ประสิทธิภาพของกรดอินทรีย์ต่อการยึดเกาะ และการอยู่รอดของเชื้อ *Salmonella* Typhimurium *Campylobacter jejuni* และ *Listeria monocytogenes* บนผิวหนังไก่

นางสาวจิตชวลี สุวรรณางกูร

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EFFICACIES OF ORGANIC ACIDS ON THE ATTACHMENT AND SURVIVAL OF *SALMONELLA* TYPHIMURIUM, *CAMPYLOBACTER JEJUNI*, AND *LISTERIA MONOCYTOGENES* ON CHICKEN SKIN

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จิตชวลี สุวรรณางกูร: ประสิทธิภาพของกรดอินทรีย์ต่อการยึดเกาะ และการอยู่รอดของเชื้อ *Salmonella* Typhimurium, *Campylobacter jejuni* และ *Listeria monocytogenes* บนผิวหนังไก่
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อ. ที่ปรึกษา : รศ.น.สพ.ดร. ธงชัย เฉลิมชัยกิจ, อ. ที่ปรึกษาร่วม : รศ.น.สพ เกรียงศักดิ์ พูนสุข, อ. ชมดาว สิกขะมณฑล, 140 หน้า. ISBN 974-17-7006-5

ประสิทธิภาพในการกำจัดเชื้อ *Salmonella* Typhimurium *Campylobacter jejuni* และ *Listeria monocytogenes* ของกรดอะซิติค กรดซิตริค และกรดแลคติคซึ่งมีความเข้มข้น 0.25, 0.5, 1, 2 และ 4 % รวมทั้งกรดอินทรีย์ผสมระหว่างกรดแลคติคกับกรดซิตริค 6 สูตรความเข้มข้น 0.5:0.5, 0.5:1, 1:0.5, 1:1, 1:2 และ 2:1 % โดยการเติมเชื้อแบคทีเรียเข้มข้น 10^8 โคโลนีต่อมล. ปริมาณ 0.5 มล. ลงในสารละลายกรดข้างต้น 4.5 มล. สังเกตการเจริญของเชื้อแบคทีเรียในอาหารเลี้ยงเชื้อเหลวภายหลังจากสัมผัสกรดเป็นเวลา 5, 10, 15 และ 20 นาที แล้วบ่มเชื้อที่อุณหภูมิ 37 องศาเซลเซียส 48 ชม. พบว่ากรดแลคติคความเข้มข้น 4 % และกรดอินทรีย์ผสมระหว่างกรดแลคติค 2 % กับกรดซิตริค 1 % มีประสิทธิภาพในการกำจัดเชื้อแบคทีเรียทั้ง 3 ชนิดได้ดีที่สุดคือ สามารถทำลายเชื้อได้ภายในเวลา 5 นาที จากนั้นทำการศึกษาความคงตัวของกรดอินทรีย์ผสมระหว่างกรดแลคติค 2 % กับกรดซิตริค 1 % ภายหลังจากเก็บที่อุณหภูมิ 4 และ 25 องศาเซลเซียส เป็นเวลา 1, 3, 5 และ 7 วัน พบว่ากรดอินทรีย์ผสมสามารถกำจัดเชื้อแบคทีเรียทั้ง 3 ชนิดได้ภายในเวลา 5 นาทีเช่นเดียวกัน

จากการทดสอบความสามารถของกรดอินทรีย์ผสมระหว่างกรดแลคติค 2 % กับกรดซิตริค 1 % ในการกำจัดเชื้อ *S.* Typhimurium *C. jejuni* และ *L. monocytogenes* เข้มข้น 10^5 ถึง 10^6 โคโลนีต่อ ตร.ซม. บนผิวหนังไก่เปรียบเทียบกับน้ำโดยการฉีดพ่นด้วยแรงดัน 40-45 psi นาน 10 วินาที และให้อุณหภูมิของกรดอินทรีย์ผสมขณะฉีดพ่นเท่ากับ 0, 25 และ 55 องศาเซลเซียส พบว่ากรดอินทรีย์ผสมที่อุณหภูมิ 55 องศาเซลเซียส สามารถลดการปนเปื้อนของเชื้อทั้ง 3 ชนิดได้ดีที่สุด คือ ลดการปนเปื้อนของเชื้อ *S.* Typhimurium *C. jejuni* และ *L. monocytogenes* ได้ 1.55 $\log_{10}$ (97.3 %), 1.76 $\log_{10}$ (98.2 %) และ 0.91 $\log_{10}$ (86.8 %) ตามลำดับ การฉีดพ่นกรดอินทรีย์ผสมสามารถลดปริมาณเชื้อแบคทีเรียทดสอบได้อย่างมีนัยสำคัญทางสถิติที่ระดับความเชื่อมั่น 95 % เมื่อเทียบกับผิวหนังไก่ที่ไม่ได้ฉีดพ่น และภายหลังเก็บผิวหนังไก่ไว้ที่อุณหภูมิ 4 องซาเซลเซียสเป็นเวลา 24 และ 48 ชม. พบว่าลดการปนเปื้อนของเชื้อ *S.* Typhimurium ได้ 1.40 $\log_{10}$ (96.0 %) และ 1.84 $\log_{10}$ (98.5 %) ลดการปนเปื้อนของเชื้อ *C. jejuni* ได้ 1.84 $\log_{10}$ (98.6 %) และ 1.99 $\log_{10}$ (99.0 %) และลดการปนเปื้อนของเชื้อ *L. monocytogenes* ได้ 1.01 $\log_{10}$ (90.1 %) และ 1.18 $\log_{10}$ (92.9 %) ตามลำดับ

การทดสอบทางประสาทสัมผัสพบว่าผิวหนังไก่ที่ฉีดพ่นกรดอินทรีย์และเก็บที่ 4 องศาเซลเซียส นาน 1 วันจะมีสีเหลืองเข้มขึ้นเล็กน้อยและมีกลิ่นของกรดตกค้างบนผิวหนังไก่ แต่หลังจากปรุงสุกกลิ่นและรสชาติของเนื้อไก่ไม่ต่างจากไก่ที่ไม่ได้ฉีดพ่นกรดอินทรีย์ จากการสำรวจผู้บริโภคโดยตัดสินจากลักษณะภายนอกของเนื้อไก่ติดหนังซึ่งฉีดพ่นกรดอินทรีย์พบว่าผู้บริโภค 94.4 % ยอมรับ แต่ทั้งนี้ เมื่อมีตัวอย่างเนื้อไก่ติดหนังซึ่งไม่ได้ฉีดพ่นกรดอินทรีย์เปรียบเทียบ มีเพียง 41.6 % ของผู้บริโภคที่เลือกซื้อเนื้อไก่ติดหนังที่ฉีดพ่นด้วยกรดอินทรีย์

สาขาวิชา จุลชีววิทยาทางการแพทย์ ลายมือชื่อนิสิต.....
ปีการศึกษา 2547 ลายมือชื่ออาจารย์ที่ปรึกษา.....
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ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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KEY WORD: *SALMONELLA* TYPHIMURIUM/*CAMPYLOBACTER JEJUNI*/ *LISTERIA MONOCYTOGENES*/ORGANIC ACID/CHICKEN

