milk base (Kang-Kati) showed the promising antibacterial activity, act as functional food, against all three food-borne pathogen.

Keywords: Food Natural Antibacterial, Thai Red Curry Paste, Kang-Kati, Salmonella sp. and Listeria monocytogenes

CONTENT

LIST OF TABLES

LIST OF FIGURES

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INTRODUCTION

Salmonella bacteria are rod-shaped, gram-negative bacteria, cell size range from 2 and 5 micrometers long, and 0.7 to 1.5 micrometers in diameter[1]. *Salmonella* is an aerobic, none spore forming microorganism that can motile because they have flagella[1]. The optimum temperature for growth of *Salmonella* is between 35 to 37°C[2]. Slow growth has been observed at 5°C, with a maximum growth temperature range of 45 to 47°C[2]. Growth may occur between pH 4 (dependent upon the acid) and 9.0; optimum pH is range of 6.5 to 7.5. *Salmonella* growth in liquid media has been observed between a_w 0.999 and 0.945, although growth at an a_w of 0.93 has been observed[2]. Salmonella is a common bacterium that can be found in many places such as in soil, water, raw food, and the bowel movements of some animals[3]. The disease that cause by *Salmonella* is called as Salmonellosis which has many symptoms, and are sometimes absent altogether[4]. The most common symptoms are diarrhea, abdominal cramps, and fever[5]. Normally, the symptom from the infection will develop within 6 to 72 hours after eating contaminated food and will last for 3 to 7 days without treatment[4], however, the using of antibiotic to cure the infection of *Salmonella* has become more difficult because they are more resistant to the antibiotic[4] . In addition, there are some additional symptoms which are bloody diarrhea, vomiting, headache, and body aches[4]. The infection from *Salmonella* in food can be prevented by cooking food thoroughly until they reach an

internal temperature of 71^{O}C[5] or keep at 4^{O}C in the refrigerator to prevent the growth[6].

Listeria monocytogenes is a rod-shaped, gram-positive, none spore forming bacteria [7]. The morphology of *L. monocytogenes* is in form of short chains, however, coccoid may appeared if they were direct smeared, which leads to misunderstand with *Streptococci*[7]. *L. monocytogenes* can be found in soil, water, or vegetables that become contaminated from the soil or from manure used as fertilizer[8]. Animals can carry the bacterium without appearing ill and can contaminate foods of animal origin, such as meats and dairy products[8]. The disease that cause by *L. monocytogenes* is called as listeriosis, which considered as a severe infection because the mortality rate is up to 25%[7]. The incubation time for listeriosis may ranges from 3 to 70 days, and averages 21 days[9]. Symptoms from the infection of *L. monocytogenes* are similar to mild-flu, fever, muscle aches, and nausea or diarrhea. These symptoms normally last up to a week and spontaneously recover. However, in some cases *L. monocytogenes* can infect the brain of the patients and cause the symptoms of meningitis which leads to stiff neck, headache, and fever, or altered mental status which leads to confusion, reduced mental activity, balance problems, and seizures develop in brain infections. In addition, pregnant women who are healthy usually will develop only minor symptoms, but some pregnant females,

the infection can cause miscarriage, stillbirth, premature birth, or cause infection, and death of the newborn[7, 9].

L. monocytogenes is a microorganism that has extraordinary ability to survive in many stress condition which are 10% NaCl solutions and a range of temperatures from −0.1 to 45°C, also be able to tolerate a pH as low as 3.5 after an adaptation phase at pH 5.5[10]. This high degree of adaptability is one reason for the difficulty in controlling the pathogen in a number of food products, since treatments used in food processing and preservation often utilize stressing agents and parameters to which *L. monocytogenes* is resistant[10, 11].

In order to prevent the infection from the *L. monocytogenes*, it was suggested that raw food from animal sources, such as beef, pork, or poultry must be washed and cooked thoroughly, the uncooked food and the ready-to-eat foods must be kept separately, wash hands, knives, and cutting boards after handling uncooked foods to prevent the cross contamination[12]. Moreover, avoid consuming unpasteurized milk or foods made from unpasteurized milk, and consume perishable and ready-to-eat foods as soon as possible[12].

The processing processes that need for destroying those food borne pathogens and ensure the food safety involve with high temperature and longtime which may alter the aesthetics of food product. As a result the using of foods, that are natural antibiotics, might be another alternative choice in food safety. Nowadays, the using of natural antibiotics food for food preservation method become more and more popular because it not only reduce the cost but also maintain the original characteristic of the food product

Antibacterial referred to substances that can kill or inhibit the growth of the microorganisms[13]. Originally, an antibiotic was a substance that produced by a microorganism that can inhibit or reduce the growth of another[14]. In addition, the synthetic antibiotics are usually chemically related to natural antibiotics which were produce to function in the same manner[14].

Curry paste is a rich aromatic mixture of freshly ground herbs and spices[15]. Curry paste is a major key ingredient in Thai foods because it is the main component in the curry that gives the characteristic of curry. The ingredients that used for making Thai curry paste are the combination of many herbs which 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)[15]. Moreover, the herbs that used for cooking curry not only work as a food

but also they can also act as a medicinal food. At this present, Thai curry paste mostly makes by the electric blender or use the ready-made in the supermarket, because of life styles changing however, traditionally Thai curry pastes were made by the granite mortar and pestle[16].

Therefore the objective of this experiment is to investigate the antibiotics activity of Thai curry paste in Thai coconut milk based curry (Kang-Kati) model on *Salmonella enterica Enteritidis*, *Salmonella enterica 4,5,12:i:-* (human) US clone, and *Listeria monocytogenes* 10403S

OBJECTIVES

- To investigate the antibacterial activity of Thai curry paste in coconut milk based curry model (Kang-Kati) on *Salmonella enterica Enteritidis*, *Salmonella enterica 4,5,12:i:-* (human) US clone, *Listeria monocytogenes* 10403S

LITERATURE REVIEW

Outbreak of *Salmonella*

Salmonella is a bacterium that causes intestinal infections in the United States which also called as Salmonellosis[4, 6]. Salmonellosis was reported to be a cause of the enteric disease and there were about 14 cases per each 100,000 persons, amounting to approximately 30,000 confirmed cases of salmonellosis yearly in the U.S.[17]. Moreover, the *Salmonella* outbreaks represent 11 times in the year of 2011 solely, with more than 300 patients and more than 30 deaths[17]. The spread of the *Salmonella* can be occurred by the feces of the people of animals through food or beverage. The symptom of infection will show within 72 hours. included fever and chills, headache, multiple bouts of diarrhea which may be bloody, nausea and vomiting, and severe abdominal pain and cramps[5]. Some recently outbreaks are the following:

- 2012, U.S., there are 160 persons infected, 26 ill patients have been hospitalized, and no deaths have been reported. The outbreak strain was *S. bareilly*, from raw Scraped ground tuna product, have been reported from 20 states and the district of Columbia[18, 19].

- 2012, U.S., 72 persons infected, 12 ill persons have been hospitalized, and no deaths have been reported, with outbreak strains of *S. sandiego*, *S. pomona*, and *S. poona* have been reported from 17 states. It was indicated exposure to turtles or their environments (e.g., water from a turtle habitat) is the cause of these outbreaks[19, 20].

- 2011, U.S., there are 11 cases occurred with 950 infected, 202 ill persons have been hospitalized, and 2 deaths have been reported. The outbreaks strains are *S. typhimurium, S. heidelberg, S. enteritidis, S. agona, S. altona, S. johannesburg, S. hadar, and S. panama.* In addition, the sources that cause the outbreaks are in the group of raw meats, vegetable, fruits, animal, and teaching of microbiology laboratory[19].

- 2010, U.S., there are 9 cases occurred with 2,642 infected, 148 ill persons have been hospitalized, and no deaths have been reported. The outbreaks strains are *Salmonella* I *4,5,12:i:-, S. enteritidis, S. chester, S. typhi (Typhoid Fever), S. hartford, S. baildon, S. newport, S. montevideo,* and *S. typhimurium.* Moreover, the sources of the outbreaks are in the group of raw food, vegetable, frozen foods, and animal[19].

