Natural Antibacterial Activity of Thai Red Curry Paste in Water based Curry Model (Kang-Pa) on *Salmonella* sp. and *Listeria monocytogenes*

BY

Ms. Supawan Rattanakom

ID.5110535

A special project submitted to

School of Biotechnology, Assumption University

In part fulfillment of the requirements of the Degree of Bachelor of Science in Biotechnology

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By	**:**	**Ms. Supawan Rattanakom**
Advisor	**:**	**Dr.Patchanee Yasurin**
Level of study	**:**	**Bachelor of Science**
Department	**:**	**Agro-Industry**
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Ms. Supawan Rattanakom
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ABSTRACT

Natural Antibacterial Activity of Thai Curry Paste in Thai Red Curry-Water Base (Kang-Pa) Model on *Salmonella* sp. and *Listeria monocytogenes*

Supawan Rattanakom

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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. and *Listeria monocytogenes* are food pathogen which has been reported about their outbreaks frequently in wide variety of foods. Thai red curry (Kang-Pa) is a Thai cultural dish and become well-known menu found worldwide. Thai curry paste, Thai red curry main ingredients, compose of many 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 model as natural antibacterial agent on *S. enteric* 4, 5, 12: i: - (human) US clone, *S. enteric* Enteritidis and *L. monocytogenes* 10403S (gift of S. Chaturongakul, MU). Thai curry paste in-vitro antibacterial activity was evaluated by cell count serial dilution method on SS media for *Salmonella* sp. and on BHI for *L. monocytogenes* every hour for 6 hrs at 30°C. Thai red curry was prepared by Thai homemade authentic cooking method. The result showed that the *S. enteric* 4, 5, 12: i: - (human) US clone level in Thai red curry 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 *S. enteric* Enteritidis in Kang-Pa showed significant lower in number compare to control up to three hours: 2^{nd} hr; 5.705±0.199 and 6.370±0.085 and 3^{rd} hr; 5.872±0.255 and 6.878±0.177 log CFU/ml. *L. monocytogenes* in Kang-Pa was significant lower than those of positive control ($P < 0.05$), since 1^{st} – 6^{th} hour: 1^{st} hr; 6.17±0.04 and 6.34±0.10, 2^{nd} hr; 6.29±0.03 and 7.03±0.04, 3^{rd} hr; 6.67±0.02 and 7.36±0.01, 4^{th} hr; 7.09±0.11 and 8.22±0.004, 5^{th} hr; 7.17±0.12 and 8.26±0.004 and 6^{th} hr; 7.31±0.003 and 8.91±0.01 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 showed promising antibacterial activity against food-borne pathogenic bacteria, *Salmonella* sp. and *L. monocytogenes*.

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

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INTRODUCTION

Nowadays, the using of natural antibiotics food for food preservation method become more and more popular thus the consumption trends are changing. People concern about health and what they eat more. Thus from the globalization the international trading on foods and ingredients expand increasingly. Food safety has become an increasingly important international concern.

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). Foodborne disease is an increasingly serious public health problem all over the world and the cause of that is determined to be microorganisms.

Salmonella Enteritidis is an important cause of human illness. A person infected with *S.* Enteritidis usually has fever, abdominal cramps, and diarrhea beginning 12 to 72 hours after consuming a contaminated food or beverage. The illness usually lasts 4 to 7 days, and most persons recover without antibiotic treatment. However, the diarrhea can be severe, and hospitalization may be required. The elderly, infants, and those with impaired immune systems may have a more serious illness. In these patients, the infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics (CDC, 2012).

Listeria monocytogenes is an opportunistic intracellular pathogen that has become an important cause of human foodborne infections worldwide (Liu, 2006). The *Listeria* species are tolerant to extreme conditions such as low pH, low temperature and high salt conditions

(Sleator *et al.,* 2003; Liu *et al.,* 2005). *L. monocytogenes* has been described as opportunistic pathogen affecting mainly children, pregnant women, the aged and immune-challenged individuals (Schlech, 2000; Liu, 2006). Also a wide variety of animals including sheep, cattle, goats, pigs, rabbits, mice, birds, and fish are also infected (Ireton *et al*., 2006). The pathogen is also responsible for listeria infections that can lead to abortion, bacteraemia, sepsis, and meningoencephalitis (Khelef *et al*., 2006; Sukhadeo *et al*., 2009).

Spices and aromatic vegetable materials have long been used in food for flavoring. Since the ancient times, they have been used for preventing food spoilage and deterioration and also for extending the shelf life of foods (Shan *et al*., 2007). 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 curry 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).

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 objectives 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 food-borne pathogens.

OBJECTIVE

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

LITERATURE REVIEW

Foodborne Diseases and Statistics

Foodborne illnesses are infections or irritations of the gastrointestinal (GI) tract caused by food or beverages that contain harmful bacteria, parasites, viruses, or chemicals. The GI tract is a series of hollow organs joined in a long, twisting tube from the mouth to the anus (HHS, 2012). Common symptoms of foodborne illnesses include vomiting, diarrhea, abdominal pain, fever, and chill however most foodborne illnesses are acute. They happen suddenly and last a short time, and most people recover on their own without treatment. Rarely, foodborne illnesses may lead to more serious complications (HHS, 2012). Each year, an estimated 48 million people in the United States experience a foodborne illness. Foodborne illnesses cause about 3,000 deaths in the United States annually (Scallan, 2011).

The majority of foodborne illnesses are caused by harmful bacteria and viruses (CDC, 2010). Some parasites and chemicals also cause foodborne illnesses. According to CDC's 2011 Estimates for Foodborne Illness Report eight known pathogens account for the vast majority of illnesses, hospitalizations, and deaths. Tables 1- 4 list the top five pathogens causing illness, hospitalization, and death.

Outbreak of *Salmonella*

Salmonella spp. is one of the top five foodborne pathogen results causing illness and hospitalized in United State. The disease called Salmonellosis. There are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths worldwide due to Salmonella (Bhunia, 2008). Nontyphoidal salmonellosis or enterocolitis is caused by at least 150 *Salmonella* serotypes with *Salmonella* Typhimurium and *Salmonella* Enteritidis being the most common serotypes in the United States. Infection always occurs via ingestion of water or food contaminated (Pui *et al*, 2011). The outbreaks of *Salmonella* Enteritidis and *Salmonella* 4,[5],12:i:- that have been reported by CDC in United State in the past 5 years are;

- 2012, A total of 46 persons infected with the outbreak strain of *Salmonella* Enteritidis linked to ground beef were reported from 9 states. The number of ill persons identified in each state was as follows: Maine (2), Massachusetts (3), New Hampshire (3), New York (20), North Carolina (1), Rhode Island (3), Vermont (11), Virginia (2), and West Virginia (1). Twelve ill persons were hospitalized, and no deaths were reported.
- 2012, Multistate outbreak of *Salmonella* Enteritidis infections which was associated with eating food from a Mexican-style fast food restaurant chain, Restaurant Chain A. A widely distributed contaminated food product might cause illnesses in a specific region and across the United States. As of January 19, 2012, a total of 68 individuals infected with the outbreak strain of *Salmonella* Enteritidis have been reported from 10 states. The number of ill persons identified in each state with the outbreak strain was as follows: Texas (43), Oklahoma (16), Kansas (2), Iowa (1), Michigan (1), Missouri (1), Nebraska (1), New Mexico (1), Ohio (1), and Tennessee (1).
- 2011, Multistate outbreak of *Salmonella* Enteritidis infections linked to Turkish pine nuts purchased from bulk bins at Wegmans grocery stores. A total of 43 individuals infected with the outbreak strain of *Salmonella* Enteritidis were reported from 5 states. The number of ill persons identified in each state with the outbreak strain was as follows: Maryland (1), New Jersey (2), New York (28), Pennsylvania (8), and Virginia (4).

- 2011, Multistate outbreak of *Salmonella* Enteritidis infections linked to alfalfa sprouts and spicy sprouts. A total of 25 persons with the outbreak strain of *Salmonella* Enteritidis have been reported from 5 states: Idaho (3), Montana (10), New Jersey (1), North Dakota (1) and Washington (10).
- 2010, Multistate Outbreak of Human *Salmonella* I 4,[5],12:i:- Infections Linked to alfalfa sprouts. 140 individuals infected with the outbreak strain of *Salmonella* serotype I 4,[5],12:i:-, whose illnesses began (onset dates) since November 1, were reported from 26 states and the District of Columbia. The number of ill persons identified in each state and the District of Columbia with the outbreak strain is as follows: Arkansas (1), California (1), Colorado (1), Connecticut (1), District of Columbia (1), Georgia (1), Hawaii (1), Iowa (1), Illinois (70), Indiana (13), Kentucky (1), Louisiana (1), Massachusetts (2), Maryland (1), Missouri (23), Nebraska (1), Nevada (1), New Jersey (1), New York (2), North Carolina (1), Oregon (1), Pennsylvania (4), South Carolina (1), South Dakota (1), Tennessee (2), Virginia (2), and Wisconsin (4).
- 2010, Multistate outbreak of Human *Salmonella* Enteritidis associated with shell eggs. Approximately 1,939 illnesses were reported that are likely to be associated with this outbreak.
- 2010, Multistate outbreak of Human *Salmonella* I 4,[5],12:i:- infections associated with frozen rodents. A total of 34 individuals infected with a matching strain of *Salmonella* serotype I 4,[5],12:i:- have been reported from 17 states since January 1, 2010. The number of ill persons identified in each state with this strain is as follows: AL (1), AZ (1), CO (1), GA (7), IA (1), IL (3), MA (3), MI (1), MO (3), NC (3), NV (1), NY (2), SC (1), TN (1), VA (1), WI (3), and WY (1).