JICHAWALEE SUWANNANGKOON: EFFICACIES OF ORGANIC ACIDS ON THE ATTACHMENT AND SURVIVAL OF *SALMONELLA* TYPHIMURIUM, *CAMPYLOBACTER JEJUNI*, AND *LISTERIA MONOCYTOGENES* ON CHICKEN SKIN.
THESIS ADVISOR: ASSOC. PROF. THONGCHAI CHALERMCHAIKIT, PH.D.,THESIS CO-ADVISORS: ASSOC. PROF. KRIENGSAK POONSUK, MISS CHOMDAO SIKKHAMONDHOL, 140 pp. ISBN: 974-17-7006-5

Bactericidal efficacies of acetic acid, citric acid, lactic acid, and mixed organic acids to eliminate *Salmonella* Typhimurium, *Campylobacter jejuni*, and *Listeria monocytogenes* were investigated. The studied concentrations were 0.25, 0.5, 1, 2, and 4 % for each organic acid and 0.5:0.5, 0.5:1, 1:0.5, 1:1, 1:2, and 2:1 % for mixed organic acids of lactic acid : citric acid. Adding 0.5 ml of each tested bacterial suspension into each concentration of acid solutions volumed 4.5 ml to yielded bacterial count 10^7 CFU/ml. After their contact time of 5, 10, 15 and 20 min, the results showed that mixed acids (2 % lactic and 1 % citric acids) and 4 % lactic acid were the most effectiveness which eliminated 3 species of tested bacteria within 5 min. The stability of mixed acids was very well, since they were still be able to eliminate all tested bacteria after keeping at 4 and 25 ^oC for 1, 3, 5 and 7 days.

A skin attachment model was used to determined bacterial eliminating efficacies of mixed organic acids on the chicken skins inoculated with *S.* Typhimurium, *C. jejuni,* or *L. monocytogenes* 10^5 to 10^6 CFU/cm^2. After 30 min firmly attached, the bacterial inoculated skins were sprayed with mixed organic acids (2 % lactic and 1 % citric acids) compared with using water. Pressure of the spray-gun was set at 40-45 psi and applied for 10 seconds. Temperature of mixed organic acids were tested at 0, 25, and 55 ^oC. Bacterial numbers were maximally reduce with mixture of 2 % lactic and 1 % citric acids at 55 ^oC the variations in the reduction in the number of bacteria followed on varied with bacterial species. Of the pathogens tested, *C. jejuni* was the most susceptible to acids which was reduced 1.76 $\log_{10}$ (98.2 % reduction) followed by *S.* Typhimurium which was reduced 1.55 $\log_{10}$ (97.3 % reduction). *L. monocytogenes* was the least susceptible to acids which was reduced 0.91 $\log_{10}$ (86.8 % reduction) after exposed 1 h and after storage 4 ^oC for 24 and 48 h, bacterial numbers continuously decreased.

Organic acid spray treatments caused slight color-change to yellow on chicken skin and left some sour odor. However, the differences of odor and taste after cooking could not be detected by sensory-trained panels. Acceptance of consumers on decision of purchasing raw acid- treated chicken was 94.4 %, but the acceptance was reduce to 41.6 % when having raw non-acid treated chicken samples for comparison.

Field of study Medical Microbiology
Academic year 2004

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Co-advisor's signature…….....
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ABBREVIATIONS

BHI	Brain heart infusion
CAMP	Christie-Atkins-Munch-Peterson
CFU	Colony forming units
cm	centimeter
cm^2	centimetersquare
°C	Degree celieus
DW	Distilled water
et al.	et alli
g	gram
h	hour (s)
L	liter
Max	maximum
Min	minimum
MOX	modified Oxford agar
mCCDA	modified Charcoal Cefoperazone Desoxycholate agar
mg	milligram (s)
ml	microliter (s)
µl	milliliter (s)
mm	millimeter (s)
min	minute (s)
N	number of sample (s)
NaCl	sodium chroride
NaOH	sodium hidroxide
No	number
PBS	phosphate buffer saline
sec	second (s)
Std	standard deviation
TSA	Trypticase soy broth
TSI	Triple sugar iron
LDA	Lysine deaminase
LDC	Lysine decarboxylase

ABBREVIATIONS (CONT.)

LSD	Fisher's least significant difference
UV	Ultraviolet
V	Volt
WHO	World Health Organization
XLD	Xylose lysine desoxycholate

CHAPTER I

INTRODUCTION

Bacterial contamination of poultry during commercial processing is undesirable but unavoidable. Chickens naturally carry a wide variety of bacteria into the processing plant. This microflora can be transferred to the surface of carcasses such as skin during processing. Most of these bacteria are non-pathogenic, but do constitue the major factor affecting poultry shelf-life. Bacterial pathogens of public health significance including *Salmonella* spp (1, 2), *Campylobacter jejuni* (3, 4), and *Listeria monocytogenes* (5, 6) are also shown to be present on processed poultry.

Cross-contamination can occur at each stage in the process of bringing the product to the consumers, beginning at the farm and continuing through processing (7). A United State Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) study that 5% of broilers entering a processing plant harbored *Salmonella*, whereas, the incidence increased to 36 % for processed carcasses from the same flock (8). Bacterial contamination during poultry processing may occur at several critical control points including scalding, picking, eviscerating and chilling (9). Cross contamination of carcasses during processing can lead to an increase in the incidence of pathogen contaminated carcasses. During processing, carcass washing and immersion chilling effectively reduce, but do not completely eliminate, bacterial contamination of poultry skin. Currently, chlorine is routinely used in other areas of poultry processing plants as well. The most commercial poultry processors in the United States use chlorinated ice water for carcass chilling, exposing them to a maximum of 50 ppm chlorine for approximately 1 h (10). There are some questions as to the effectiveness of chlorine to lower numbers of pathogens (11) and concerns exist regarding creation of dangerous by products on reaction with organic material (12). One report suggested that a buildup of microorganism in the chiller tank occurs and that pathogenic bacteria may also be accumulated (13). Data from Conner and Bilgili indicated that 47 to 80% of broilers from retail sources harbored *C. jejuni*, whereas 17 to 77% of fresh processed broilers harbored *Salmonella* (8).

Contamination of raw processed poultry continues to be of concern to consumers and to regulatory and health officials. Attention has focused on the

microbiological quality of broiler carcasses and how processing procedures influence cross-contamination of carcasses. Research is needed to find effective, safe, and affordable methods to reduce bacterial contamination of fresh poultry. Physical and chemical methods have been tested with varying degree of success. Physical methods include air scrubbing, UV, microwaves, high voltage pulsed electric field, air ions, high pressure, ultrasonic energy, stream or hot water sprays and gamma irradiation (14-18). Chemical treatments include chlorine, chlorine dioxide, glutaraldehyde, trisodium phosphate, sodium hypochlorite, sodium metabisulfite, sodium chloride, sodium hydroxide, grapefruit seed extract, Tween 80, cetylpyridinium chloride, ozone, a combination of hydrogen peroxide and sodium bicarbonate, and organic acids (8, 9, 19-31).