- 2009, U.S., there are 3 cases occurred with 240 infected approximately, 8 ill persons have been hospitalized, and no deaths have been reported. The outbreaks are *involved with many strains and the sources of the outbreaks are alfalfa sprout, peanut butter, and pistachio[19].*

- 2008, U.S. and Canada, there are 3 cases occurred with 1,500 infected approximately, 310 ill persons have been hospitalized, and no deaths have been reported. The outbreaks strains are *S saintpaul, S. agona,* and *S. Litchfield.* In addition, the sources of the outbreaks are raw produce, cantaloupe, and wheat cereals[19].

Outbreak of *Listeria monocytogenes*

Outbreaks of *L. monocytogenes* were occurred many times in the past until now. In addition, *L. monocytogenes* which has ability to invade from cell to cell that can cause the death to the patient who got infection when the invasion reaches to the nervous system [8]. Moreover, it was reported about 1,600 cases annually in the United States[21]. However, compared to 1996-1998, the incidence of listeriosis had declined by about 38% by 2003. However, illnesses and deaths continue to occur. On average from 1998-2008, 2.4 outbreaks per year were reported. Before 2011, the largest outbreak occurred in 2002, when 54 illnesses, 8 deaths, and 3 fetal deaths in 9 states[21]. The few recently outbreaks are the following:

- 2011, U.S., 146 persons infected, 30 death and 1 miscarriage, with any of the four outbreak-associated strains of *L. monocytogenes* from 28 states. The cause of outbreaks was indicated that it came from whole or pre-cut cantaloupe harvested in 2011 from Jensen Farms[22].

- 2011, England, 3 persons infected and hospitalized, no death reported, with outbreak strains of *L. monocytogenes.* The investigation reported that the source of the outbreaks was the pre-packed sandwiches and salads manufactured in compliance with regulations. While breaches in cold chain and shelf life controls at hospital level were identified as key contributing factors[23].

- 2010, U.S., 10 persons infected, and 5 death, with outbreak strains of *L. monocytogenes.* The investigation indicated that the source of outbreaks was fresh cut celery from local manufacturer[24].

- 2009, Austria and Germany, 389 persons infected, and 2 deaths, with outbreak strains of *L. monocytogenes.* serotype 1/2a. The investigation indicated that the source of the outbreaks is acid curd cheese 'QUARGEL'[25].

- 2008, Canada, 57 persons infected, and 22 deaths, with outbreak strains of *L. monocytogenes.* The investigation indicated that the sources of the outbreaks are deli meat consumption and the consumption of Maple leaf product[26].

Coconut milk

Coconut milk is a very important ingredient that used for making curry. Coconut milk, mostly, compost of water and fat[27], so that using the coconut milk as a extractor will enhance both polar and non-polar active compound in the chili paste released to the curry because of its property. The main fatty acid that found in the coconut milk is lauric acid, which is a medium chain fatty acid[28]. The lauric acid can transform to monolaurin in the human body, which can act as antiviral, antibacterial, and antiprotozoal monoglyceride used by the human[29]. The Monolaurin will works effectively when it was combined with ethylenediaminetetraacetic acid (EDTA), or nisin, it can work against *Esherichia coli*, *Bacillus subtilis*[30] and *Candida Albicans*[31].

***Cuminum cyminum* L.**

The previous study showed that the chemicals that contain in the cumin, steam distillation technique are cuminic, cymene, dipentene, limonene, phellandrene and pinene[32]. In addition, the major component in *C. cyminum* L. (Cumin) oil was cuminaldehyde (20-72%) and monoterpene hydrocarbons, which showed that they can inhibit the growth of about 20 serotypes of *Salmonella* sp. (inhibition zone range of 8-10 mm.) by the ethanolic extracts and *E. coli* O157:H7 with the methanolic extract [33, 34]. In addition, they also has antimycotic properties on *A. fumigatus* and *A. niger* by hydro distilled method[35]. The cumin oil also can be used as a medicinal plant for curing the diarrhea and cholera which are infected by bacteria[32].

Capsicum annuum

Chilli is a very good source of various vitamins and minerals such as vitamin C, vitamin B, vitamin B6, potassium, magnesium, and iron[36].The main ingredient of chilli paste is *C. annuum* (chili) which was investigated that the main chemical component was capsaicin[37]. Capsaicin is a hydrophobic molecule with boiling point of 210-220°C[38], with broadly antimicrobial activities on both bacterial and fungal such as *Fusarium* [39], *Helicobacter pylori* [40], *Botrytis cinerea*, and *Aspergillus niger* [41]. Capsaicin also can be used as a medicine for anti-inflammatory agent because it inhibits substance P, which is associated with inflammatory processes; much like it relieves headaches and migraines, listed earlier. Capsaicin may also help to protect the heart because it can reduce

cholesterol, triglycerides and platelet aggregation and the body dissolve fibrin, which is necessary for blood clots to form[36].

Allium sativum

Garlic is an important food ingredient for many countries, especially in Asia. It also known as a herb that have variety of active compound, including seventeen amino acids, at least 33 sulfur compounds, eight minerals which are germanium, calcium, copper, iron, potassium, magnesium, selenium, and zinc and the vitamins A, B1 and C. It also comprises fiber and water[42]. An allicin is a chemical component from *A. sativum* (Garlic) which work as an antibacterial and antifungal[43-45]. Allicin was proved that they can inhibit the growth of *E.coli*, *Shigella* sp., *Salmonella* sp., *Proteus mirabilis*[46], *Klebsiella pneamoniae*[47], *Aeromonas caviae, A. hydrophila, A. sobria, Chromobacterium violaceum, Enterobacter faecalis, Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus aureus*[44, 48] by partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target[43, 45, 48]. In addition, they also has antimycotic properties on *A. fumigatus* and *A. niger* by hydro distilled method[35]. However, allicin will not present unless the barriers of enzyme alliin alkyl-sulfenate-lyase (EC 4.4.1.4) and non-protein amino acid S-allylcysteine S-oxide (alliin) were broken-down[43, 45], or only generated after the cloves are injured and the enzyme alliinase reacts with its substrate alliin[49]. The effectiveness of the garlic extract was proof that it is increased as the temperature increase, the optimum temperature is about $80^{O}C$, aqueous extraction, and decreased or maintain after this range of temperature, because the higher temperature will increase the solubility of the chemical

compounds[44, 50]. However, comparing between the dried and fresh form, it showed that fresh extracts had more antimicrobial properties than dried autoclaved extracts[51].

***Allium ascalonicum* L.**

Shallot is the herb that has many biologically active compounds of antioxidant properties which included flavonoids and phenolic acids[52]. The flavanols that found in shallot are quercetin and kaempferol. However, it was also proof that the bulbs part contains only quercetin, but the leaves part contains both quercetin and kaempferol[52]. The main active compound of *A. ascalonicum* L. (Shallot), flavanols and phenolic compounds[52], have broad spectrum against both fungal and bacterial such as *Syncephalastrum*, *A. niger*, *Penicillium* sp., *Paecilomyces* sp., *Scopulariopsis* sp.[53-55], *B. cereus*, *E. coli* O157:H7, and *S. enterica* [56]. The previous study also showed that shallot extract, by column chromatography, was active against microbes at pH range of 4-8. In addition, the relative activities of shallot extract at temperature –7 to 121°C were 88 to 100 %. Extract of shallot only was soluble in dimethyl sulphoxide, dimethyl formamide and water which means that the compound present in the shallot is polar compounds[54]. However, the previous study also indicated that the oil extraction method provide a better antibacterial activity than the fresh extraction by manual extractor and squeezing[57].