Outbreak of *Listeria monocytogenes*

Listeria monocytogenes is in the top five foodborne pathogen resulting in Death. Even though *L. monocytogenes* doesn't appear in top five foodborne pathogen resulting in Hospitalize, it shows high fatal rate. The invasive form causes life-threatening disease in persons belonging to a specific risk group. This risk group comprises the elderly, pregnant women and people with impaired immune status due to organ transplants or severe underlying disease such as cancer or human immunodeficiency virus (Schlech III, 2000; Vazquez-Boland *et al.*, 2001). The non-invasive disease causes a self-resolving febrile gastroenteritis, with no predisposing underlying disease detected (Salamina *et al.*, 1996; Miettinen *et al.*, 1999; Frye *et al.*, 2002). The outbreaks of *Listeria monocytogenes* that have been recently reported by CDC in United State are;

- 2012, Multistate outbreak of Listeriosis linked to imported Frescolina Marte Brand Ricotta Salata Cheese. A total of 22 persons infected with the outbreak-associated strain of Listeriamonocytogenes have been reported from 13 states and the District of Columbia. 20 ill persons reported being hospitalized. Four deaths have been reported. Listeriosis contributed to at least 2 of these deaths. One fetal loss has also been reported.
- 2011, 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.

The outbreaks of *Listeria monocytogenes* that have been recently reported in Europe and others are;

- 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 (Coetzee *et al.*, 2011).
- 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'(Fretz *et al.*, 2010).
- 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 (Canada, 2009).

Extraction solvent: Water

Solvents differ in their extraction capabilities depending on their own chemical properties and the properties of the extraction substance. Most widely used solvents are water. Water is often referred to as a *universal solvent.* In this project Kang-Pa food model using water in cooking can refer as using water to extract the compounds out of the herbs inside curry paste which is one of the Kang-Pa's condiment.

Water is referred to as *the universal solvent*, dissolving many types of substances. Hydrophilic (water-loving) substances mix and dissolve well with water (Cowan, 1999). Water is a highly polar solvent with polarity index value of 9 (Cheremisinoff, 2003). In the presence of miscible organic solvents, water might display less polarity and hydrogen bonding character.

Antimicrobial properties of herbs

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted (Ríos and Recio, 2005). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant (Nascimento *et al*., 2000). Herbs and spices have been used in cooking for long times. The antimicrobial properties of herbs and spices that have been used in curry paste have been reported about their antimicrobial properties which are;

Chilli

Chili has been used since ancient times; Amerindians recognized the capability of chilli and used them as therapeutic. Also form Ethnobotanical data Capsicum species harbor many potentially economically significant compounds yet to be discovered (Cichewicz and Thorpe, 1996). However it may have been in response to their therapeutic properties as antimicrobial and anti-hemolytic agents. The presence of the secondary metabolite capsaicin in these species has long been associated with strong analgesic properties (Cordell and Araujo, 1993), alterations in the pH of gastrointestinal tract epithelial cells, prevention of microbial infections (Tellez *et al*., 1993) and possible anticarcinogenic effects (Surh and Lee, 1995). From the work of Leuschner and 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

Lemongrass

Duan and Zhao (2009) 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. Nanasombat and Lohasupthawee (2005) studied crude extracts and essential oils of many herbs including crude ethanolic extract of lemongrass which was active against 17 strains of *salmonella* spp. (7-11 mm) from total 25 strains including *S.* Enteritidis. 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. Use of fresh lemongrass is typical for Southeast Asia and Sri Lanka. Lemongrass is most popular in Thailand, Vietnam, Cambodia and Indonesia. In Thailand, finely ground fresh lemongrass is added to curry pastes. Lemongrass plant is generally recognized as safe (GRAS) for human consumption and as a plant extract/essential oil (21 CFR section 182.20) (Simon *et al.*, 1984).

Garlic

Garlic is one of the herbs that have a lot of scientific report about its antimicrobial properties which comes from the substance called allicin (allyl 2-propene thiosulphinate). Alicin inhibits various thiol-dependent enzymatic systems of bacteria (Ankri and Mirelman, 1999). 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 curry paste, mechanic mortar will be able to extract allicin out. Also from the work of Ross and co-authors (2001) 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℃. While from the work of Leuschner and Zamparini (2002) noticed 2-log reduction in *S. enteri*ca concentration with 1% v/v garlic oil at 37℃. In term of fresh garlic, raw garlic extract is a more effective antimicrobial agent than antibiotics currently in use; Ciprofloxacin and Ampicillin when testing on *Salmonella* spp. (Eja *et al.*, 2007). Also the effect of garlic extract is most pronounced on enteric bacterial pathogens. Garlic was also found to be effective against *L. monocytogenes* (Kumar and Berwal, 1998; Singh *et al.*, 2001). Abubarka (2009) studied the effect of raising the temperature on the effectiveness of garlic. He found that the activity of garlic increased with increase in temperature up to 80℃, beyond which the activity remained constant or decreased, similar to the reports also presented (Roy *et al.*, 2006).

Shallot

The bulb of shallot has been used in African cooking and in salads (Adeniyi and Anyiam, 2004). Shallot also has been used 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 bacteriostatic effect against *S. enterica* and have bactericidal effect against *L. monocytogenes* (Rattanachaikunsopon and Phumkhachorn, 2009). Fresh crude juice of shallot bulbs from the work of Mahmoudabadi and Gharib Nasery (2009) has been reported about its anti-fungal effect.

Galangal

Galangal can be also used not only for food ingredients but also for medical purposes, such as forcarminative, stomachic, antispasmodic, antichloristicand antibacterial drugs (Mayachiew and Devahastin, 2007). The most active compound of *A. galangal* (Galangal) was terpinen-4-ol (Janssen and Scheffer, 1985). It can inhibit the growth of many fungal and bacteria, both gram positive and gram negative. 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). Galangal is also used as a medicine for curing stomachache in China and Thailand (Yang and Eilerman, 1999). 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).

Kaffir lime peel

Nanasombat and Lohasupthawee (2005) studied crude ethnolic extracts form of kaffir lime peel and eseential oil form, found out that the extract and oil was active against *S.* Enteritidis. By using of pressurized hot water extraction on kaffir lime fruit peel, found out that when increase temperature in extraction the phenolic compound content increasing (Khuwijitjaru *et al.*, 2008). This means that the use of Kang-Pa cooking model in heating red curry paste might extract the phenolic compound content in kaffir lime peel out. 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 %) (Lawrence *et al.*, 1970).

Cumin

Cumin seed 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 chemicals that contain in the cumin, steam distillation technique are cuminic, cymene, dipentene, limonene, phellandrene and pinene (Services, 2012). 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 (Nanasombat and Lohasupthawee, 2005).

Table 1: Estimated annual number of domestically acquired, foodborne illnesses, hospitalizations, and deaths due to 31 pathogens and unspecified agents transmitted through food in United States (From CDC, 2011).

Foodborne Agents	Estimated annual number of illnesses (90% credible interval)	%	Estimated annual number of hospitalizations (90% credible interval)	%	Estimated annual number of deaths (90% credible interval)	%
31 known pathogens	9.4 million (6.6–12.7 million)	20	55,961 (39,534–75,741)	44	1,351 (712–2,268)	44
Unspecified agents	38.4 million (19.8–61.2 million)	80	71,878 (9,924–157,340)	56	1,686 (369–3,338)	56
Total	47.8 million (28.7–71.1 million)	100	127,839 (62,529–215,562)	100	3,037 (1,492–4,983)	100

***Known foodborne pathogens**: 31 pathogens known to cause foodborne illness. Many of these pathogens are tracked by public health systems that track diseases and outbreaks.

***Unspecified agents**: Agents with insufficient data to estimate agent-specific burden; known agents not yet identified as causing foodborne illness; microbes, chemicals, or other substances known to be in food whose ability to cause illness is unproven; and agents not yet identified. Because you can't "track" what isn't yet identified, estimates for this group of agents started with the health effects or symptoms that they are most likely to cause acute gastroenteritis.

*** 90% credible interval**

Table 2: Top five pathogens contributing to domestically acquired foodborne illnesses (From CDC, 2011).

Pathogen	Estimated number of illnesses	90% Credible Interval	%
Norovirus	5,461,731	3,227,078–8,309,480	58
Salmonella, nontyphoidal	1,027,561	644,786–1,679,667	11
Clostridium perfringens	965,958	192,316–2,483,309	10
Campylobacter spp.	845,024	337,031–1,611,083	9
Staphylococcus aureus	241,148	72,341–529,417	3
Subtotal			91

Table 3: Top five pathogens contributing to domestically acquired foodborne illnesses resulting in hospitalization (From CDC, 2011).

Pathogen	Estimated number of hospitalizations	90% Credible Interval	%
Salmonella, nontyphoidal	19,336	8,545–37,490	35
Norovirus	14,663	8,097–23,323	26
Campylobacter spp.	8,463	4,300–15,227	15
Toxoplasma gondii	4,428	3,060–7,146	8
E.coli (STEC) O157	2,138	549–4,614	4
Subtotal			**88**

Table 4: Top five pathogens contributing to domestically acquired foodborne illnesses resulting in death (From CDC, 2011).

Pathogen	Estimated number of deaths	90% Credible Interval	%
Salmonella, nontyphoidal	378	0–1,011	28
Toxoplasma gondii	327	200–482	24
Listeria monocytogenes	255	0–733	19
Norovirus	149	84–237	11
Campylobacter spp.	76	0–332	6
Subtotal			**88**

METHODOLOGY

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 Pattanakarn Rd. local market, Bangkok, Thailand. The raw materials were hand grinded by the mortar. In grinding, the raw materials were added in order and time as following; chili and salt for 5 min, garlic and shallot 4 min, galangal and lemongrass for 4 min, kaffir lime peel and cumin powder for 3 min, shrimp paste 2 min.

Table 5: 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 6: Order for adding the ingredients and time for grinding

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

Preparation of curry

The 500 ml of water mix with 45 gram of curry paste and boil for 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 (Gift of S. Chaturongakul, MU) was prepared by inoculating one loopful of *S. enterica* Enteritidis, *S. enterica* 4,5,12:i:- (human) US clone 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-Pa. Then, inoculated Kang-Pa was incubated at room temperature. The cell count serial dilution method was used to evaluate antibacterial activity by using the

Salmonella- Shigella agar and Brian Heart Infusion agar. The Kang-Pa 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 analysis

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.