Organic acids have been investigated because of their bactericidal activity and because they are generally recognizes as safe (GRAS) and therefore are utilized for preservation in many food application (32-35). In a study where 13 acids were evaluated, acetic and propionic acids were found to have the most inhibitory effect against salmonellae, whereas malic and lactic acids exhibited intermediate activity, and tartaric and citric were least inhibitory (36). In 1987 Lillard et al (37) showed that the use of acetic acid in scalded water reduced the number of microorganisms in the water by almost 100% but had no effect on the carcass itself. It has also been documented that most organic acids alter the visual appearance of the carcass by bloating it and either bleaching or darkening the finished carcass (38, 39). In 1990 Blankenship reported that the use of acetic and citric acids in concentration above 3% and lactic acid in concentration of 1% or higher resulted in flavor changes of cooked broiler meat (40). Dickens and Whittemore used air agitation with and without acetic acid to investigate moisture pick-up, chilling time and temperature, subjective appearance changes, and microbiological quality of processed poultry. Their research showed lower microbiological counts with the addition of acetic acid. Only minor changes to the appearance of the treated carcasses were noted (41).

In this study, the influence of 0, 25, and 55 ^{o}C of mixed organic acids spraying to survival of *Salmonella* Typhimurium, *Campylobacter jejuni*, and *Listeria monocytogenes* on chicken skin and evaluate sensory properties of mixed organic acids sprayed chicken including consumer acceptability by trained and consumer panels.

CHAPTER II

OBJECTIVE

1. To study the efficiencies of edible organic acids those be able to reduce *Salmonella* Typhimurium, *Campylobacter jejuni*, and *Listeria monocytogenes* on the chicken skin.
2. To find the suitable edible organic acids to be use as disinfectant on chicken carcasses in broiler processing.

CHAPTER III

LITERATURE REVIEW

Large amounts of poultry meat products are consumed in all countries and transported in international trade. Processors and governmental officials in importing countries are interested in the microflora of these products as indices of previous sanitation and storage, remaining shelf-life, and danger to public health. In addition to the nutrients in poultry meat, other properties influence the growth of microorganisms. Water activity (a_w) of meat is about 0.98 to 0.99 depending on whether and how long the meat has been stored in dry air. Because of their composition and other properties, both poultry muscle and skin are excellent substrates for a wide variety of microorganism. Birds infected with pathogen spread the causative microorganisms to their pen mates and to other carcasses during processing. Many microorganisms, including pathogenic species, are shed in feces, such as *Campylobacter* spp., *Salmonella* spp., *Escherichia coli*, *Streptococci, Proteus* spp. Fecalborne organisms can adhere to feathers or feet when birds walk, roost, or set on contaminated surfaces and can also be inhaled with air that contains feces-contaminated dust or aerosols. In general, poultry is processed as follows figure 1 (42): birds are removed from cages, hung by the feet on shackles of a conveyer, stunned by electric shock, killed by cutting the carotid arteries, and allowed to bleed. Next, they are scalded, defeathered, and washed. Head, hocks, shanks, and oil glands are cut off, and viscera are drawn, inspected, and removed, and neck are cut off. The carcasses are then usually spray-washed and chilled. After chilling they are graded and either packaged, packed in crates with ice, or further processed. Cleaned, edible viscera and the neck are sometimes stuffed into the body cavity, and the carcass is packaged and stored in a cold room. Some processing operations promote a significant increase of contamination or even permit multiplication of contaminating microorganism. Certain steps of the processing operation transfer microorganisms from heavily contaminated sites to lightly contaminated sites or introduce additional contaminants. Bacterial contamination during poultry processing may occur at several critical control points including scalding, picking, eviscerating and chilling (9). Five Percentage of broilers entering a processing plant harbored *Salmonella*,

Campylobacter and incidence increased to 36% for processed carcasses from the same flock (43). During picking or defeathering operations that many fecal and other types of bacteria become attached to surfaces or enter feather folicles, and therefore are difficult to remove during subsequent processing. The levels of organism that remain on the skin of processed birds reflect the bacterial population of the equipment surfaces and of the washing and chilling suspensions to which carcasses are exposed. During evisceration, microoraganisms can be transfered from carcass to carcass by worker and equipment (42). The one of the most critical steps for inhibiting the growth of microorganism is carcass chilling. However, during the immersion chilling process, pathogen cross-contamination from one carcass to another may occur because of limitation of chlorine is its rapid inactivation by organic material (42). Organic matter rapidly inactivates chlorine, and organisms such as *Salmonella* spp. and *Campylobacter* spp. may protected on the surface of the skin of poultry carcasses even if chlorinated water has been used (44). Several authors have suggested that immersion chilling is unhygienic, considering that pathogens such as *Salmonella* spp. and *C. jejuni*, which may be present in a large numbers on relatively few carcasses before chilling, may be homogeneously distributed to other carcasses by cross-contamination with direct contact between broilers and the water used for chilling (45, 46).