Citrus hystrix

The previous study also report that Kaffir lime can be used for both food ingredient and also a medicine for traditional Indonesian medicines or used as a cleaner, shampoo and the natural deodorizer in Thailand[58]. For *C. hystrix* (Kaffir lime), the main compound

in kaffir lime leaves is citronellal (65.4 %) whereas the major constituents in essential oil of kaffir lime peels are β-pinene (30.6 %), limonene (29.2 %), and sabinene (22.6 %)[59]. The previous study also reported that the essential oil form *C. hystrix* can inhibit the growth of many microorganisms such as *S. Enteritidis*[33], *S. aureus*, *B. cereus*, *L. monocytogenes, Saccharomyces cerevisiae* var. sake, *Aspergillus fumigatus* TISTR 3180[60], *E. coli* TISTR 292, *P. aeruginosa*, *C. jejuni*, and *C. perfringens* DMST 1591 with the inhibition zone range from 6 -90 mm.(hydro-distillation technique)[61]. However, the previous study also indicated that the oil extraction method provide a better antibacterial activity than the fresh extraction by manual extractor and squeezing[57].

Cymbopogon citratus

For *C. citratus* (Lemongrass), it contains myrcene, traces of limonene, geranyl acetate, nerol, citronellal, geraniol, citral and neral[62]. The previous study also reported that the major chemical constituents of *C. citratus* from chemical analysis, GC analysis, of lemon grass oil contains geranial (citral a) about 28.93% and neral (citral b) about 18.49%, respectively[61]. Citral which is a major compound in almond grass is a mixture of two geometric isomers of compounds in lemongrass oil. Geranial is the *trans* isomer of citral in lemongrass, which accounts for 40-62%, and neral is the *cis* isomer of citral in lemongrass, which accounts for 25-38%[63] .The previous study also reported that the essential oil from *C. citratus* can inhibit the growth, inhibitory affect, of about 17 serotypes of *Salmonella* sp. (inhibition zone range of 7-11 mm.) by the ethanolic extracts[33], and *E. coli* TISTR 292, *P. aeruginosa*, *S. aureaus, B. cereus,*

Campylobacter jejuni, and *Clostridium perfringens* DMST 1591 with the inhibition zone range from 7.5 -90 mm.(hydrodistillation technique) [61].The previous study also reported that with the methanol extraction the extract from lemon grass has antibacterial activity on *E. coli, K. pneumoniae, P. aeruginosa, P. vulgaris* and two gram positive bacteria which are *B. subtilis* and *S. aureus*[64]. In addition, they also has antimycotic properties on *A. fumigatus* and *A. niger* by hydro distilled method[35].

Alpinia galangal

Alpinia galangal (Galangal) can be also used not only for food ingredients but also for medical purposes, such as forcarminative, stomachic, antispasmodic, antichloristicand antibacterial drugs[65]. The most active compound of *A. galangal* (Galangal) was terpinen-4-ol[66], it can inhibit the growth of many fungal and bacteria, both gram positive and gram negative. The active compound that contains in the *A. galangal* was proof that it will provide a highest antimicrobial affect with the non-polar extraction by the non-polar extractant such as hexane[67]. By the scanning electron microscopy observations, it showed that *A. galanga* oil has a mechanism that can modify of the bacterial cell membrane, disrupting the membrane's permeability which make the spill out of cell materials and the death of pathogens cell[68]. The previous study al so showed that the extract from *A. galangal* has antimicrobial activity on many microorganisms such as *S. typhimurium*, *S. aureus*, *F. solani*, *B. cinera* KCTC 6973[69], and *L. monocytogenes*[67]. However, the previous study also indicated that the oil extraction method provide a better antibacterial activity than the fresh extraction by manual extractor and squeezing[57].

MATERIALS AND METHODS

Preparation of 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), 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 road local market, Bangkok, Thailand. 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 5 min, garlic and shallot 5 min, galangal and lemongrass for 4 min, kaffir lime peel and cumin powder for 2 min, shrimp paste 4 min.

Table 1: List of ingredients and the percentage used for making curry paste.

Scientific name	Common name	Plant part	% (W/W)
Capsicum annuum	Chili	Fruit	40
Cymbopogon citratus	Lemongrass	Stem	20
Allium sativum	Garlic	Tuber	15
***Allium ascalonicum* L.**	Shallot	Tuber	10
Alpinia galangal	Galangal	Tuber	10
Citrus hystrix	Kaffir lime	Peel	1
***Cuminum cyminum* L.**	Cumin	Seed	0.5
Salt			0.5
Shrimp paste			3

Table 2: Order for adding the ingredients and time for grinding

Order	Materials	Time (min)
1	Chilli and Salt	5
2	Garlic and Shallot	5
3	Lemongrass and Galangal	4
4	Kaffir lime peel and Cumin powder	2
5	Shrimp paste	4

Preparation of curry

The 200 ml of ready-to-used pasteurized coconut milk was boiled for 5 minutes by using the hot plate (VELP SCIETIFICA, model Are2), which oil and water phase in coconut milk separated then 45 g of 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 was controlled in the range of 90 - 92 °C.

Preparation of the culture

The stock culture was prepared by inoculating one loopful of *S. enterica Enteritidis*, *S. enterica 4,5,12:i:-* (human) US clone (Gift of S. Chaturongakul, MU) into 50 ml fresh NB and *L. monocytogenes* 10403S in fresh BHI medium and shake on the shaker (IKA LABORTECHNIK, model KS 501 Digital) with 100 rpm overnight. Then 1 % v/v overnight culture was inoculated into 50 ml of fresh NB for *Salmonella spp.* and 50 ml of fresh BHI for *L. monocytogenes*, at 37 °C by Culture tube Rotator SCI (Stuart Scientific), until OD600 reach 0.1 (SPECTRONIC, model GENESYS 5) which is early log phase.

Antibacterial Assay

1% v/v of 0.1 OD_{600}, as the early log phase of *S. enterica Enteritidis*, *S. enterica 4,5,12:i:-* (human) US clone and *L. monocytogenes* 10403S was inoculated in 100 ml Kang-Kati. Then, inoculated Kang-Kati then incubated at room temperature. The cell count serial dilution method was used to evaluate antibacterial activity by using the Salmonella- Shigella agar and BHI agar. The Kang-Kati was taken every hour for 6 hrs. The colony forming unit was observed after 24 hours. The control was done in the same way in NB for *Salmonella* sp. and BHI for *L. monocytogenes* 10403S, inoculated at room temperature, 100 rpm, to show the real growth pattern of *S. enterica Enteritidis*, *S. enterica 4,5,12:i:-* (human) US clone and *L. monocytogenes* 10403S.

Statistical analyses

The experiment was performed in duplicate and repeated three times independently. The independent two-sample t-test was used to study the effect of the antibiotic from the curry paste on the growth of *S. enterica Enteritidis*, *S. enterica 4,5,12:i:-* (human) US clone and *L. monocytogenes* 10403S, at different time by using SAS program.

RESULT

The results from Table 3-6 and figure 1-4 showed that the antibacterial activity promising of curry paste extracted by real food model compared with growth pattern of control