RESULTS

The results from statistical program showed that the levels of *L. monocytogenes* in Kang-Pa was significant lower than those of positive control (BHI), since 1st – 6th 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* in Kang-Pa and in control (BHI) showed in table 7.

Table 7: Mean and SD of *L. monocytogenes* growth in Kang-Pa and Control (BHI) up to six hour

Hour	**Mean ± SD**	
	Kang-Pa (log CFU/ml)	**Control (BHI) (log CFU/ml)**
1	5.95±0.056 [a]	6.02±0.015 [a]
2	6.17±0.040 [a]	6.34±0.100 [b]
3	6.29±0.030 [a]	7.03±0.040 [b]
4	6.67±0.020 [a]	7.36±0.010 [b]
5	7.09±0.110 [a]	8.22±0.004 [b]
6	7.17±0.120 [a]	8.26±0.004 [b]

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

The result showed that the *S. enteric* in Kang-Pa was significant lower than those of positive control (NB). The values in Kang pa and NB at 4th hour were 7.09±0.11 and 8.22±0.004 log CFU/ml, respectively. At 5th hour were 7.17±0.12 and 8.26±0.004 log CFU/ml, respectively. At 6th hour were 7.31±0.003 and 8.91±0.01177 log CFU/ml, respectively. The comparison between Thai red curry and culture at 4th, 5th and 6th are different in 1 log cycle and more.

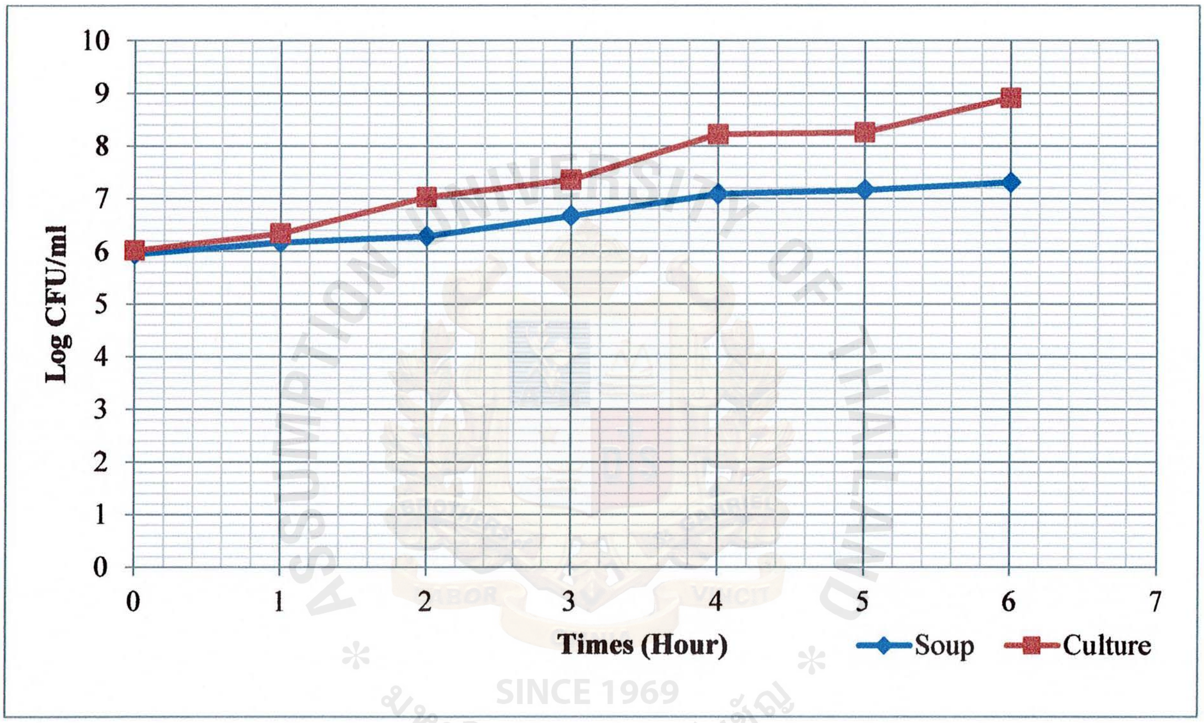

Figure 1: The histogram chart of *L. monocytogenes* (log CFU/ml) between Thai red curry and culture from 0 to 6 hours

The result showed that the *Salmonella enteric* Enteritidis (Human) level in Thai red curry was significantly lower than in nutrient broth, positive control, ($P < 0.05$) at 2 & 3 hr. The comparison between mean and SD growth of *S.* Enteritidis in Kang-pa versus control were presented in table 8.

Table 8: Mean and SD of *S.* Enteritidis growth in Kang-Pa and Control (NB) up to six hour

Hour	**Mean ± SD**	
	Kang-Pa (log CFU/ml)	**Control (NB) (log CFU/ml)**
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

The result showed that the *S. enteric* in Kang-Pa was significant lower than those of positive control (NB). The values in Kang pa and NB at 3 hr were 5.872±0.255 and 6.878±0.177 log CFU/ml, respectively. The comparison between Thai red curry and culture at 3 hr is different almost in 1 log

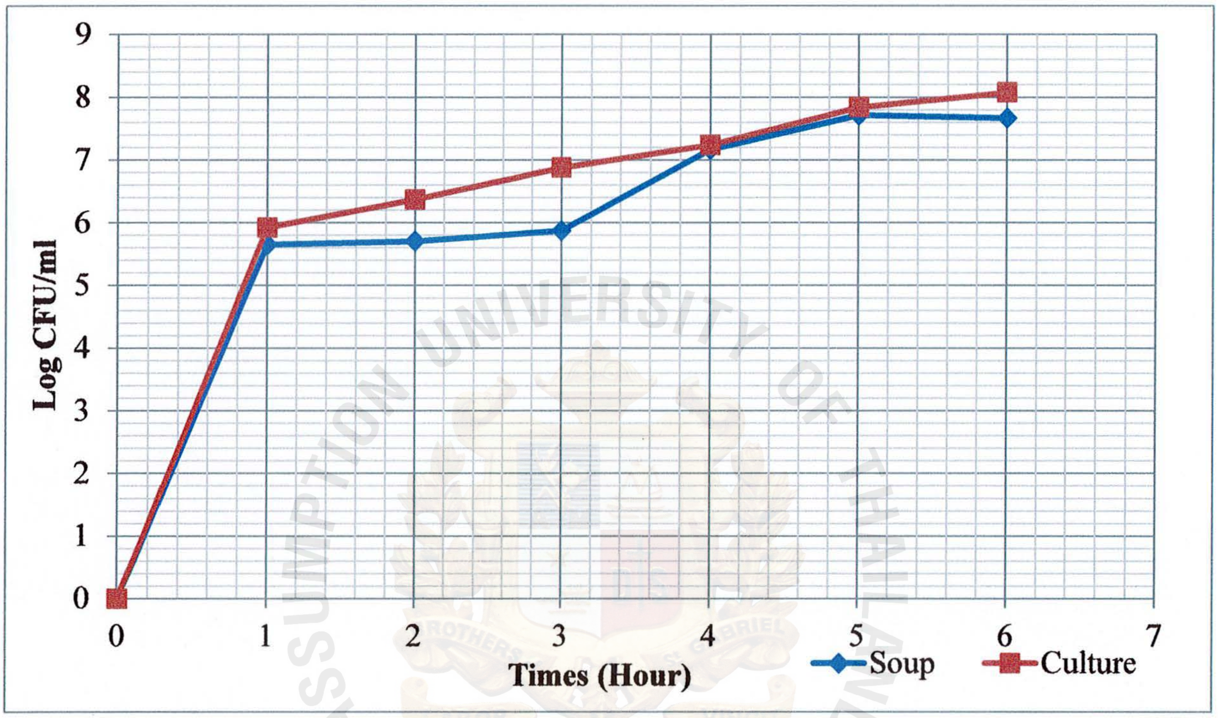

Figure 2: shows histogram chart of *Salmonella enteric* Enteritidis (Human) in Thai red curry and culture from 0 to 6 hours

The result showed that the *S. enterica* 4, 5, 12: i level in Thai red curry was significantly lower than in nutrient broth, as positive control, ($P < 0.05$) since 2^{nd} - 6^{th} hour. The comparison between mean and SD growth of *S. enterica* 4, 5, 12: i in Kang-pa versus control presented in table 9.

Table 9: Mean and SD of *S. enterica* 4, 5, 12: i growth in Kang-Pa and Control (NB) up to six hour

Hour	Mean ± SD	
	Kang-Pa (log CFU/ml)	**Control (NB) (log CFU/ml)**
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

The result showed that the *S.* 4, 5, 12: i in Kang-Pa was significant lower than those of positive control (NB). The values in Kang pa and NB at 4 hr were 5.85±0.16 and 6.97±0.6 log CFU/ml, respectively. At 5 hr were 5.92±0.22 and 6.26±0.27 log CFU/ml, respectively. The comparison between Thai red curry and culture at 4th and 5th are different almost in 1 log cycle.