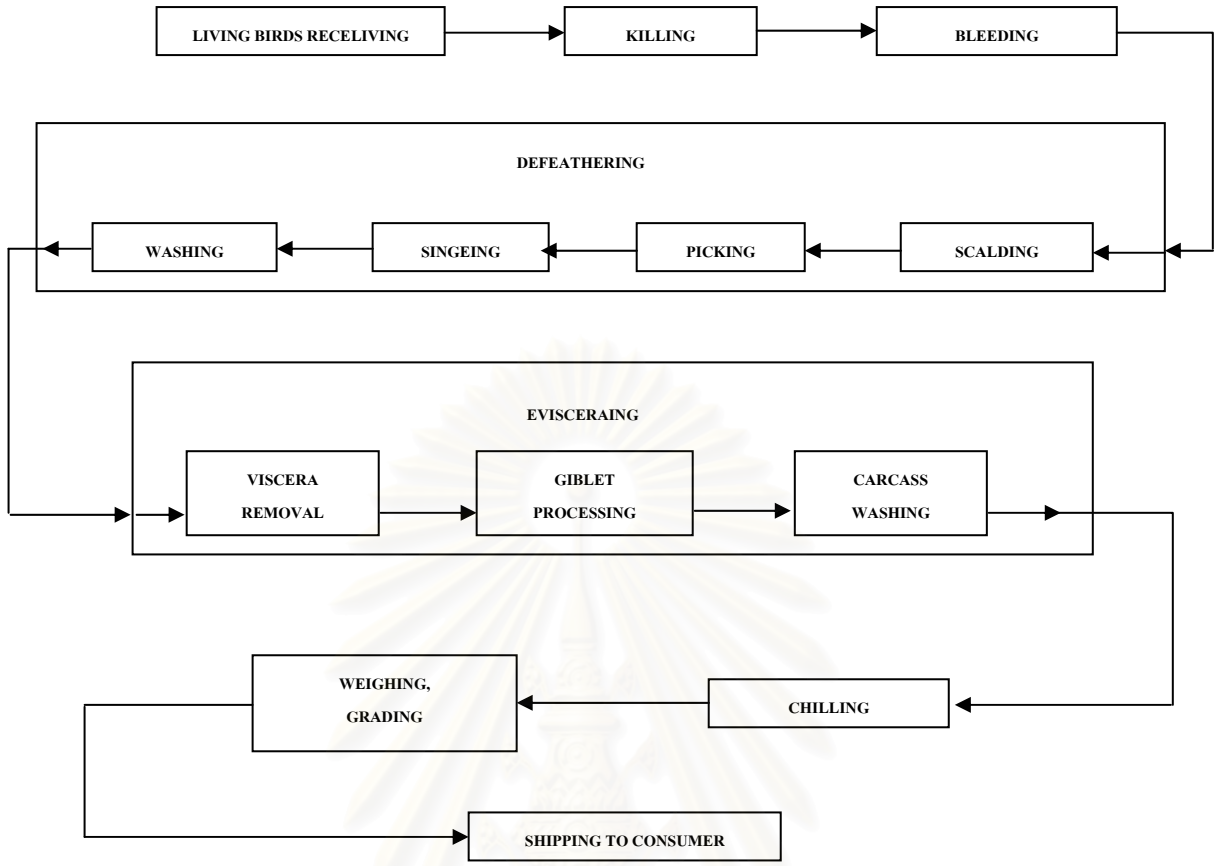

Figure 1 General process flowchart for poultry processing.(42)

Bacterial attachment

The attachment mechanism initially involves retention of bacteria in a liquid film on the skin from which they migrate and become entrapped in ridges and crevices (26, 47). The process of retention begins with the live birds and is exacerbated during scalding. It continues during processing, and the level of contamination is directly related to the microbial concentration in the processing water (48). The scalding operation opens feather follicles to aid feather removal, and the follicles remain open throughout processing until chilling where they close thereby retaining microorgamism. Later, because of water uptake during immersion, certain microorganisms such as *Salmonella*, *Campylobacter* adhere to polysaccharide material and material surrounding collagen fibers (49). Lillard proposed that adherence is rapid within 15 s of exposure, but attachment is a time dependent process (50). On the other hand McMeekin and Thomas (48) reported that time did not influence attachment but depend on bacterial population size. Finally, Conner and

Bilgili (8) stated that there was no significant difference for attachment concerning culture temperature (23 or 37 ^{o}C), inoculum level, or contact time. These conflicting results indicate that the nature of bacterial attachment to skin is complex and involves many elusive factors.

Campylobacter jejuni

Importance

Several *Campylobacter* species can cause human gastroenteritis, however, *Campylobacter jejuni* and *C. coli* are considered the most common causative agents of human diarrheal disease in many countries worldwide. In many countries, the number of cases of campylobacteriosis probably far exceeds the combined number of salmonellosis and shigellosis cases. Epidermiological datas have confirmed this in Canada, the U.K., and Scotland. Isolation of *Campylobacter spp*. From a suspected sample requires specific methods. After developing this method and in cooperating it to isolate suspected foodborne pathogens. *C. jejuni* has been implicated in 53 foodborne outbreaks in the U.S. between 1979 and 1987, affecting 1547 individuals and resulting in 2 deaths. The foods implicated most often in campylobacteriosis were raw milk, raw chicken and improperly cooked chicken. Although severals *Campylobacter spp*. have been associated with foodborne campylobacteriosis, *C. jejuni* has been isolated in most incidents (51).

Characteristics

Campylobacter jejuni is a gram – negative, nonsporulating, rod – shaped bacterium. The cells are small, fragile, and spirally curved (0.5-8μm in length and 0.2-0.5 μm in width). Log-phase cell have a slender, curved or spiral shape and one or more polar or amphitrichous flagella which confer a rapid, darting motility and maybe an important feature in pathogenesis. The strains are microaerophilic and catalase and oxidase positive. The strains require a microaerophilic environment of 5-10 % O_2, 3-5 % CO_2, and 87 % N_2 for growth. Growth temperature range between 32 and 45 ^{o}C, with optimum temperature is 42 ^{o}C. They grow better in amino acids than carbohydrates. They generally grow slowly and are not a good competitor while growing with other bacteria. They do not generally grow well in many foods. They are sensitive to many environmental parameters, including oxygen (in air), Nacl

(above 2.5 %), low pH (below pH 5.0), temperature (below 30 ^{o}C), heat (pasteurization), and drying. However, they survive well under refrigeration and for months in the frozen state. (51)

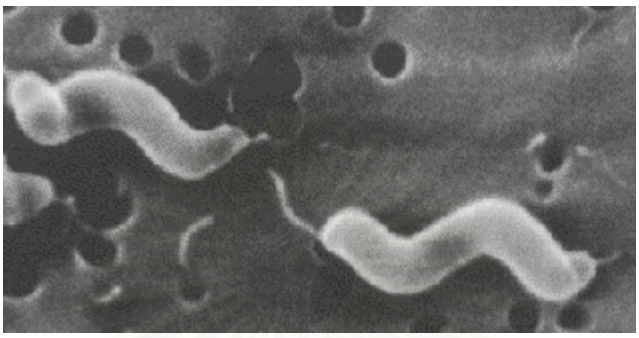

Figure 2 Electron micrograph image *of Campylobacter jejuni* (52)

Habitat

Campylobacter jejuni is an enteric organism. It has been isolated in high frequency from feces of animals and birds. Human carries were also found to shed the organisms in feces. Fecal materials from poultry were found to contain more than 10^6 cells/g in some instances. Water, sewage, vegetables, and foods of animal origin are easily contaminated with *C. jejuni* excreted through feces. (51)

Food association

This organism is present in high frequency in animals, birds, and the environmental, many foods, both from plant and animal sources, can be contaminated with *C. jejuni*. The foods can be contaminated directly because *Campylobacter* is carried in the intestinal tract of a wide variety of wild and domestic animals, especially birds. *Campylobacter* can be easily spread from bird to bird through a common water source or through contact with infected feces. When an infected bird is slaughtered, *Campylobacter* can be transferred from the intestines to the meat. *Campylobacter* is also present in the giblets, especially the liver. The foods can be contaminated indirectly from sewage and contaminated water. *C. jejuni* has been isolated at a very high frequency from raw meat such as beef, lamp, pork, chicken, and turkey including milk, eggs, vegetables, mushrooms, and clams. *C. jejuni* can

survive 2-4 weeks under moist, reduced-oxygen conditions at 4 °C, often outlasting the shelf life of the product (except in raw milk products). They can also survive 2-5 months at -20 °C, but only a few days at room temperature (53-56) *C. jejuni* is widely thought of as an environmentally-fragile organism, indicating that it would not persist for long periods of time on fruits and vegetables. It is sensitive to drying, acidity, freezing, salting, osmotic stress, oxygen (>5 %), chemical rinses and disinfectants. Although the organism is a poor competitor against other microorganism present in food and generally does not grow well in food, enough cell can survive in a contaminated food to provide the dose required for the disease. (51)