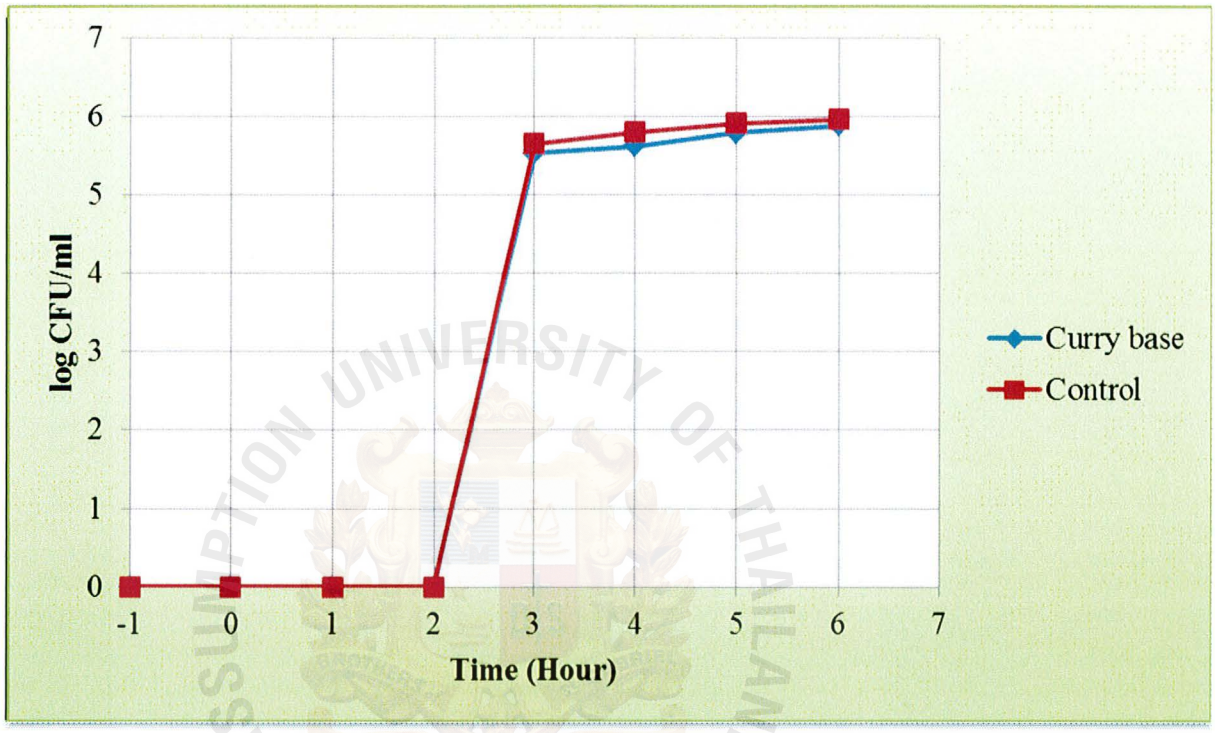

Figure 1: log CFU/ml of *S. enterica Enteritidis (human)* growth in Kang-Kati and control

Table 3: log CFU/ml count of *S. enterica Enteritidis (human)* growth in Kang-Kati and control

Time(hr.)	log CFU/ml	
	Kang-Kati	Control
-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

*: there is significantly different ($P < 0.05$)

ND: less than 30 colonies

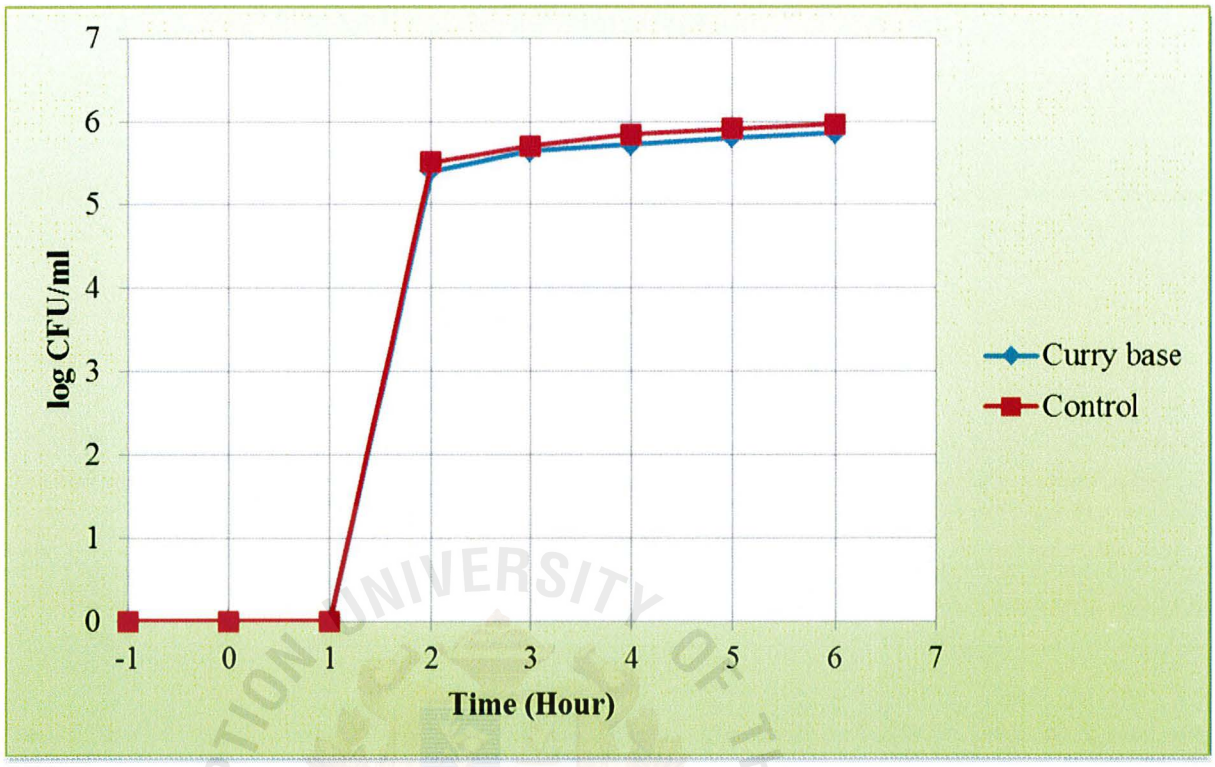

Figure 2: log CFU/ml of *S. enterica 4,5,12:i:-*(human) growth in Kang-Kati and control

Table 4: log CFU/ml count of *S. enterica 4,5,12:i:-*(human) growth in curry and control

Time(hr.)	log CFU/ml	
	Kang-Kati	Control
-1	0	0
0	ND	ND
1	ND	ND
2	3.72±3.22	5.51±0.02
3	5.64±0.07	5.70±0.02
4*	5.72±0.06	5.84±0.01
5*	5.80±0.04	5.91±0.03
6*	5.85±0.04	5.96±0.01

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

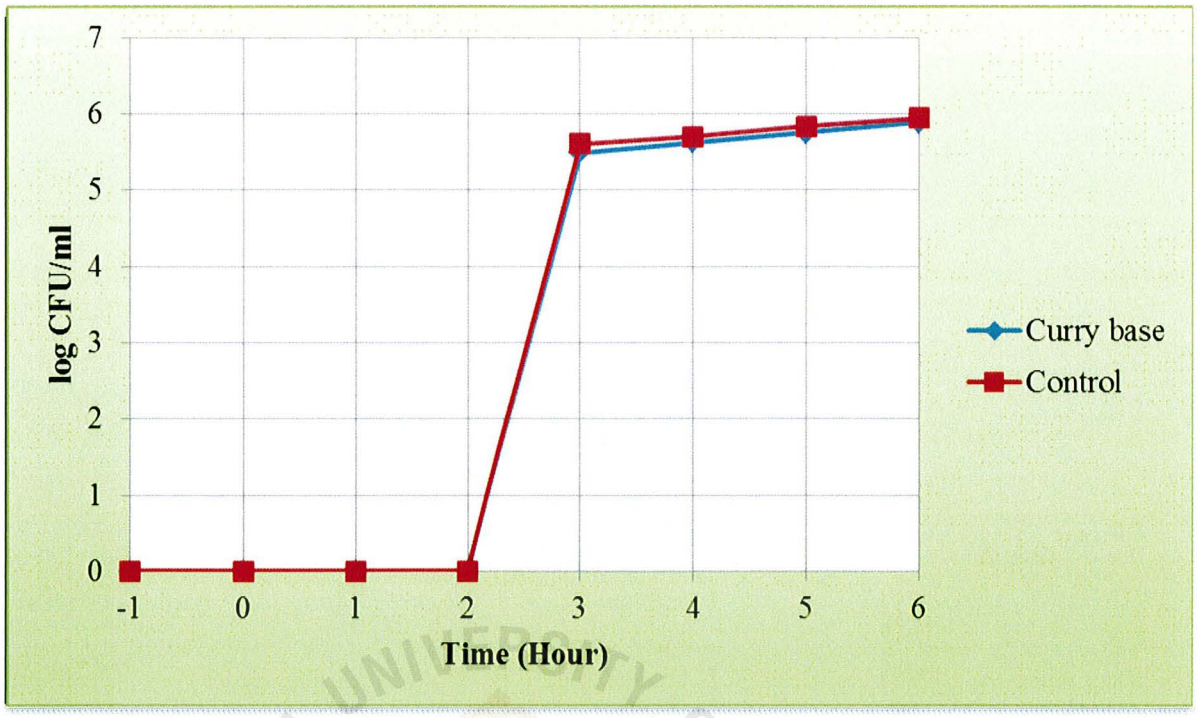

Figure 3: log CFU/ml of *L. monocytogenes* 10403S growth in Kang-Kati and control