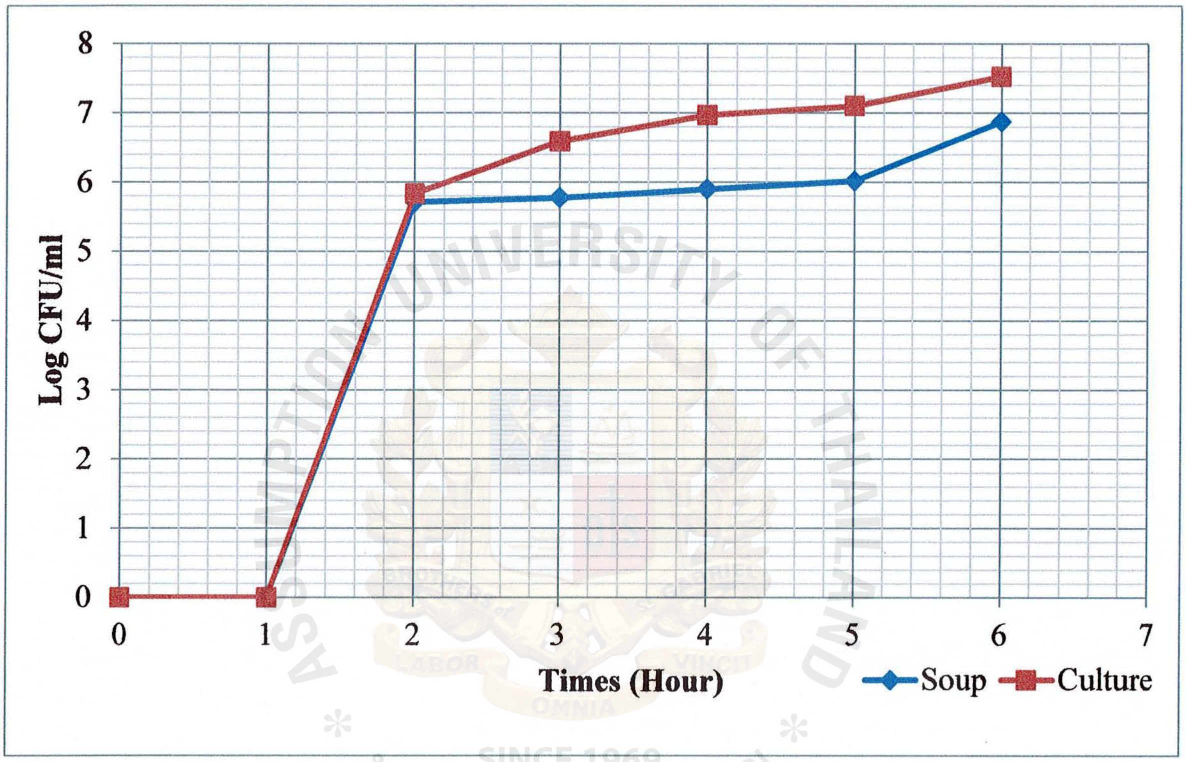

Figure 3: The histogram chart of *S. enterica* 4, 5, 12: i (log CFU/ml) between in Thai red curry and in control (NB) from 0 to 6 hours

Table 10: Comparison of specific growth rate of *S. enterica* Enteritidis (human), *S. enterica* 4,5,12:i:-(human), *L. monocytogenes* 10403S growth in Kang-Pa and control

Specific Growth Rate	($hour^{-1}$)	
	Kang-Pa	Control
S. enterica* Enteritidis *(human)	0.41	1.22
***S. enterica* 4,5,12:i:-(*human*)**	0.25	0.96
***L. monocytogenes* 10403S**	0.92	1.36

The result showed that specific growth rate of three foodborne pathogens compare to control. Kang-Pa shows lower specific growth rate than in control for all three pathogens.

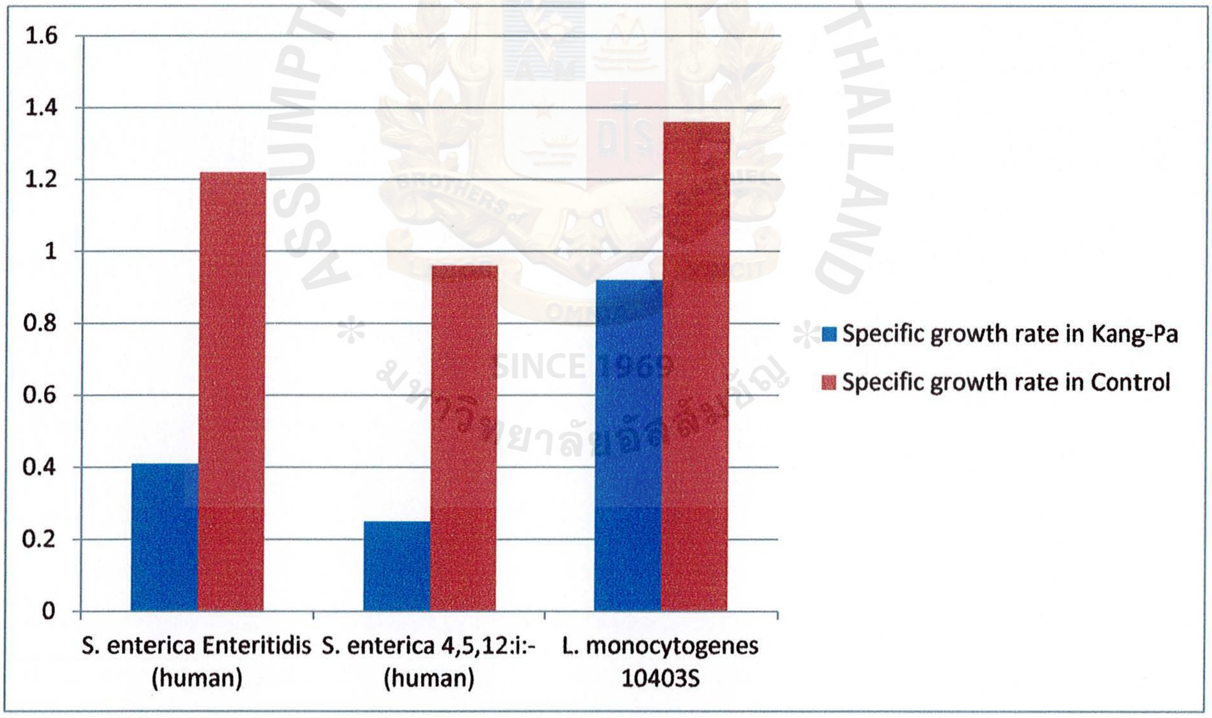

Figure 4: specific growth rate of *S. enterica* Enteritidis (human), *S. enterica* 4, 5, 12:i (human), *L. monocytogenes* 10403S growth in Kang-Pa and control

DISCUSSION

Listeria monocytogenes 10403S

Growth of *L. monocytogenes* was monitored at ambient temperature for 6 hr in nutrient broth and Kang-Pa food model. 1% v/v of *L. monocytogenes* was inoculated into both BHI and Kang-Pa. The result is presented in Figure 1. Kang-Pa effectively reduced the cell number of *L. monocytogenes* compared to broth nutrient broth in approximately 1 log cycle. Kang-Pa showed promising bacteriostatic effects up to 6 hr might come from the antimicrobial activities inside the curry paste ingredients.

Garlic which is one of the condiments in 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 curry paste, mechanic mortar will be able to extract allicin out. Also from the work of Kumar & Berwal (1998) and Singh and others (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 curry paste ingredient also has been studied about its antimicrobial properties. It has been reported to have an heat stable antimicrobial activity against bacteria and fungi by Amin and Kapadnis (2005). Thus by cooking, Kang-Pa was not reducing the potential of antimicrobial agents inside shallot. The oil of shallot also has been reported to have bactericidal effect against *L. monocytogenes* (Rattanachaikunsopon & Phumkhachorn, 2009).

Chili is main ingredient used to make 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 Leuschner and 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 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 curry paste. It contains antimicrobial compounds. The work of Khuwijitjaru and others (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 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 reported of cumin seed extracts or essential oil on *L. monocytogenes* inhibition has not yet been found. From previous information, the ingredients use in making curry paste using Kang-Pa food model show promising antimicrobial activity. Although the combination of above spices & herbs that have been used as food not yet been investigated. The result from this experiment shows that when cooking herbs & spices using food model, the spices & herbs still have antimicrobial properties.

The different in the growth of *L. monocytogenes* between normal media (BHI) and Kang Pa shows significantly different in growth level. However the function of the combination of herbs & spices in food-model need further investigate on the active molecular level of how antimicrobial agents react to subjected microorganism.

***Salmonella enterica* 4, 5, 12: i and *Salmonella enterica* Enteritidis**

Growth of *S. enterica* 4, 5, 12: i was monitored at room temperature for 6 hr. in Kang-Pa food model and in nutrient broth (control). The 1% v/v of *S. enterica* 4, 5, 12: i was inoculated into both Kang-Pa and NB. Results that were presented in Figure I showed that Kang-Pa statistically reduced the cell number of *S. enterica* 4, 5, 12: i when compared to nutrient broth (NB) in almost 1 log cycle at 3, 4 and 5 hour. Kang-Pa showed potentiality antimicrobial effects from 2 hr. to 6 hr. might come from the antimicrobial activities inside the red curry paste ingredients.

Growth of *S. enteric* Enteritidis was monitored at room temperature for 6 hr. in Kang-Pa food model and in nutrient broth (control). The 1% v/v of *S. enteric* Enteritidis was inoculated into both Kang-Pa and NB. Results that were presented in Figure I showed that Kang-Pa statistically reduced the cell number of *S. enteric* Enteritidis when compared to nutrient broth (NB) in almost 1 log cycle at 2 and 3 hour. Kang-Pa showed potentiality antimicrobial effects in retarding the growth of *S. enteric* Enteritidis 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. Garlic (Allium 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 (Ankri and Mirelman, 1999). 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 curry paste, mechanic mortar will be able to extract allicin out. Also from the work of Ross and co-authors (2001) 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℃. While from the work of Leuschner and Zamparini (2002) noticed 2-log reduction in *S. enteri*ca concentration with 1% v/v garlic oil at 37℃. In term of fresh garlic Eja and others (2007) found that raw garlic extract is a more effective antimicrobial agent than antibiotics currently in use; Ciprofloxacin and Ampicillin when testing on *Salmonella* spp. Also the effect of garlic extract is most pronounced on enteric bacterial pathogens. Abubarka (2009) studied the effect of raising the temperature on the effectiveness of garlic. He found that the activity of garlic increased with increase in temperature up to 80℃, beyond which the activity remained either constant or decreased, similar to the reports presented by Roy and others (2006). It is known that raising the temperature increases the solubility of chemical compounds (Abubarka, 2009). Thus from the founding, heating process during cooking wouldn't destroy the antimicrobial properties inside garlic.