Pathogenesis

Pathogenesis of *Campylobacter jejuni* is depend on pathogen-specific and host related factors including age, health, and immune response. Due to these factor, the minimum infectious dose has not been established although in a volunteer study, 500 to 800 cell was enough to cause illness (57, 58). With in 1-7 days of infection by *C. jejuni* (59) the clinical response can be broad (60). On the mild side, the infection can be a transient asymptomatic colonization or symptoms lasting about 24 hours, resembling viral gastroenteritis. Typically, illness includes diarrhea, fever, malaise, and abdominal cramping. The illness last about a week and can also include nausea and vomiting. Having at least 1 day with eight or more bowel movements in not uncommon (61). Diarrhea can be bloody due to the penetration and proliferation of *Campylobacter* with in intestinal epithelium. Chronic illness can occur within 1-2 weeks in approximately 1 % of patients appearing in the form of reactive arthritis (59). An estimated one in every 1,000 patients contract Guillian-Barre syndrome (peripheral polyneuropathy) (57)and a more serious side effect. A *Campylobacter* infection has been implicated in up to 40 % of the syndrome cases (57). Seasonal trends in cambacteriosis show a rise in May peaking in July and leaving off to base level by December. The summer rise occurs about eight weeks before the summer rise of *Salmonella* infections. A small secondary peak is seen in autumn (59)

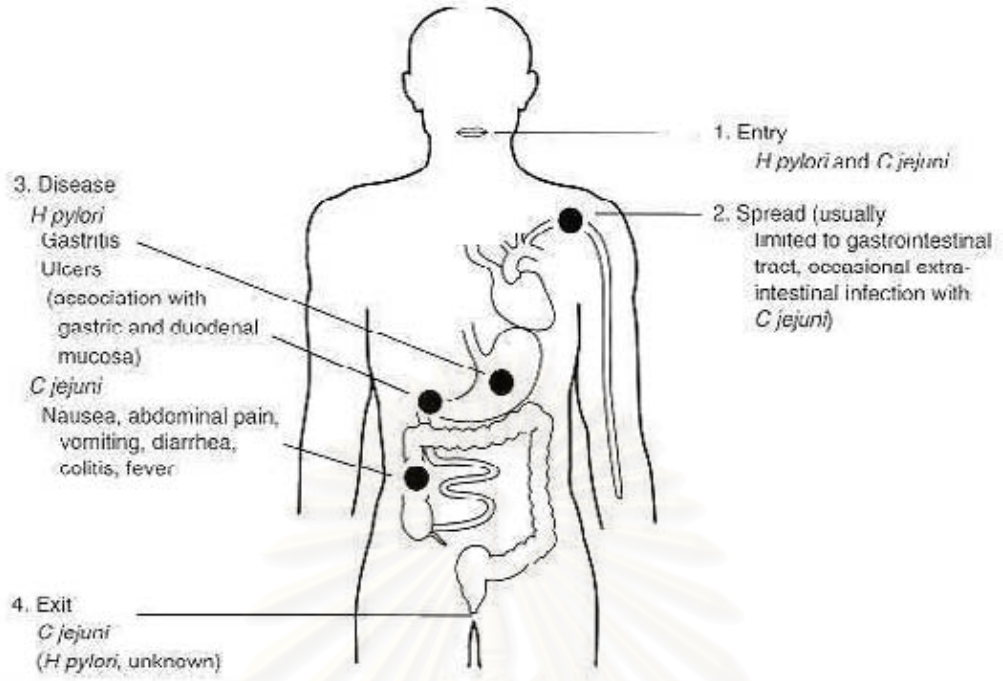

Figure 3 Phathogenesis of *C. jejuni* infection in human (62)

Listeria monocytogenes

Importance

Human listeriosis had been recognized for a long time, However, the presence of *Listeria monocytogenes* in many foods of animal and plant origin and illnesses resulting from consumption of contaminated food was recognized rather recently (51, 63-67). In addition, its ability to grow in many foods at refrigerated temperature helps the organism reach from a low initial level to an infective dose level during storage of refrigerated foods. It is clear that many of the conditions given have an advantage to *L. monocytogenes* to become a newly emerging foodborne pathogen in many countries (51).

The genus *Listeria* contains several species, of which *L. monocytogenes* is considered to be a pathogenic. The species has several serogroups: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c and 4b. Whereas 1/2a and 1/2b were the predominant serogroups isolated in foodborne human listeriosis in Europe, 4b was predominant in Canada and the U.S. (51)

Characteristics

Listeria monocytogenes is a gram–positve, psychrotrophic, facultative anaerobic, catalase-positive, oxidase-negative, non- sporulating, small rod (0.4-0.5 μm x 0.5-2.0 μm) cultured at 20-25^{o} C posses peritrichous flegella and exhibit a characteristic tumbling motility. In fresh culture, the cells may form short chains. It is hemolysic and ferments rhamnose but not xylose. *L. monocytogenes* is a psychrotroph and grows between 1 to 44^{o}C, with optimum growth at 35 and 37 ^{o}C. It ferments glucose without producing gas. It can grow in many foods and environment. The cells are relatively resistant to freezing, drying, high salt, and pH 5.0. They are sensitive to pasteurization temperature that is 71.7^{o}C for 15 s. or 62.8 ^{o}C for 30 min. But when inside the white blood cells, a temperature of 76.4 to 77.8 ^{o}C for 15 s. is required to kill the cells (51, 64-66).

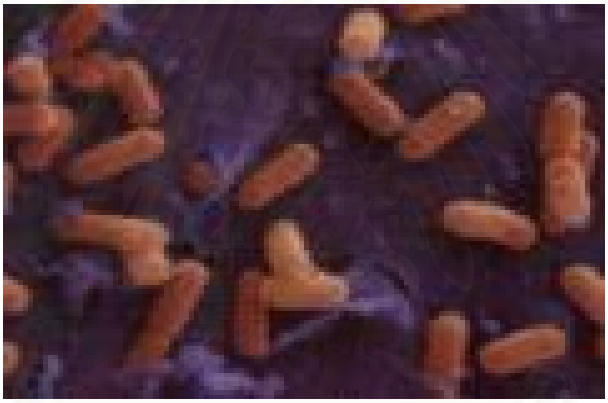

Figure 4 Electron micrograph image *of Listeria monocytogenes* (68)

Habitat

Listeria monocytogenes is isolated from many environmental samples, such as soil, sewage, water, and dead vegetation. It is isolated from the intestinal contents of domesticated animals and birds. Humans can also carry the organism in the intestine without any symptoms. A large proportion of uncooked meat, milk, egg, seafoods, and fish, as well as vegetables and tubers, contain *L. monocytogenes.* Many heat – processed foods, such as pasteurized milk and dairy products, and ready-to-eat meat preparation also contain the organism. *L. monocytogenes* is isolated in high frequency from different places of food processing and storage areas. (51)