Table 5: log CFU/ml count of *L. monocytogenes* 10403S growth in Kang-Kati and control

Time(hr.)	log CFU/ml	
	Kang-Kati	**Control**
-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

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

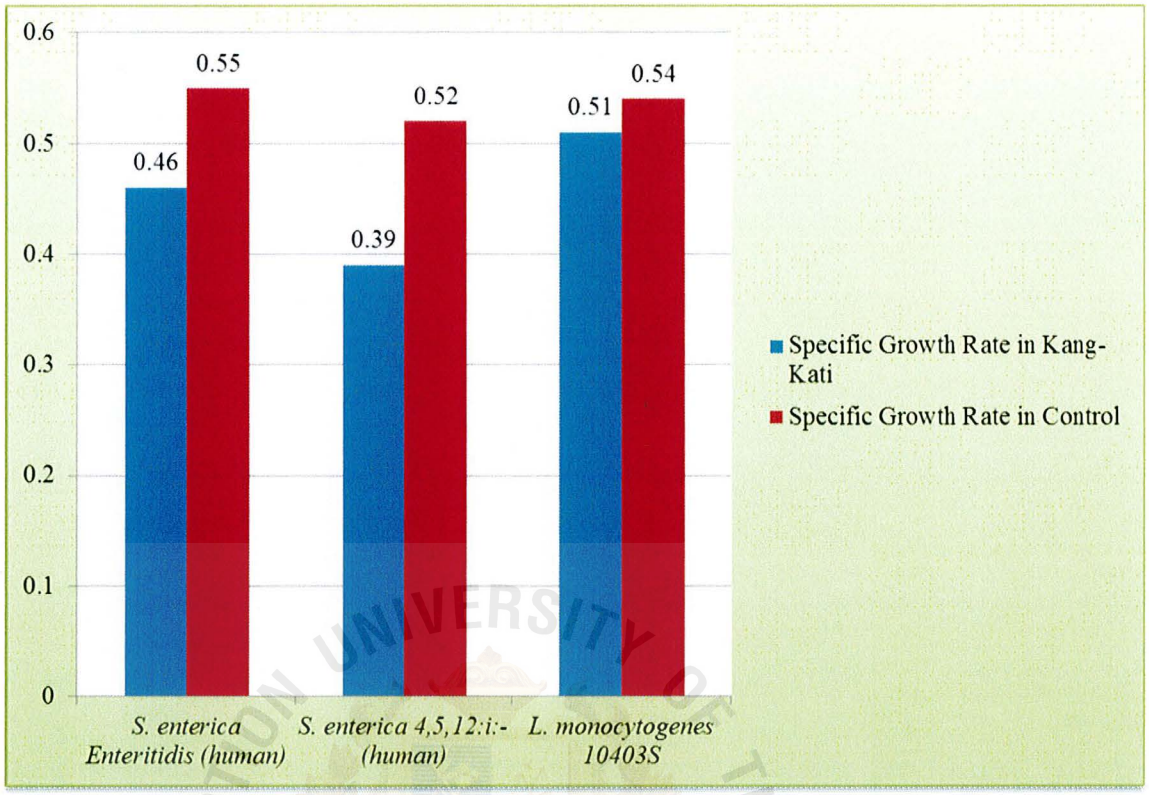

Figure 4: specific growth rate of *S. enterica Enteritidis (human), S. enterica 4,5,12:i:-*(human), *L. monocytogenes* 10403S growth in Kang-Kati and control

Table 6: Comparison of specific growth rate of *S. enterica Enteritidis (human), S. enterica 4,5,12:i:-*(human), *L. monocytogenes* 10403S growth in Kang-Kati and control

Specific Growth Rate	**($hour^{-1}$)**	
	Kang-Kati	**Control**
S. enterica Enteritidis (human)	0.46	0.55
S. enterica 4,5,12:i:-(human)	0.39	0.52
***L. monocytogenes* 10403S**	0.51	0.54

DISCUSSION

From Table 3-6 showed that curry paste showed that curry paste showed in antibacterial activity promising.

For Table 3 and figure 1, the result showed that there is no significantly different of *S. enterica Enteritidis (human)* level (P>0.05) between Kang-Kati and control during the first 2 hours. By the way, the third to the sixth hour, showed the significantly different of *S. enterica Enteritidis (human)* level (P>0.05) between Kang-Kati comparing with control at 3rd hour 5.53±0.03 and 5.65±0.02, at 4th hour 5.62±0.07 and 5.80±0.03, at 5th hour 5.79±0.11 and 5.91±0.01, and at 6th hour 5.88±0.08 and 5.96±0.00 log CFU/ml, respectively.

According to Table 4 and figure 2, the result indicated that there is no significantly different of *S. enterica 4,5,12:i:-*(human*)* level (P>0.05) between Kang-Kati and control during first 3 hours. However, since the fourth to the sixth hour, the result showed that there is significantly different of *S. enterica 4,5,12:i:-*(human*)* level (P>0.05) between Kang-Kati comparing with control at 4th hr.; 5.72±0.06 and 5.84±0.01, at 5th hr.; 5.80±0.04 and 5.91±0.03, and at 6th hr.; 5.85±0.04 and 5.96±0.01 log CFU/ml, respectively.

For Table 5 and figure 3, the result indicated that there is no significantly different of *L. monocytogenes* 10403S level (P>0.05) between Kang-Kati and control during first 3 hours. And last two hours. However, during the third and the fourth hour, the result showed that there is significantly different of *L. monocytogenes* 10403S level (P>0.05) between Kang-Kati comparing with control at 3rd hr.; 5.49±0.01and 5.61±0.02, and at 4th hr.; 5.63±0.02 and 5.70±0.04 log CFU/ml, respectively.

Moreover, the result from Table 6 and figure 4 also showed that the specific growth rate, the increase in cell mass per unit time, of *S. enterica Enteritidis*, *S. enterica 4,5,12:i:-* (human), and *L. monocytogenes* 10403S in Kang-Kati was smaller than the control, which are 0.46 $hour^{-1}$ and 0.55 $hour^{-1}$, 0.39 $hour^{-1}$ and 0.52 $hour^{-1}$, and 0.50 $hour^{-1}$ and 0.54 $hour^{-1}$ respectively. The reducing of specific growth rate indicated that curry paste inhibited *S. enterica Enteritidis*, *S. enterica 4,5,12:i:-* (human), and *L. monocytogenes* 10403S.

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 curry paste. The previous study 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, and antibacterial against *E. coli*, *B. subtilis* and *C. Albicans*[28-31]. With the procedure that used in this

experiment the coconut milk was boiled until the water phase and the oil phase is separate so that the chemical that obtain from the extraction process would be both polar and non-polar substances. However, the active compound that contain in the extract, including lauric acid, should be identified and tested in the further experiment for both of oil and water phases.

C. hystrix (Kaffir lime), was report by the previous experiment that the major constituents in essential oil of kaffir lime peels are β-pinene (30.6 %), limonene (29.2 %), and sabinene (22.6 %)[59]. The previous study also reported that the essential oil form *C. hystrix* can inhibit the growth of many microorganisms such as *S. Enteritidis*[33], *S. aureus*, *B. cereus*, *L. monocytogenes, Saccharomyces cerevisiae* var. sake, *Aspergillus fumigatus* TISTR 3180[60], *E. coli* TISTR 292, *P. aeruginosa*, *C. jejuni*, and *C. perfringens* DMST 1591 with the inhibition zone range from 6 -90 mm.(hydro-distillation technique)[61]. However, the previous study also indicated that the oil extraction method provide a better antibacterial activity than the fresh extraction by manual extractor and squeezing[57] so that the chemical compound that contain in the extract is possible to have active compound 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* (chili) was investigated by the previous experiment and reported that the most profuse component was capsaicin[37]. Capsisin is a hydrophobic molecule, that has wide range of antimicrobial activities over bacterial and fungal such as *Fusarium*, *Helicobacter pylori*, *Botrytis cinerea*, and *Aspergillus niger* [38-41]. Capsaicin can be absorbed to human body both ingestion pathway and via skin, however, the using should be in the appropriate dose[70]. The boiling point of capsaicin is about 210 -220 ^{O}C, so it is possible that capsaicin will also in the oil phase extract, which is non-polar phase, obtain after the extraction process and work as one of antibacterial agent[70]. However, the amount and quality of capsaicin should be investigated in the further experiment because using too much amount of capsaicin may lead to adverse effect.