From the work of Duan and Zhao (2009) 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. Nanasombat and Lohasupthawee (2005) studied crude extracts and essential oils of many herbs including crude ethanolic extract of lemongrass which was active against 17 strains of *salmonella* spp. (7-11 mm) from total 25 strains.

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 bacteriostatic effect against *S. enterica* (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). There also have been studies about different levels of dietary capsaicin, either natural or synthetic on broilers and leghorn; has demonstrated reductions in *Salmonella enteritidis* organ invasion with no adverse effects on body weight (McElroy *et al*., 1994; Tellez *et al*., 1993).

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 and others (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).

Fresh Extracts

Kang-Pa itself using water and heat to cooking Kang-Pa could be one of the extraction forms that might bring the antimicrobial inside herbs in curry paste out. Also in the process of making curry paste, grinding by mortar, is another steps in which antimicrobial inside herbs could be extracted from the applying of the force to the herbs. From the works of Ikigai and others (1993) and Otake and others (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. The ingredients use in making red curry paste using Kang-Pa food model show promising antimicrobial activity.

Although numerous studies have been done in-vitro to evaluate the antimicrobial activity of plant extracts, very few studies are available for food products (Negi, 2012). The reduced effectiveness may be attributed to the use of crude extracts in most studies (Negi, 2012). As the crude extracts generally contain flavonoids in glycosidic form, where the sugar present in them decreases effectiveness against some bacteria (Kapoor *et al.*, 2007; Parvathy *et al.*, 2009; Rhee *et al.*, 1994).

Mode of action

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 (Cowan, 1999). 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 (Davidson, 2001) 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 (Negi, 2012). Storage temperature may also influence the effectiveness of antimicrobial as diffusibility of compounds is related to the temperature (Friedman *et al.*, 2004). 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.

Synergistic effect

There're few studied about the results of the combined effect of plants. From the study of Gutierrez and others (2008) 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 . 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* (Das, Anjeza and Mandal, 2012). Thus from that results the synergistic or additive effects of those plants extracts give an alternative way of using plants and giving higher effectiveness.

According to Cain and others (2003) synergistic activity suggest different mode of actions of the combining compounds. 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.

CONCLUSION

The curry paste that was made by the traditional cooking model has an antimicrobial activity against *Salmonella enterica Enteritidis*, *Salmonella enterica 4,5,12:i:-* (human) US clone, and *Listeria monocytogenes* 10403S. The Log CFU/ml number of all three pathogens show statistically different in amount of cells compare between Kang-Pa and control; at 2^{nd} hour and 3^{th} hour for *Salmonella enterica Enteritidis*, from 2^{nd} hour to 6^{th} hour for *Salmonella enterica 4,5,12:i:-* (human) US clone, and from 1^{st} hour up to 6^{th} hour for *Listeria monocytogenes* 10403S.. Kang-pa showed promising antibacterial activity as in real food model. This might be another explanation as food safety aspect that why Kang-Pa was kept in food cabinet at room temperature without food poisoning. In addition,

References

Abubakar, E.-m.M. 2009. Efficacy of crude extracts of garlic *(Allium sativum Linn.)* against nosocomial *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniea and Pseudomonas aeruginosa.* Journal of Medicinal Plants Research. 3(4): 179-185.

Adeniyi, B.A. and Anyiam, F. M. 2004. In vitro anti-Helicobacter pylori potential of methanol extract of Allium ascalonicum Linn. (Liliaceae) leaf: susceptibility and effect on urease activity. Phytother. Res. 18: 358- 361.

Amin, M. and Kapadnis, B. P. 2005. Heat stable antimicrobial activity of *Allium ascalonicum* against bacteria and fungi. Ind. J. Exp. Biol. 43: 751–754.

Ankri, S. and Mirelman, D. 1999. Antimicrobial properties of allicin from garlic. Microbes Infect. 1: 125–129.

Bhunia, A. K. 2008. Foodborne microbial pathogens: Mechanisms and pathogenesis. United States of America: Springer Science + Business Media, LLC.

Cain, C. C., Lee, D., Waldo III, R. H., Henry, A. T., Casida Jr, E. J., Wani, M. C., Wall, M. E., Oberlies, N. H. and Falkinham III, J. O. 2003. Synergistic antimicrobial activity of metabolites produced by a nonobligate bacterial predator. Antimicrobial Agents and Chemotherapy. 47(7): 2113-2117.

Canada, H.G.o. 2009. 2008 Listeriosis Outbreak in Ontario, Epidemiologic Summary 1, O.M.o.H.a.L.-T. Care, Editor. Queen's Printer for Ontario.

CDC. 2012. Reports of selected *salmonella* outbreak investigations. Centers for Diseases Control and Prevention, Available:http://www.cdc.gov/salmonella/outbreaks.html. Access 13 October 2012

CDC. 2011. Multistate Foodborne Outbreaks. Centers for Diseases Control and Prevention, Available: http://www.cdc.gov/outbreaknet/outbreaks.html#2011. Access 5 May 2012.

CDC. 2010. Surveillance for foodborne disease outbreaks-United States, 2007. Morbidity and Mortality Weekly Report. 59(31):973-979.

Cheremisinoff, N. P. 2003. Industrial solvents Handbook. Second Edition. Marcel Dekker, Inc. New York.

Cichewicz, R. H. and Thorpe, P. A. 1996. The antimicrobial properties of chile peppers (Capsicum species) and their uses in Mayan medicine. Journal of Ethnopharmacology. 52: 61-70.

Cordell, G. A. and Araujo, O. E. 1993. Capsaicin: identification, nomenclature and pharmacotheraphy. Ann. Pharmacother. 27: 330-336.

Coetzee, N., Laza-Stanca, V., Orendi, J. M., Harvey, S., Elviss, N.C. and Grant, K. A. 2011. A cluster of *Listeria monocytogenes* infections in hospitalised adults, Midlands, England. Euro Surveill. 16(20): 19869.

Cowan, M. M. 1999. Plant products as antimicrobial agents. Clinical Microbiology Review. 12: 564–582.

Das, S., Anjeza, C. and Mandal, S. 2012. Synergistic or additive antimicrobial activities of Indian spice and herbal extracts against pathogenic, probiotic and food-spoiler microorganisms. International Food Research Journal. 19(3): 1185-1191.

Davidson, P. M. 2001. Chemical preservatives and natural antimicrobial compounds, In: Doyle, M.P., Beuchat, L.R., Montville, T.J. (Eds.), Food Microbiology: Fundamental and Frontiers, 2nd ed. ASM Press, Washington DC: 593–627.

De, M., AK, D., Mukhopadhyay, R. and Banerjee, A. B. 2003. Antimicrobial activity of *Cuminum cyminum* L. Ars Pharmaceutica. 44: 257-69.

Duan, J. and Zhao, Y. 2009. Antimicrobial efficiency of essential oil and freeze–thaw treatments against *Escherichia coli* O157:H7 and *Salmonella enterica* Ser. Enteritidis in Strawberry Juice. J Food Sci. 74: 131-137.

Eja, M. E., Asikong, B. E., Abriba, C., Arikpo, G. E., Anwan, E. E. and Enyi-idoh, K. H. 2007. A comparative assessment of the antimicrobial effects of garlic (*Allium sativum*) and antibiotics on diarrheagenic organisms. Southeast Asian J Trop Med Public Health. 38(2): 343-348.

El-Kamil, H. H., Ahmed,A. H., Mohammed, A. S., Yahia, A. A. M., El-Tayeb, I. H. and Ali, A. A. 1998. Antibacterial properties of essential oils from Nigella sativa seeds, *Cymbopogon citratus* leaves and *Pulicaria undulata* aerial parts. Fitoterapia. 69: 77-78.

Farnsworth, N. R. and Bunyapraphatsara, N. 1992. Thai medicinal plants. Recommended for primary health care system. Bangkok: Prachachon.

Fretz, R., Sagel, U., Ruppitsch, W., Pietzka, A. T., Stöger, A., Huhulescu, S., Heuberger, S., Pichler, J., Much, P., Pfaff, G., Stark, K., Prager, R., Flieger, A., Feenstra, O. and Allerberger, F. 2010. Listeriosis outbreak caused by acid curd cheese 'Quargel', Austria and Germany 2009. Euro Surveill. 15(5): 1947.

Friedman, M., Henika, P. R. and Mandrell, R. E. 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica.* Journal of Food Protection. 65: 1545-1560.

Frye, D. M., Zweig, R., Sturgeon, J., Tormey, M., LeCavalier, M., Lee, I., Lawani, L. and Mascola, L. 2002. An outbreak of febrile gastroenteritis associated with delicatessen meat contaminated with *Listeria monocytogenes*. Clin. Infect. Dis. 35: 943-949.

Gutierrez, J., Barry-Ryan, C. and Bourke, P. 2008. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. International Journal of Food Microbiology 124: 91-97.

HHS. 2012. U. S. department of health and human services: National Institutes of Health. NIH Publication. 12- 4730.

Hughes, B. G. and Lawson, L. D. 1991. Antimicrobial effects of *Allium sativum* L. (garlic), *Allium ampeloprasum* (elephant garlic) and *Allium cepa* L. (onion), garlic compounds and commercial garlic supplement products. Phytother. 5:154-8.

Ikigai, H., Nakae, T., Hara, Y. and Shimamura, T. 1993. Bactericidal catechins damage the lipid bilayer. Biochemistry Biophysics Acta. 1147: 132-136.

Ireton, K. 2006. *Listeria monocytogenes.* In: Bacterial Genomes and Infectious Diseases. Chan VL, Sherman PM, Bourke B (eds). Totowa New Jersey: Humana Press Inc: 125-149.

Janssen, A. M. and Scheffer, J. J. C. 1985. Acetoxychavicol acetate, an antifungal component of *Alpinia galanga.* Planta Medica. 6: 507-511.