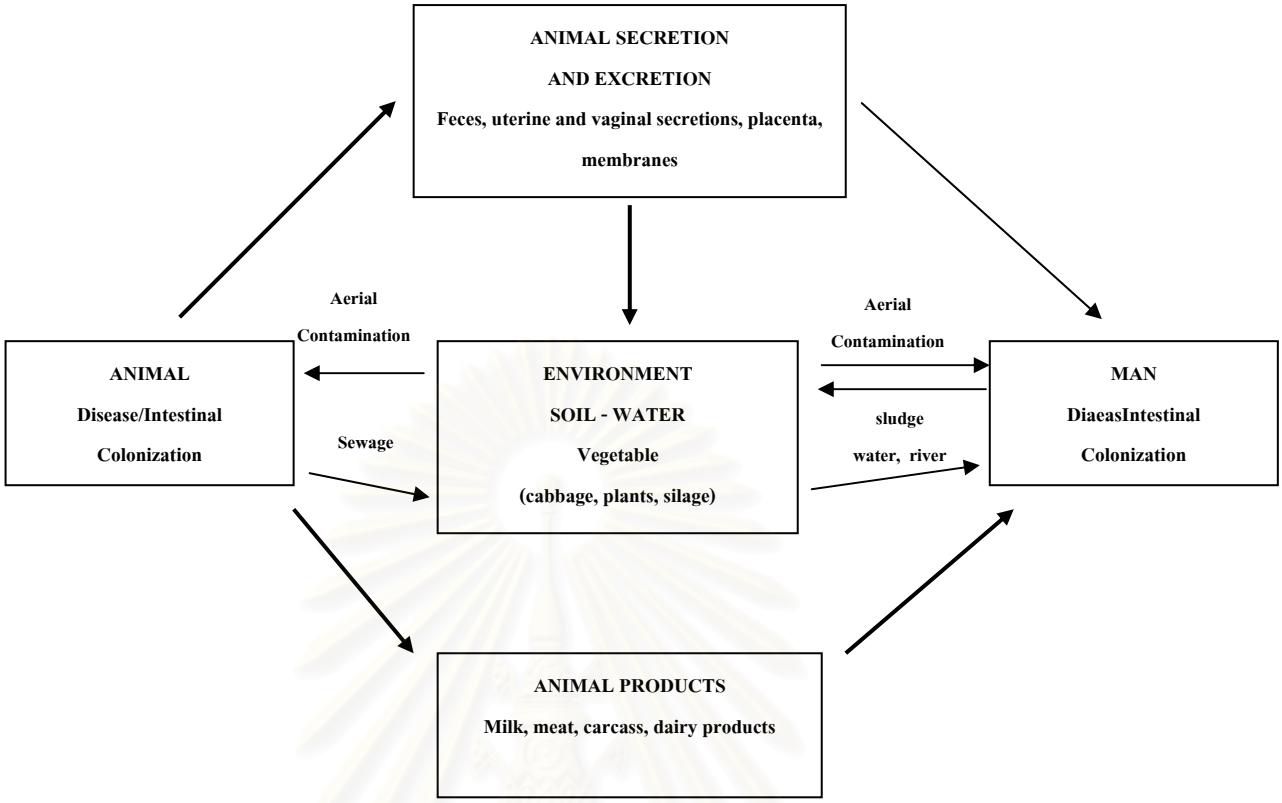

Figure 5 Ways in which *L. monocytogenes* is disseminated in the environment, animals, foods, and human (69)

Food association

Listeria monocytogenes has been recognized as an emerging and important foodborne pathogen during the last decade. There are many opportunities for contamination with *Listeria* during the process of food production because *L. monocytogenes* is ubiquitous in the environment (soil, surface water, plants). Also humans and animals can be asymptomatic excretors, thus introducing the pathogen in the environment. *L. monocytogenes* in particular contribute to its widespread distribution becase of it can survive for long periods of time in many different environments and it is psychrotrophic. Food can contaminated at any step of food chain, and cold storage does not inhibit the growth of *Listeria* spp.

Listeria monocytogenes is an important human pathogen that is a well-known problem in production environment including abattoirs and meat processing plants (70-73). Entry of *L. monocytogenes* into food processing plants occurs through soil on worker's shoes, clothing and on transport equipment, animals which excrete the

bacterium or have contaminated hides or surfaces, raw plant tissue, raw food of animal origin, and possibly healthy human carries. *L. monocytoenes* is most often deteted in moist areas such as floor drains, condensed and stagnant water, floors, residues, and processing equipment. *L. monocytoenes* can attach to various kinds of surfaces including stainless steel, glass, rubber and biofilms have been found in meat and daily processing environment (45). *Listeria spp.* Survive on fingers after hand washing and in aersols. The presence of *L. monocytoenes* in food processing chain is evidenced by the widespread distribution of the organism in processed products. Poultry such as broiler, ready-to-eat, precooked, chilled, or frozen chicken are also frequently contaminated, with up to 60 % of samples *L. monocytoenes* positive in some studies. In review article by Johnson et al. (1990) *L.* monocytogenes contamination of 23-60 % for poultry was reported (74). Hudson and Mead (1989), Pini and Gilmour (1988) and Lewrence and Gilmour (1994) found *L. monocytogenes* in 50, 60 and 59 %, respectively, of oven-ready poultry (70, 75, 76). In 1992-1993, the study of Uyttendaele et al found that 32.1 % and 27.2 % of poultry products from Belgian and French abattoirs (77). The population of *L. monocytoenes* present in raw or processed meat products are usually low, with 80 % to 90 % of samples contaminating less than 10 to 100 CFU/g. However, high populations have been reported for some ready-to-eat products, including those implicated in outbreaks of listeriosis (64, 65, 78).

Pathogenesis

Listeria monocytoenes is transmitted via three main routes; contact with animals, cross-infection of new borne babies in hospital and foodborne infection (foodborne pathogen). The listerial infection most frequently reported in non-pregnant adults is that affecting the CNS (55 to 70 % of cases). Pure meningeal forms are observed in some cases, but infection normally develops as a meningoencephalitis accompanied by severe changes in consciousness, movement disorders, and, in some cases, paralysis of the cranial nerves (Fig. 6). An association between clinical episodes of invasive listeriosis and a history of gastrointestinal symptoms, including diarrhea, vomiting, and fever, was noticed some time ago (79-81). Investigations of recent food-borne outbreaks have provided compelling evidence that a febrile gastroenteritis syndrome may indeed be the main clinical manifestation of

L. monocytogenes infection (82-85). *L. monocytogenes* should be sought as a possible etiologic agent in cases of diarrheagenic disease in humans. The potential enteropathogenicity of *L. monocytogenes* has also been recognized in animals, with outbreaks of diarrhea and gastroenteritis having been reported in sheep (86)

The pathophysiology of *Listeria* infection in humans and animals is still poorly understood. Most of the available information is derived from interpretation of epidemiological, clinical, and histopathological findings and observations made in experimental infections in animals, particularly in the murine model. As contaminated food is major source of infection in both epidemic and sporadic cases (87, 88) the gastrointestinal tract is thought to be the primary site of entry of pathogenic *Listeria* organisms into the host. The clinical course of infection usually begins about 20 h after the ingestion of heavily contaminated food in cases of gastroenteritis (83), whereas the incubation period for the invasive illness is generally much longer, around 20 to 30 days (80, 89).