The major active compound in *C. cyminum* L. (Cumin) oil was identified and reported by the previous experiment as 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. (inhibition zone range of 8-10 mm.) by the ethanolic extracts[33]. In addition, it also reported by the previous study that it can be used to cure the diarrhea and cholera which are infected by bacteria[32], which is the group of microorganism in this study. In this experiment, the oil phase extraction might be source

of the cuminaldehyde, however, the further investigation is required to improve the understanding about the extract from cumin.

The main active compound of *A. ascalonicum* L. (Shallot) was investigated and reported by the previous experiment as flavanols and phenolic compounds[52]. Moreover, they also has wide range of antimicrobial activities over bacterial and fungal such as *Syncephalastrum*, *A. niger*, *Penicillium* sp., *Paecilomyces* sp., *Scopulariopsis* sp., *B. cereus*, *E. coli* O157:H7, and *S. enterica*[53, 56]. In addition, the active compound that also compromise to be a antimicrobial agent is the compound in the group of Sulfide compound that also contain in shallot. Diallyl monosulfide, diallyl disulfide, diallyl trisulfide, and diallyl tetrasulfide are the sulfide compound that found in shallot[56]. Kang-Kati is good model for shallot extracted against food-borne pathogens. In addition, cooking temperature doesn't affect antibacterial activity so that the curd extract from the shallot might act as one of the antibacterial agent in the extract. However, the active compounds in shallot need the further investigation.

An allicin is a chemical component from *A. sativum* (Garlic) that was identified and reported by the previous experiment, which work as an antibacterial and antifungal[43]. Allicin was proved that they can inhibit the growth of *E. coli*, *Shigella* sp., *Salmonella* sp. and *Proteus mirabilis*[46] by partially inhibiting DNA and protein synthesis and also

totally inhibiting RNA synthesis as a primary target[43]. However, allicin will not present unless the barriers of enzyme alliin alkyl-sulfenate-lyase (EC 4.4.1.4) and non-protein amino acid S-allylcysteine S-oxide (alliin) were broken-down[43], however, the effects of temperature on the activity of crude aqueous extract of garlic showed that optimum temp is $80^{O}C$ and may decrease or maintain when the temperature is increased. The temperature that use for cooking Kang-Kati in this experiment is about $90^{O}C$ so it might decrease antibacterial activity of garlic under aqueous extraction, but it need the further investigation for the activity of the extract from the garlic in the extract.

For *C. citratus* (Lemongrass), it was reported by the previous study that its major chemical component contains myrcene, traces of limonene, geranyl acetate, nerol, citronellal, geraniol, citral and neral[62], which can inhibit the growth of about 17 serotypes of *Salmonella* sp. (inhibition zone range of 7-11 mm.) by the ethanolic extracts[33]. The previous study also reported that the oil extraction method provide a better antibacterial activity than the fresh extraction by manual extractor and squeezing[57] so that the oil phase of the extract might be a source of antibacterial compound(s) in lemongrass extracted. However, the active compound(s) in lemongrass need the further investigation.

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The most active compound of *A. galangal* (Galangal) was investigated by the previous study and reported that the major compound was terpinen-4-ol[66]. The active compound that contain in *A. galangal* is more effective when they were extracted by the non-polar

extractant, compare between hexane and water, which means that most of the active compound are non-polar[67]. In addition, They also reported that it can inhibit the growth of many fungal and bacterial such as *S. typhimurium*, *S. aureus*, *F. solani* KACC 40384, and *B. cinera* KCTC 6973[69]. The oil extraction phase might give higher antibacterial compound than in aqueous extraction against food-borne pathogens. However, the active compound(s) in galangal need the further investigation.

Nevertheless, plants that used in this experiment also contain the active compound in a group of flavonoid. Flavonoids are water soluble polyphenolic molecules, normally containing 15 carbon atoms. Flavonoids are classified as the polyphenol family which can be visualized as two benzene rings which are joined together with a short three carbon chain. The flavonoids consist of 6 major subgroups: chalcone, flavone, flavonol, flavanone, anthocyanins and isoflavonoids. Flavonoids have antioxidant activity. Flavonoids are becoming very popular because they have a lot of health benefit. In addition, some of the activities attributed to flavonoids including anti-allergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral[71]. However, these compounds need the further investigation for more understanding.

In addition, by the "2 hour rule", general food safety rule for ready-to-eat food, foods must be discarded when they were left out at room temperature for longer than two hours. Moreover, when temperatures are above 90° F (32° C), discard food after one hour[72]. This experiment shows that Thai curry, which used curry paste as main ingredient, seem to be able to inhibit the *S. enterica Enteritidis*, *S. enterica 4,5,12:i:-* (human), and *L. monocytogenes* 10403S growth in ready-to-eat food longer than 2 hours even though the foods were kept in Thai traditional style, room temperature, approximately 32 °C. Curry paste is the main source of antibacterial compound against *S. enterica Enteritidis*, *S. enterica 4,5,12:i:-* (human), and *L. monocytogenes* 10403S growth.

By the result, it indicated that the curry paste which was extracted by the real food model can inhibit both gram positive, *Salmonella sp.*, and gram negative microorganism, *L. monocytogenes.*. Normally, the synthetic antibiotic that available in the market such as penicillins, cephalosporins, and carbapenems has the same characteristic which is the structure of beta-lactams[73]. The beta-lactams all work by interrupting the synthesis of the bacterial cell wall. The walls of bacteria are made of a complex polymeric material called as peptidoglycan, which is produce by N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM)[73]. The mechanism that beta-lactam antibiotics kill bacteria is that it will bind and inhibit enzymes required for the synthesis of the peptidoglycan wall. Whenever the antibiotic bind with the bacterial cell wall, it will leave the gap which

make all of the cell material in the bacteria spill out and cause the death of the bacteria[74]. While they have little effect on resting bacteria, they are lethal to dividing bacteria as defective walls cannot protect the organism form bursting in hypotonic surroundings[73]. Eventhough, the using of synthetic antibiotic is effective to kill the microorganism, however, they are costly and may alter the original characteristic of the food and may destroy the aesthetic of the food.

CONCLUSION

The curry paste that was made by the traditional model has an antimicrobial activity against *Salmonella enterica Enteritidis*, *Salmonella enterica 4,5,12:i:-* (human) US clone, and *Listeria monocytogenes* 10403S since the third to the fourth hour, fourth to sixth, and third to fourth hour, respectively. This might provide a new and alternative treatment approach for *Salmonella enterica Enteritidis*, *Salmonella enterica 4,5,12:i:-* (human) US clone, and *Listeria monocytogenes* 10403S since the third to the fourth hour, fourth to sixth, and third to fourth hour, respectively. Kang-Kati showed promising antibacterial activity as in real food model. This might be another explanation as food safety aspect that why Kang-Kati was kept in food cabinet at room temperature (about 32°C all year around) without food poisoning. In addition, Kang-Kati has high potential to be functional food.