Kapoor, N., Narain, U., Misra, K., 2007. Bioactive conjugates of curcumin having ester, peptide, thiol and disulphide links. Journal of Scientific and Industrial Research. 66: 647-650.

Khelef, N., Lecuit, M., Buchrieser, C., Cabanes, D., Dussurget, O. and Cossart, P. 2006. The *Listeria monocytogenes* and the Genus *Listeria*. Prokaryotes. 4: 404-476.

Khuwijitjaru, P., Chalooddong, K. and Adachi, S. 2008. Phenolic content and radical scavenging capacity of kaffir lime fruit peel extracts obtained by pressurized hot water extraction. Food Sci. Technol. Res. 14: 1 – 4.

Kumar, M. and Berwal, J. S. 1998. Sensitivity of food pathogens to garlic (*Allium sativum*). J. Appl. Microbiol. 84: 213 - 215.

Lawrence, B. M., Hogg, J. W., Tehune, S. T. and Podimuang, V. 1970. The Leaf and Peel Oils of *Citrus hystrix* DC. Phytochemistry. 10(6): 1404-1405.

Liu, D. 2006. Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. Journal of Medical Microbiology. 55: 645-659.

Liu, D., Lawrence, M., Austin, F. W. and Ainsworth, A.J. 2005a. Comparative assessment of acid, alkali and salt tolerance in Listeria monocytogenes virulent and avirulent strains. FEMS Microbiology Letters. 243: 373–378.

Leuschner, R. and Zamparini, J. 2002. Effects of spices on growth and survival of *Escherichia coli* 0157 and *Salmonella enterica* serovar Enteritidis in broth model systems and mayonnaise. Food Control. 13: 399–404.

Leuschner, R. G. and Lelsch, V. 2003. Antimicrobial effects of garlic, clove and red hot chilli on *Listeria monocytogenes* in broth model systems and soft cheese. International Journal of Food Sciences and Nutrition. 54: 127-133.

Lis-Balchin, M. and Dean, S. G. 1997. Bioactivity of selected plant essential oils against *Listeria monocytogenes*. J. Appl. Microbiol. 82: 759-762.

Mahmoudabadi, A. Z. and Gharib Nasery, M. K. 2009. Anti-fungal activity of Shallot, *Allium ascalonicum* Linn. (Liliaceae), *in vitro*. Journal of Medicinal Plants Research. 3(5): 450-453.

Mayachiew, P. and Devahastin, S. 2007. Antimicrobial and antioxidant activities of Indian gooseberry and galangal extracts. Food Science and Technology. 41(7): 1153-1159.

Miettinen, M. K., Siitonen, A., Heiskanen, P., Haajanen, H., Björkroth, K. J. and Korkeala, H. 1999. Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in cold-smoked rainbow trout. J. Clin. Microbiol. 37: 2358-2360.

McElroy, A. P., Manning, J. G., Jaeger, L. A., Taub, M., Williams, J. D. and Hargis B.M. 1994. Effect of prolonged administration on dietary capsaicin on broiler growth and *Salmonella enteritidis* susceptibility. Avian Dis. 38: 329-333.

Molina, T. J., Garcia, C. A. and Ramiriz, C. E. 1999. Antimicrobial properties of alkamides present in flavouring plants traditionally used in Meso-America: affinin and capsaicin. Journal of Ethnopharmacology. 64: 241 - 248.

Nanasombat, S. and Lohasupthawee, P. 2005. Antibacterial activity of crude ethanolic extracts and essential oils of spices against *Salmonellae* and other Enterobacteria. KMITL Science and Technology Journal. 5(3): 527-538.

Negi, P. S. 2012. Plant extracts for the control of bacterial growth: Efficacy, stability and safety issue for food application. Int J. Food Microbiol. 156: 7-17

Onawunmim G. O. 1989. Evaluation of the antimicrobial activity of citral. Lett. Appl. Microbiol. 9: 105-108.

Otake, S., makimura, M., Kuroki, T., Nishihar, Y. and Hirasawa, M. 1991. Anticaries effects of polyphenolic compounds from Japanese green tea. Caries Research. 25: 438–443.

Pui, C. F., Wong, W. C., Chai, L. C., Tunung, R., Jeyaletchumi, P., Noor Hidayah, M. S., Ubong, A., Farinazleen, M. G., Cheah, Y. K. and Son, R. 2011. Review article *Salmonella* : A foodborne pathogen. International Food Research Journal. 18: 465-473.

Parvathy, K.S., Negi, P.S., Srinivas, P., 2009. Antioxidant, antimutagenic and antibacterial activities of curcumin-β-diglucoside. Food Chemistry. 115: 265–271.

Rattanachaikunsopon, P. and Phumkhachorn, P. 2009. Shallot (*Allium ascalonicum* L.) oil: Diallyl sulfide content and antimicrobial activity against food-borne pathogenic bacteria. African Journal of Microbiology Research. 3: 747-750.

Rhee, J., Diaz Ricci, J.C., Bode, J., Schugerl, K., 1994. Metabolic enhancement due to plasmid maintenance. Biotechnology Letters. 16: 881–884.

Rocourt, J. and Cossart, P. 1997. *Listeria moncytogenes*. In Doyle, M. P., Beuchat, L. R., & Montville, T. J. Food Microbiology: Fundamentals and Frontiers. Washington, D. C.: ASM Press.

Ross, Z., O'Gara, E., Hill, D., Sleightholme, H. and Maslin, D. 2001. Antimicrobial properties of garlic oil against human enteric bacteria: evaluation of methodologies and comparisons with garlic oil sulphides and garlic powder. Appl Environ Microbiol. 67(1): 475–480.

Roy, J., Shakaya, D. M., Callery, P. S. and Thomas, J. G. 2006. Chemicalconstituents and antimicrbila activity of a traditional herbal medicine containing garlic and black cumen. Afr. J. Trad. CAM. 3(20): 1-7.

Salamina, G., Dalle Donne, E., Niccolini, A., Poda, G., Cesaroni, D., Bucci, M., Fini, R., Maldini, M., Suchuchat, A., Swaminathan, B., Bibb, W., Rocourt, J., Binkin, N. and Salmaso, S. 1996. A foodborne outbreak of gastroenteritis involving *Listeria monocytogenes*. Epidemiol. Infect. 117: 429-436.

Scallan, E., Grifin, P. M., Angulo, F. J., Tauxe, R. V. and Hoekstra, R. M. 2011. Foodborne illness acquired in the United States - unspecified agents. Emerging Infectious Diseases. 17(1):16-22.

Schlech III, W. F. 2000. Foodborne listeriosis. Clin. Infect. Dis. 31: 770-775.

Scwarz, K., Bertelsen,G., Nissen, L. R., Gardner, P. T., Heinonen, M. I., Hopia, A., Huynh-Ba, T., Lambelet, P., McPhai, D., Skibsted, L. H. and Tijburg, L. 2001.

Investigation of plant extracts for the protection of processed food against lipid oxidation. Comparison of antioxidant assays based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. European Food Research and Technology. 212: 319-328.

Services, R. 2012. Health Benefits of Cumin Essential Oil. Available: http://www.organicfacts.net/health-benefits/essential-oils/health-benefits-of-cumin-essential-oil.html. Access 14 August 2012.

Shahidi, F., Amarowicz,R., Abou-Gharbia, H. A., Shehata, A. and Adel, Y. 1997. Endogenous antioxidants and stability of sesame oil as affected by processing and storage. Journal of the American Oil Chemists' Society. 74: 143-148.

Shan B., Cai Y., Brooks J., Corke H. 2007. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. Int. J. Food Microbiol. 117(1): 112-119.

Shan, B., Yi-Zhong, C. Brooks, J. D. and Corke, H. 2009. Antibacterial and antioxidant effects of five spice and herb extracts as natural preservatives of raw pork. Journal of the Science of Food and Agriculture. 89: 1879-1885.

Simon, J.E., Chadwick, A. F. and Craker, L.E. 1984. Herbs: An Indexed Bibliography. 1971-1980. The Scientific Literature on Selected Herbs, and Aromatic and Medicinal Plants of the Temperate Zone. Archon Books, 770 pp., Hamden, CT.

Singh, B., Falahee, M. B. and Adams, M. R. 2001. Synergistic inhibition of Listeria monocytogenes by nisin and garlic extract. Food Microbiol. 18: 133 - 139.

Sleator, R. D., Gahan, C. G. M. and Hill, C. 2003. A postgenomic appraisal of osmotolerance in *Listeria monocytogenes*. Applied and Environmental Microbiology. 69: 1–9.

Sobel, J. and Watson , J. 2009. Intentional Terrorist Contamination of Food and Water. Beyond Anthrax. Humana Press. 207-224.

Sukhadeo, B. B. and Trinad, C. 2009. Molecular mechanisms of bacterial infection via the gut. Cur. Topics in Microbiol. Immunol. 337: 173-195.

Surh, Y.J. and Lee, S. S. 1995. Capsaicin a double-edged sword: toxicity. Metabolism and chemopreventive potential. Life Sci. 56: 1845-1855.

Tanabe, H., Yoshiad, M. and Tomita, N. 2002. Comparison of the antioxidant activities of 22 commonly used herbs and spices on the lipid oxidation of pork meat. Animal Science Journal. 73: 389-393.

Tellez, G., Jaeger, L., Dean, C. E., Corrier, D. E., DeLoach, J. R., Williams, J. D. and Hargis, B. M. 1993. Effect of prolonged administration of dietary capsaicin on *Salmonella enteritidis* infection in leghorn chicks. Avian Dis. 37: 143-148.

Vazquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T., Dominguez-Bernal, G., Goebel, W., Gonzalez-Zorn, B., Wehland, J. and Kreft, J. 2001. *Listeria* pathogenesis and molecular virulence determinants. Clin. Microbiol. Rev. 14: 584-640.

Yang, X. and Eilerman, R. G. 1999. Pungent principle of *Alpinia galanga* (L.) Swartz and its applications. Journal of Agricultural and Food Chemistry. 47: 1657–1662.