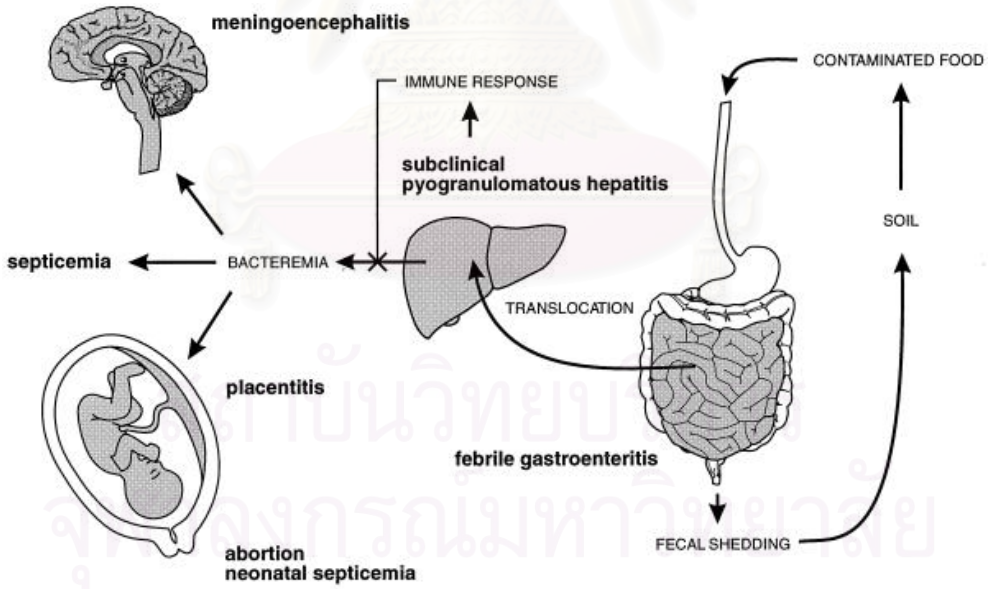

Figure 6 Schematic representation of the pathophysiology of *Listeria* infection in human. (90)

Salmonella Typhimurium

Importance

Since the 1950s, Foodborne salmonellosis has been recognized to be the major cause of all foodborne diseases by pathogenic bacteria, both in number of incidences and number of cases. Although, at present, scientific information about the habitats, mode of transmission in foods, growth characteristics, and survival parameters of the pathogen are available, and methods to control its contamination of foods have been developed, foodborne salmonellosis is still the leading cause of foodborne bacterial diseases in the U.S. and the other developed countries (51).

Tauxe (91) indicated that the present increase in salmonellosis, including foodborne salmonellosis in the U.S. could be related to four facters :1. increase in number of antimicrobial-resistant Salmonella isolates, 2. increase in immunodeficient individuals who are extremely susceptible to Salmonella, 3. increase in egg-associated *Salmonella* Enteritidis contamination due to increase in laying hens with infected ovaries, and 4. food production in centralized facilities that can lead to, if contamination occurs, extremely large and wide spread outbreaks.

There are more than 2000 serovars of *Salmonella*, potentially capable of causing salmonellosis in humans. Along with feacal-oral direct transmission, contaminated food and water can cause salmonellosis. However, two of the more than 2000 serotypes, serotype Typhimurium and serotype Enteritidis, are involed in higher frequencies worldwide. Many animals, including food animals and pets, Harbor serotype Typhimurium in a carrier state, whereas serotype Enteritidis can infect the ovaries of poultry, especially chicken, and can be transmitted through eggs. Because of increase in antibiotic use in feed of food animals and birds, there is an increase in multidrug-resistant (MDR) strains among *S. enterica* serotypes. One that has created concern is the MDR *Salmonella* Typhimurium definitive phage type (DT) 104. The strains are resistant to several antibiotics, including ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline. It is suspected that they are transferred among strains or serovars by different methods of genetic recombinations, especially under antibiotic selective pressure (92).

During the least 10 years, foodborne salmonellosis from *Salmonella* Enteritidis has increased greatly, and, at present, the frequency of incidence from it is as high as that caused by *Salmonella* Typhimurium. One of the reason for this could be the way the poultry is raised (51).

Chracteristics

Salmonella spp. Is member of the Enterobacteriaceae. *Salmonella* cells are Gram–negative, nonsporulating, facultative anaerobic, rod-shape (typically 0.5 μm by 1-3 μm), motile with peritricous flagella. They form gas while growing in media containing glucose. Generally, they not ferment lactose, utilize citrate as carbon source, produce hydrogensulfide, decarboxylate lysine, and ornithine, do not produce indole, and are negative for urease. They are mesophilic, with optimum growth temperature between 35 and 37 ^{o} C, but generally have a growth range of 5 to 46 ^{o} C. They are killed by pasteurization temperature and time, sensory to low pH (4.5 or below), and do not multiply at an a_w of 0.94 especially in combination with a pH 5.5 and below, the survival rate increasing as the a_w is reduced. The cells survive in frozen and dried states for a long time. They can multiply in many foods without affecting the acceptance qualities (93, 94).

Figure 7 Electron microscope image of Salmonella (95)

Habitat

Salmonellae are natural inhabitants of the gastrointestinal tracts of domesticated and wild animals, birds, and pets, and insects. In animals and birds, they can cause salmonellosis and then persist in a carrier state. Humans can also be carriers following and infection and shed the pathogens through feces for a long time. They have also been isolated from soil, water, and sewage contaminated with fecal matters (93, 94).

Food association

The ubiquity of *Salmonella* spp. In the natural environment, coupled with the intensive husbandary practices used in the meat, fish, and shellfish industries and the recycling of offal and inedible raw materials into animal feeds, has favored the continued prominence of this human bacterial pathogen in the global food chain (94, 96). Foods of animal origin have been associated with the large numbers of outbreaks. These include beef, chicken, turkey, pork, eggs, milk, and product made from them. These foods were contaminated directly or indirectly with fecal matters from carrier (animals, birds, and human) and eaten either raw or improperly cooked, or contaminated following adequate heat treatment. Cross-contamination at home and at food services are the major sits of contamination of heated foods with *Salmonella* (97, 98) .