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APPENDIXES

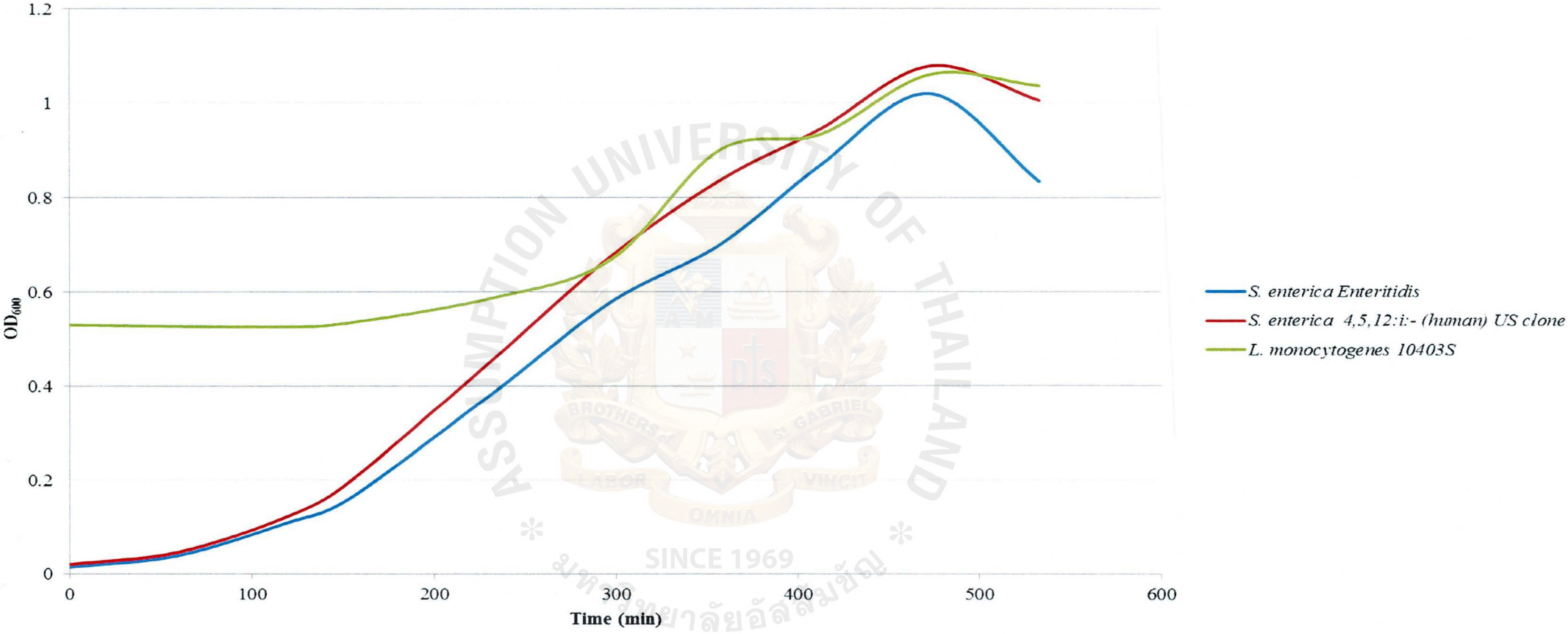

Figure 5: Growth curve of *S. enterica Enteritidis*, *S. enterica 4,5,12:i:-* (human) US clone, and *L. monocytogenes* 10403S with NB medium

Raw Data

The CFU/ml information of *Salmonella enterica Enteritidis*

Table 7: CFU/ml of *S. enterica Enteritidis*, control, from each replication

Time (Hr.)	Replication 1	Replication 2	Replication 3	Average	Log CFU/ml
0	0	0	0	0	0
1	0	0	0	0	0
2	0	0	0	0	0
3	480000	450000	440000	456666.7	5.65
4	680000	620000	590000	630000	5.8
5	820000	830000	840000	830000	5.91
6	910000	900000	900000	903333.3	5.96

Table 8: CFU/ml of *S. enterica Enteritidis*, Kang-Kati, from each replication

Time (Hr.)	Replication 1	Replication 2	Replication 3	Average	Log CFU/ml
0	0	0	0	0	0
1	0	0	0	0	0
2	0	0	0	0	0
3	350000	360000	320000	343333.3	5.53
4	400000	510000	370000	426666.7	5.62
5	480000	790000	620000	630000	5.79
6	620000	840000	860000	773333.3	5.88

The CFU/ml information of *Salmonella enterica 4,5,12:i:-* (human) US clone

Table 9: CFU/ml of *Salmonella enterica 4,5,12:i:-* (human) US clone, control, from each replication

Time (Hr.)	Replication 1	Replication 2	Replication 3	Average	Log CFU/ml
0	0	0	0	0	0
1	0	0	0	0	0
2	310000	330000	340000	326666.70	5.51
3	510000	480000	520000	503333.30	5.70
4	680000	690000	720000	696666.70	5.84
5	770000	810000	880000	820000.00	5.91
6	900000	950000	940000	930000.00	5.97

Table 10: CFU/ml of *S. enterica 4,5,12:i:-* (human) US clone, Kang-Kati, from each replication

Time (Hr.)	Replication 1	Replication 2	Replication 3	Average	Log CFU/ml
0	0	0	0	0	0
1	0	0	0	0	0
2	380000	0	380000	253333.33	5.40
3	460000	360000	490000	436666.67	5.64
4	540000	450000	600000	530000.00	5.72
5	640000	580000	700000	640000.00	5.81
6	700000	670000	800000	723333.33	5.86

The CFU/ml information of *Listeria monocytogenes* 10403S

Table 11: CFU/ml of *L. monocytogenes* 10403S, control, from each replication

Time (Hr.)	Replication 1	Replication 2	Replication 3	Average	Log CFU/ml
0	0	0	0	0	0
1	0	0	0	0	0
2	0	0	0	0	0
3	400000	400000	430000	410000.00	5.61
4	570000	500000	470000	513333.33	5.70
5	800000	650000	620000	690000.00	5.84
6	970000	860000	820000	883333.33	5.95

Table 12: CFU/ml of *L. monocytogenes* 10403S, Kang-Kati, from each replication

Time (Hr.)	Replication 1	Replication 2	Replication 3	Average	Log CFU/ml
0	0	0	0	0	0
1	0	0	0	0	0
2	0	0	0	0	0
3	300000	310000	320000	310000.00	5.49
4	410000	420000	440000	423333.33	5.63
5	550000	560000	580000	563333.33	5.75
6	800000	740000	800000	780000.00	5.89

Statistical analysis for *Salmonella enterica Enteritidis*

The TTEST Procedure

Statistics

Variable	trt	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev	Upper CL Std Dev	Std Err
colony	cul33	3	5.6106	5.6593	5.708	0.0102	0.0196	0.1233	0.0113
colony	soup	3	5.4688	5.5352	5.6015	0.0139	0.0267	0.1679	0.0154
colony	Diff (1-2)		0.071	0.1241	0.1773	0.014	0.0234	0.0673	0.0191

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	6.49	0.0029
colony	Satterthwaite	Unequal	3.67	6.49	0.0039

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	1.85	0.7007

The TTEST Procedure

Statistics

Variable	trt	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev	Upper CL Std Dev	Std Err
colony	cul34	3	5.7209	5.7986	5.8763	0.0163	0.0313	0.1967	0.0181
colony	soup	3	5.4454	5.6259	5.8065	0.0378	0.0727	0.4568	0.042
colony	Diff (1-2)		0.0458	0.1726	0.2995	0.0335	0.056	0.1608	0.0457

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	3.78	0.0195
colony	Satterthwaite	Unequal	2.72	3.78	0.0385

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	5.40	0.3127

The TTEST Procedure

Statistics

Variable	trt	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev	Upper CL Std Dev	Std Err
colony	cul35	3	5.9061	5.9191	5.9321	0.0027	0.0052	0.0329	0.003
colony	soup	3	5.5216	5.7904	6.0592	0.0563	0.1082	0.68	0.0625
colony	Diff (1-2)		-0.045	0.1286	0.3023	0.0459	0.0766	0.2201	0.0625

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	2.06	0.1089
colony	Satterthwaite	Unequal	2.01	2.06	0.1754

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	427.61	0.0047

The TTEST Procedure

Statistics

Variable	trt	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev	Upper CL Std Dev	Std Err
colony	cul36	3	5.949	5.9558	5.9627	0.0014	0.0028	0.0174	0.0016
colony	soup	3	5.6868	5.8837	6.0806	0.0413	0.0793	0.4981	0.0458
colony	Diff (1-2)		-0.055	0.0721	0.1992	0.0336	0.0561	0.1611	0.0458