Yanishlieva, N.V., Marinova, E. and Pokorny, J. 2006. Natural antioxidants from herbs and spices. European Journal of Lipid Science and Technology. 108: 776-793.

APPENDIX

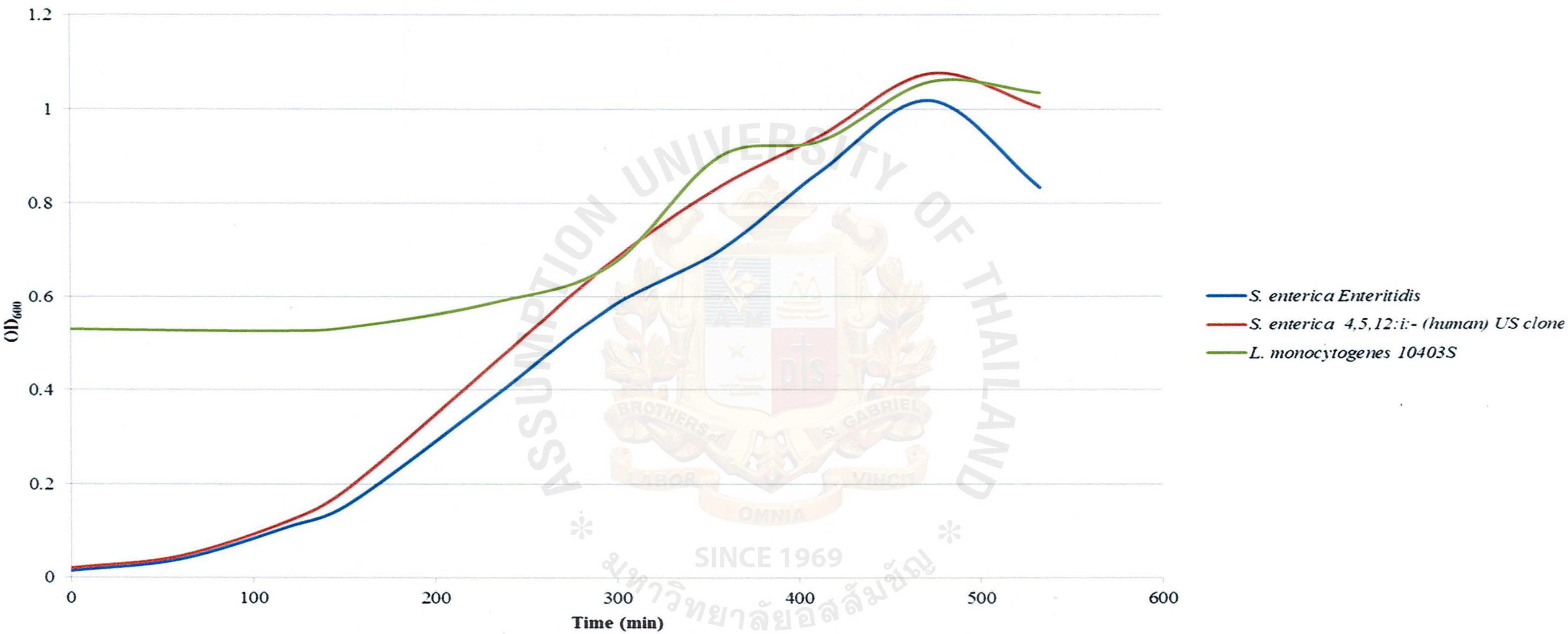

Figure 5: Growth curve of *S. enterica* Enteritidis, S. enterica *4,5,12:i:-* (human) US clone, and *L. monocytogenes* 10403S with NB medium

Raw Data

Table 11: CFU/ml of *S. enterica* Enteritidis

Time (Hr.)	Kang-Pa					Control (NB)				
	Replication 1	Replication 2	Replication 3	Average	Log CFU/ml	Replication 1	Replication 2	Replication 3	Average	Log CFU/ml
0	0	0	0	0	0	0	0	0	0	0
1	450000	470000	410000	443333.3	5.65	890000	860000	760000	836666.7	5.92
2	800000	320000	520000	546666.7	5.74	2920000	2000000	2200000	2373333	6.38
3	1130000	380000	960000	823333.3	5.92	5880000	12100000	6070000	8016667	6.90
4	15900000	510000	13600000	10003333	7	25400000	13700000	15000000	18033333	7.27
5	47000000	6800000	57000000	36933333	7.57	77000000	43000000	111000000	77000000	7.87
6	97000000	15800000	66000000	59600000	7.78	98000000	101000000	104000000	101000000	8.00

Table 12: CFU/ml of *S. enterica* 4,5,12:i:- (human) US clone

Time (Hr.)	Kang-Pa					Control (NB)				
	Replication 1	**Replication 2**	**Replication 3**	**Average**	**Log CFU/ml**	**Replication 1**	**Replication 2**	**Replication 3**	**Average**	**Log CFU/ml**
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	.0	0
2	470000	570000	560000	520000	5.72	830000	560000	696000	695000	5.84
3	510000	700000	605000	605000	5.78	5000000	3000000	4000000	4000000	6.60
4	920000	690000	805000	805000	5.91	10200000	8500000	9350000	9350000	6.97
5	1440000	760000	1100000	1100000	6.04	12000000	13300000	12650000	12650000	7.10
6	7100000	8000000	7550000	7550000	6.88	28000000	40000000	34000000	34000000	7.53

Table 13: CFU/ml of *L. monocytogenes* 10403S

Time (Hr.)	Kang-Pa					Control (BHI)				
	Replication 1	Replication 2	Replication 3	Average	Log CFU/ml	Replication 1	Replication 2	Replication 3	Average	Log CFU/ml
0	912000	1000000	776000	896000	5.95	1000000	1050000	1070000	1040000	6.02
1	1445000	1622000	1380000	1482333	6.17	2240000	2140000	2190000	2190000	6.34
2	1905000	2090000	1820000	1938333	6.29	12000000	10230000	10000000	10743333	7.03
3	4467000	4790000	4900000	4719000	6.67	22400000	23400000	22900000	22900000	7.36
4	14791000	13490000	9330000	12537000	7.09	169000000	167000000	168000000	168000000	8.23
5	19054000	14800000	11220000	15024667	7.17	181000000	183000000	182000000	182000000	8.26
6	20323000	20500000	20430000	20417667	7.31	832000000	794000000	813000000	813000000	8.91

SAS Output: *Salmonella enterica Enteritidis* (human)

2 Hour

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	Culture	3	6.1573	6.3693	6.5814	0.0444	0.0853	0.5364	0.0493
colony	Soup	3	5.211	5.7053	6.1997	0.1036	0.199	1.2507	0.1149
colony	Diff (1-2)		0.3169	0.664	1.0111	0.0917	0.1531	0.44	0.125

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	5.31	0.0060
colony	Satterthwaite	Unequal	2.71	5.31	0.0168

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	5.44	0.3107

Conclude Reject Ho significant different

3 Hour

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	Culture	3	6.4377	6.8783	7.319	0.0924	0.1774	1.1148	0.1024
colony	Soup	3	5.238	5.8717	6.5053	0.1328	0.2551	1.6031	0.1473
colony	Diff (1-2)		0.5086	1.0067	1.5047	0.1316	0.2197	0.6313	0.1794

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	5.61	0.0050
colony	Satterthwaite	Unequal	3.57	5.61	0.0069

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	2.07	0.6519

Conclude Reject Ho significant different

4 Hour

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	Culture	3	6.8807	7.239	7.5973	0.0751	0.1442	0.9064	0.0833

colony	Soup	2	6.7418	7.1675	7.5932	0.0211	0.0474	1.5118	0.0335
colony	Diff (1-2)		-0.28	0.0715	0.4227	0.0685	0.1209	0.4507	0.1104

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	3	0.65	0.5632
colony	Satterthwaite	Unequal	2.57	0.80	0.4927

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	1	9.27	0.4525

Conclude Accept Ho NO significant different

5 Hour

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	Culture	2	5.2215	7.839	10.456	0.13	0.2913	9.2963	0.206
colony	Soup	2	7.1803	7.714	8.2477	0.0265	0.0594	1.8954	0.042
colony	Diff (1-2)		-0.78	0.125	1.0296	0.1095	0.2102	1.3213	0.2102

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	2	0.59	0.6124
colony	Satterthwaite	Unequal	1.08	0.59	0.6522

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	1	1	24.06	0.2561

Conclude Accept Ho NO significant different

SAS Output: *Salmonella enterica* 4,5,12:i:- (human) US clone

2 Hour

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	Culture	3	6.049	6.7367	7.4243	0.1441	0.2768	1.7398	0.1598
colony	Soup	3	5.3871	5.8567	6.3262	0.0984	0.189	1.188	0.1091
colony	Diff (1-2)		0.3427	0.88	1.4173	0.142	0.237	0.6811	0.1935

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	4	4.55	0.0104
colony	Satterthwaite	Unequal	3.53	4.55	0.0139

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	2	2	2.14	0.6360

Conclude Reject Ho significant different

3 Hour

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	Culture	2	5.1776	6.588	7.9984	0.07	0.157	5.0092	0.111
colony	Soup	2	4.8993	5.776	6.6527	0.0435	0.0976	3.1138	0.069
colony	Diff (1-2)		0.2497	0.812	1.3743	0.068	0.1307	0.8214	0.1307

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	2	6.21	0.0249
colony	Satterthwaite	Unequal	1.67	6.21	0.0378

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	1	1	2.59	0.708

Conclude Reject Ho significant different

4 Hour

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	Culture	2	6.4608	6.969	7.4772	0.0252	0.0566	1.8051	0.04
colony	Soup	2	5.1074	5.9015	6.6956	0.0394	0.0884	2.8205	0.0625
colony	Diff (1-2)		0.7482	1.0675	1.3868	0.0386	0.0742	0.4664	0.0742

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	2	14.39	0.0048
colony	Satterthwaite	Unequal	1.7	14.39	0.0089