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	1.58	0.1904
colony	Satterthwaite	Unequal	2	1.58	0.2556

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	818.37	0.0024

Statistical analysis for *S. enterica 4,5,12:i:-* (human) US clone

The TTEST Procedure

Statistics

Variable	trt	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev	Upper CL Std Dev	Std Err
colony	cul42	3	5.4629	5.5138	5.5646	0.0107	0.0205	0.1287	0.0118
colony	soup	3	-4.283	3.7199	11.722	1.6773	3.2215	20.246	1.8599
colony	Diff (1-2)		-3.37	1.7939	6.958	1.3648	2.278	6.5459	1.86

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	0.96	0.3894
colony	Satterthwaite	Unequal	2	0.96	0.4366

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	24761.3	<.0001

The TTEST Procedure

Statistics

Variable	trt	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev	Upper CL Std Dev	Std Err
colony	cul43	3	5.6556	5.6987	5.7419	0.0091	0.0174	0.1092	0.01
colony	soup	3	5.4607	5.6364	5.8121	0.0368	0.0707	0.4445	0.0408
colony	Diff (1-2)		-0.054	0.0623	0.1791	0.0309	0.0515	0.148	0.042

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	1.48	0.2125
colony	Satterthwaite	Unequal	2.24	1.48	0.2636

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	16.56	0.1139

The TTEST Procedure

Statistics

Variable	trt	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev	Upper CL Std Dev	Std Err
colony	cul44	3	5.8109	5.8429	5.8749	0.0067	0.0129	0.0811	0.0074
colony	soup	3	5.5642	5.7213	5.8783	0.0329	0.0632	0.3973	0.0365
colony	Diff (1-2)		0.0182	0.1216	0.2251	0.0273	0.0456	0.1311	0.0372

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	3.27	0.0309
colony	Satterthwaite	Unequal	2.17	3.27	0.0740

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	24.02	0.0799

The TTEST Procedure

Statistics

Variable	trt	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev	Upper CL Std Dev	Std Err
colony	cul45	3	5.8404	5.9132	5.9859	0.0152	0.0293	0.184	0.0169
colony	soup	3	5.7034	5.8049	5.9064	0.0213	0.0409	0.2567	0.0236
colony	Diff (1-2)		0.0277	0.1083	0.1888	0.0213	0.0355	0.1021	0.029

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	3.73	0.0203
colony	Satterthwaite	Unequal	3.63	3.73	0.0242

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	1.95	0.6787

The TTEST Procedure

Statistics

Variable	trt	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev	Upper CL Std Dev	Std Err
colony	cul46	3	5.9375	5.9684	5.9993	0.0065	0.0124	0.0782	0.0072
colony	soup	3	5.7584	5.8581	5.9577	0.0209	0.0401	0.2521	0.0232
colony	Diff (1-2)		0.0429	0.1103	0.1776	0.0178	0.0297	0.0853	0.0243

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	4.55	0.0104
colony	Satterthwaite	Unequal	2.38	4.55	0.0322

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	10.39	0.1756

Statistical analysis for *L. monocytogenes* 10403S

The TTEST Procedure

Statistics

Variable	trt	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev	Upper CL Std Dev	Std Err
colony	cul63	3	5.5675	5.6125	5.6576	0.0094	0.0181	0.114	0.0105
colony	soup	3	5.4564	5.4912	5.526	0.0073	0.014	0.0881	0.0081
colony	Diff (1-2)		0.0846	0.1213	0.1581	0.0097	0.0162	0.0466	0.0132

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	9.17	0.0008
colony	Satterthwaite	Unequal	3.76	9.17	0.0010

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	1.67	0.7479

The TTEST Procedure

Statistics

Variable	trt	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev	Upper CL Std Dev	Std Err
colony	cul64	3	5.6027	5.709	5.8152	0.0223	0.0428	0.2688	0.0247
colony	soup	3	5.5878	5.6265	5.6652	0.0081	0.0156	0.098	0.009
colony	Diff (1-2)		0.0095	0.0825	0.1555	0.0193	0.0322	0.0925	0.0263

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	3.14	0.0349
colony	Satterthwaite	Unequal	2.52	3.14	0.0653

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	7.53	0.2345

The TTEST Procedure

Statistics

Variable	trt	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev	Upper CL Std Dev	Std Err
colony	cul65	3	5.6898	5.8361	5.9824	0.0307	0.0589	0.3701	0.034
colony	soup	3	5.7215	5.7507	5.7798	0.0061	0.0117	0.0737	0.0068
colony	Diff (1-2)		-0.011	0.0855	0.1817	0.0254	0.0425	0.122	0.0347

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	2.47	0.0693
colony	Satterthwaite	Unequal	2.16	2.47	0.1234

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	25.21	0.0763

The TTEST Procedure

Statistics

Variable	trt	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev	Upper CL Std Dev	Std Err
colony	cul66	3	5.8516	5.945	6.0384	0.0196	0.0376	0.2363	0.0217
colony	soup	3	5.8432	5.8918	5.9404	0.0102	0.0195	0.1229	0.0113
colony	Diff (1-2)		-0.015	0.0532	0.1212	0.018	0.03	0.0861	0.0245

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	2.18	0.0952
colony	Satterthwaite	Unequal	3.01	2.18	0.1176

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	3.70	0.4255

Formula for calculating Specific Growth rate

$$\mu = \frac{0.693}{t_d}$$, While t_d is doubling time

Specific Growth rate of *S. enterica Enteritidis*

Control

Cfu/ml at 200000 = 2.25 hr

Cfu/ml at 400000 = 3.75 hr

Doubling time (t_d) = time that microorganism used to increase their population from N to 2N

= 3.75 – 2.25

= 1.5 hour

Kang-Kati

Cfu/ml at 200000 = 1.25 hr

Cfu/ml at 400000 = 2.55 hr

Doubling time (t_d) = time that microorganism used to increase their population from N to 2N

= 2.5 - 1.25

= 1.25 hour

Calculate for the specific growth rates under the two conditions

Control

$$\mu = \frac{0.693}{1.5}$$

$= 0.46 \text{ hour}^{-1}$

Kang-Kati

$$\mu = \frac{0.693}{1.25}$$

$= 0.55 \text{ hour}^{-1}$

Specific Growth rate of *S. enterica 4,5,12:i:-* (human) US clone

Control

Cfu/ml at 200000 = 1.00 hr

Cfu/ml at 400000 = 2.32 hr

Doubling time (t_d) = time that microorganism used to increase their population from N to 2N

= 2.32 – 1.00

= 1.32 hour

Kang-Kati

Cfu/ml at 200000 = 1.2 hr

Cfu/ml at 400000 = 3.0 hr

Doubling time (t_d) = time that microorganism used to increase their population from N to 2N

= 3.0 – 1.2

= 1.8 hour

Calculate for the specific growth rates under the two conditions

Control

$$\mu = \frac{0.693}{1.32}$$

$= 0.53 \text{ hour}^{-1}$

Kang-Kati

$$\mu = \frac{0.693}{1.80}$$

$= 0.39 \text{ hour}^{-1}$

Specific Growth rate of *L. monocytogenes* 10403S

Control

Cfu/ml at 200000 = 1.80 hr

Cfu/ml at 400000 = 3.08 hr

Doubling time (t_d) = time that microorganism used to increase their population from N to 2N

= 3.08 – 1.80

= 1.28 hour

Kang-Kati

Cfu/ml at 300000 = 2.40 hr

Cfu/ml at 600000 = 3.76 hr

Doubling time (t_d) = time that microorganism used to increase their population from N to 2N

= 3.76 – 2.4

= 1.36 hour

Calculate for the specific growth rates under the two conditions

Control

$$\mu = \frac{0.693}{1.28}$$

$$= 0.54 \text{ hour}^{-1}$$

Kang-Kati

$$\mu = \frac{0.693}{1.36}$$

$$= 0.51 \text{ hour}^{-1}$$