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	1	1	2.44	0.7249

Conclude Reject Ho significant different

5 Hour

The TTEST Procedure

Statistics

Variable	trt	Lower CL N	Mean	Upper CL Mean	Lower CL Mean	Std Dev	Upper CL Std Dev	Std Dev	Std Err
colony	Culture	2	6.8156	7.1015	7.3874	0.0142	0.0318	1.0154	0.0225
colony	Soup	2	4.2597	6.0195	7.7793	0.0874	0.1959	6.2502	0.1385
colony	Diff (1-2)		0.4783	1.082	1.6857	0.0731	0.1403	0.8818	0.1403

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	2	7.71	0.0164
colony	Satterthwaite	Unequal	1.05	7.71	0.0745

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	1	1	37.89	0.2051

Conclude Reject Ho significant different

6 Hour

The TTEST Procedure

Statistics

Variable	trt	Lower CL N	Mean	Upper CL Mean	Lower CL Mean	Std Dev	Upper CL Std Dev	Std Dev	Std Err
colony	Culture	2	6.5398	7.5245	8.5092	0.0489	0.1096	3.4974	0.0775
colony	Soup	2	6.5466	6.877	7.2074	0.0164	0.0368	1.1733	0.026
colony	Diff (1-2)		0.2958	0.6475	0.9992	0.0426	0.0817	0.5137	0.0817

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
colony	Pooled	Equal	2	7.92	0.0156
colony	Satterthwaite	Unequal	1.22	7.92	0.0534

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
colony	Folded F	1	1	8.88	0.4121

Conclude Reject Ho significant different

SAS Output: *Listeria monocytogenes* 10403S

The TTEST Procedure

Variable: colony

trt	N	Mean	Std Dev	Std Err	Minimum	Maximum
cul6	3	6.0167	0.0153	0.00882	6.0000	6.0300
soup	3	5.9500	0.0557	0.0321	5.8900	6.0000
Diff (1-2)			0.0667	0.0408	0.0333	

trt	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
cul6		6.0167	5.9787	6.0546	0.0153	0.00795	0.0960
soup		5.9500	5.8117	6.0883	0.0557	0.0290	0.3499

trt	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Diff (1-2)	Pooled	0.0667	-0.0259	0.1592	0.0408	0.0245	0.1173
Diff (1-2)	Satterthwaite	0.0667	-0.0603	0.1936			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	4	2.00	0.1161
Satterthwaite	Unequal	2.2994	2.00	0.1666

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	2	2	13.29	0.1400

The TTEST Procedure

Variable: colony

trt	N	Mean	Std Dev	Std Err	Minimum	Maximum
cul6	3	6.3400	0.0100	0.00577	6.3300	6.3500
soup	3	6.1700	0.0361	0.0208	6.1400	6.2100
Diff (1-2)			0.1700	0.0265	0.0216	

trt	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
cul6		6.3400	6.3152	6.3648	0.0100	0.00521	0.0628
soup		6.1700	6.0804	6.2596	0.0361	0.0188	0.2266
Diff (1-2)	Pooled	0.1700	0.1100	0.2300	0.0265	0.0159	0.0760
Diff (1-2)	Satterthwaite	0.1700	0.0879	0.2521			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	4	7.87	0.0014
Satterthwaite	Unequal	2.3059	7.87	0.0103

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	2	2	13.00	0.1429

The TTEST Procedure

Variable: colony

trt	N	Mean	Std Dev	Std Err	Minimum	Maximum
cul6	3	7.0300	0.0436	0.0252	7.0000	7.0800
soup	3	6.2867	0.0306	0.0176	6.2600	6.3200
Diff (1-2)			0.7433	0.0376	0.0307	

trt	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
cul6		7.0300	6.9217	7.1383	0.0436	0.0227	0.2739
soup		6.2867	6.2108	6.3626	0.0306	0.0159	0.1920
Diff (1-2)	Pooled	0.7433	0.6580	0.8287	0.0376	0.0226	0.1082
Diff (1-2)	Satterthwaite	0.7433	0.6539	0.8327			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	4	24.19	<.0001
Satterthwaite	Unequal	3.5829	24.19	<.0001

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	2	2	2.04	0.6588

The TTEST Procedure

Variable: colony

trt	N	Mean	Std Dev	Std Err	Minimum	Maximum
cul6	3	7.3600	0.0100	0.00577	7.3500	7.3700
soup	3	6.6733	0.0208	0.0120	6.6500	6.6900
Diff (1-2)		0.6867	0.0163	0.0133		

trt	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
cul6		7.3600	7.3352	7.3848	0.0100	0.00521	0.0628
soup		6.6733	6.6216	6.7250	0.0208	0.0108	0.1308
Diff (1-2)	Pooled	0.6867	0.6496	0.7237	0.0163	0.00978	0.0469
Diff (1-2)	Satterthwaite	0.6867	0.6432	0.7301			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	4	51.50	<.0001
Satterthwaite	Unequal	2.8764	51.50	<.0001

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	2	2	4.33	0.3750

The TTEST Procedure

Variable: colony

trt	N	Mean	Std Dev	Std Err	Minimum	Maximum
cul44	2	8.2240	0.00424	0.00300	8.2210	8.2270
soup	3	7.0900	0.1058	0.0611	6.9700	7.1700
Diff (1-2)		1.1340	0.0864	0.0789		

trt	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
cul44		8.2240	8.1859	8.2621	0.00424	0.00189	0.1354
soup		7.0900	6.8271	7.3529	0.1058	0.0551	0.6651
Diff (1-2)	Pooled	1.1340	0.8829	1.3851	0.0864	0.0490	0.3223
Diff (1-2)	Satterthwaite	1.1340	0.8720	1.3960			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	3	14.37	0.0007
Satterthwaite	Unequal	2.0096	18.54	0.0028

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	2	1	622.22	0.0567

The TTEST Procedure

Variable: colony

trt	N	Mean	Std Dev	Std Err	Minimum	Maximum
cul44	2	8.2595	0.00354	0.00250	8.2570	8.2620
soup	3	7.1667	0.1150	0.0664	7.0500	7.2800
Diff (1-2)		1.0928	0.0939	0.0858		

trt	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
cul44		8.2595	8.2277	8.2913	0.00354	0.00158	0.1128
soup		7.1667	6.8809	7.4524	0.1150	0.0599	0.7230
Diff (1-2)	Pooled	1.0928	0.8199	1.3658	0.0939	0.0532	0.3503
Diff (1-2)	Satterthwaite	1.0928	0.8076	1.3780			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	3	12.74	0.0010
Satterthwaite	Unequal	2.0057	16.44	0.0036

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	2	1	1058.67	0.0435

The TTEST Procedure

Variable: colony

trt	N	Mean	Std Dev	Std Err	Minimum	Maximum
cul44	2	8.9100	0.0141	0.0100	8.9000	8.9200
soup	2	7.3100	0.00283	0.00200	7.3080	7.3120
Diff (1-2)			1.6000	0.0102	0.0102	

trt	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
cul44		8.9100	8.7829	9.0371	0.0141	0.00631	0.4513
soup		7.3100	7.2846	7.3354	0.00283	0.00126	0.0903
Diff (1-2)	Pooled	1.6000	1.5561	1.6439	0.0102	0.00531	0.0641
Diff (1-2)	Satterthwaite	1.6000	1.4910	1.7090			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	2	156.89	<.0001
Satterthwaite	Unequal	1.0799	156.89	0.0028

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	1	1	25.00	0.2513

Formula for calculating generation times

$$G = \frac{t}{3.3 \log \frac{b}{B}}$$

G (generation time) = (time, in minutes or hours)/n(number of generations)

G = t/n

t = time interval in hours or minutes

B = number of bacteria at the beginning of a time interval

b = number of bacteria at the end of the time interval

Generation time of *S. enterica Enteritidis*

Control: $$G = \frac{t}{3.3 \log \frac{b}{B}} = \frac{60\ minutes}{3.3 \log \frac{8016667}{2373333}} = 34.39\ min = 0.57\ hr.$$

Kang-Pa: $$G = \frac{t}{3.3 \log \frac{b}{B}} = \frac{60\ minutes}{3.3 \log \frac{823333}{546666}} = 102.23\ min = 1.7\ hr.$$

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

$$\mu = \frac{0.693}{0.57} = 1.22\ \text{hour}^{-1}$$

Kang-Pa

$$\mu = \frac{0.693}{1.7} = 0.41\ \text{hour}^{-1}$$

Generation time of *S. enterica* 4,5,12:i:- (human) US clone

Control: $$G = \frac{t}{3.3 \log \frac{b}{B}} = \frac{180\ minutes}{3.3 \log \frac{12650000}{695000}} = 43.2\ min = 0.72\ hr.$$

Kang-Pa: $$G = \frac{t}{3.3 \log \frac{b}{B}} = \frac{180\ minutes}{3.3 \log \frac{1100000}{520000}} = 167.63\ min = 2.79\ hr.$$

Formula for calculating Specific Growth rate

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

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

Control

$$\mu = \frac{0.693}{0.72} = 0.96 \text{ hour}^{-1}$$

Kang-Pa

$$\mu = \frac{0.693}{2.79} = 0.25 \text{ hour}^{-1}$$

Generation time of *L. monocytogenes*

Control: $$G = \frac{t}{3.3 \log \frac{b}{B}} = \frac{120\, minutes}{3.3 \log \frac{168000000}{10715193}} = 30.42\, min = 0.51\, hr.$$

Kang-Pa: $$G = \frac{t}{3.3 \log \frac{b}{B}} = \frac{120\, minutes}{3.3 \log \frac{12302688}{1934936}} = 45.27\, min = 0.75\, hr.$$

Formula for calculating Specific Growth rate

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

Specific Growth rate of *L. monocytogenes*

Control

$$\mu = \frac{0.693}{0.51} = 1.36 \text{ hour}^{-1}$$

Kang-Pa

$$\mu = \frac{0.693}{0.75} = 0.92 \text{ hour}^{-1}$$