Pathogenesis

The presence of viable salmonellae in the human intestinal tract confirms the successful evasion of ingested organisms from nonspecific host defenses. The human diarrheagenic response to foodborne salmonellosis result from the migration of the pathogen in the oral cavity to intestinal tissues and mesenteric lymph follicles. The event coincides with bacterial enterotoxin production, extensive leukocyte in flux into the infected tissues, increased mucus secretion by globlet cells, and mucosal inflammation triggered by the leukocytic release of prostaglandins. The latter occurrence also activates the adenyl cyclase in intestinal epithelial cells, resulting in increased fluid secretion into the intestinal lumen (99, 100). The failure of host defense systems to hold the invasive salmonellae in check can degenerate into septicemia and other clinical conditions.

Recent publications on the dynamics of human *Salmonella* infections are of singular interest (101, 102). An in-depth epidermiological study of a large outbreak of *S.* Typhiurium involving chicken served to delegates at a medical conference showed that the clinical course in patients was directly related to the number of ingested salmonellae (101). The incubation period for the onset of symptoms was inversely related to the infectious dose. Patients with short periods (less than 22 h.) of incubation suffered more frequent diarrheal bowel movements, higher maximum body

temperatures, greater persistence of clinical symptoms, and greater frequency of hospitalization. Interestingly, no association between the age of infected individuals and the length of the incubation period was noted. Similar findings were reported in retrospective dose-response studies of foodborne salonellosis (102, 103).

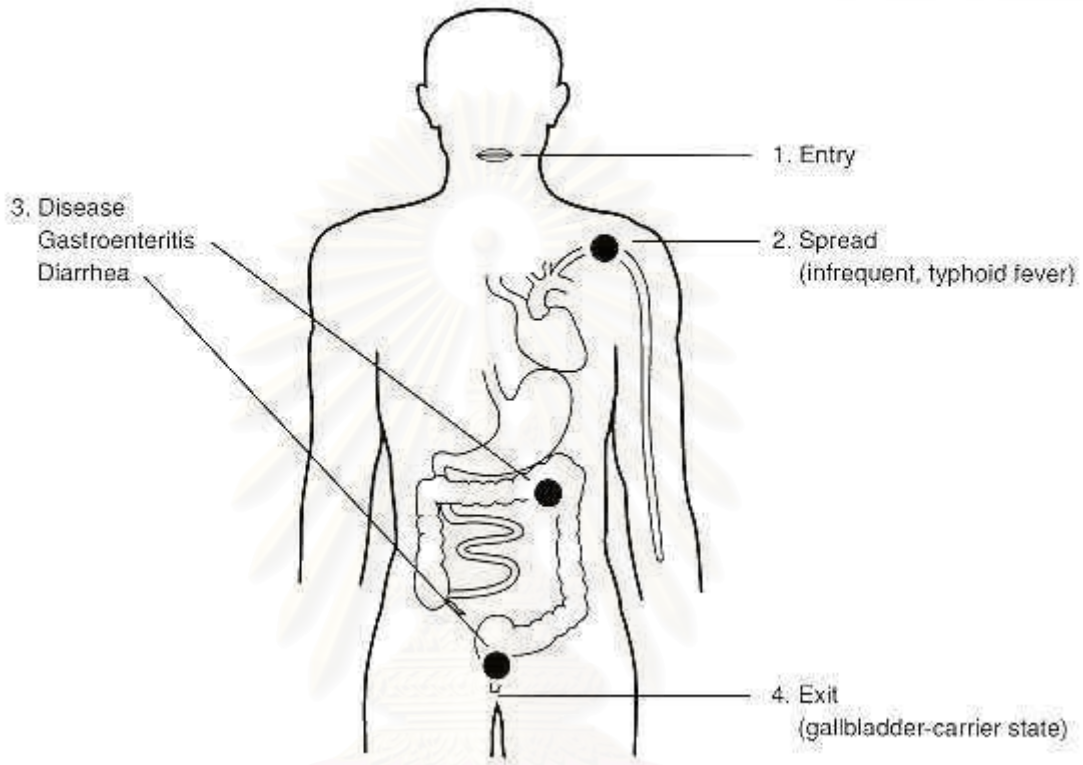

Figure 8 Pathogenesis of Salmonella spp. in human. (104)

General and special aspects for cleaning and disinfection in the area of food processing and handling

Factors to be considered:

1. The choice of suitable and compatible surfactants and antimicrobial agents in the right combination in a two step or even single-step procedure; the latter is preferred in recent time even in the meat processing area.

2. The applicability on the very different types of premises, fitting and equipment. It must be distinguished between open or closed processing lines and between surface which may be smooth or porous, plain or with irregular shape and void spaces, non- corrosive or corroded, horizontal or vertical. In addition, one has to consider whether the type of dirt to be treated is water soluble or more tenacious.

Furthermore, it must be differentiated between ‘clean’ and ‘dirty’ conditions even after a cleaning procedure.

3. The efficacy in a relatively short time of up to 30 min if possible and in the lower temperature rang of about 4-10 ^{o}C.

4. The agents within disinfectants must have no organoleptical (sensorical) influences onto the food if this comes into contact with the surfaces treated.

5. The application safety must also be regarded in a special way. The predisposition for explosion, burning, inflammation at quick disinfection procedure in the vicinity of electricial equipment e.g. with alcohols must be considered.

6. Aggressive features for human tissues must be excluded as far as possible.

7. Corrosive impacts onto equipment and fitting on premises must be considered, too.

8. The composition of the very complex microflora within different foods, which all represent different habitats, has to be taken into consideration as well; are Gram–nagatives or Gram-positives or yeasts and moulds the main targets of disinfection procedures. In special cases the predominant occurrence of spore formers

9. The costs are an important point of consideration because disinfectants must be use regularly and in adequate effective concentration. (105)

Organic acids

Many organic acids are used as food additives, but not all have antimicrobial activity. The most active antimicrobials are acetic, lactic, propionic, sorbic, and benzoic acids. Citric, caprylic, malic, fumaric, and other organic acids have limited activity but are used for flavorings.

The activity of organic acids are highly pH dependent. Early research demonstrated that the activity of organic acids are related to pH and the undissociated from of the acid is primarily responsible for antimicrobial activity (106-109). The use of organic acids are generally limited to foods with pH less than 5.5, since most organic acids have pK_a of pH 3.0 to 5.0 (35, 108, 110)

Mechanisms of action of organic acids

The mechanism of action of organic acids have some common element. Some evidence that organic acids influence cell wall synthesis in prokaryotes or that they

significantly interfere with protein synthesis or genetic mechanism. As previously, in the undissociated form, organic acid can penetrate the cell membrane lipid bilayer more easily. In this some phenomenon could be caused by interference with membrane permeability as well. Other related mechanisms involving the cytoplasmic membrane were studied by Freese, Sheu, and coworkers in the 1970s. Sheu and Freese (111) suggested that short-chain organic acids interfere with energy metabolism by altering the structure of cytoplasmic membrane proteins. They further hypothesized that the interference with membrane protein reduces ATP regeneration by uncoupling the electron transport side the cell, the acid dissociates because the cell interior has a higher pH than the exterior (112). Bacteria maintain internal pH to prevent conformational changes to the cell structural proteins, enzymes, nucleic acids, and phospholipids. Protons generated from intracellular dissociation of the organic acid acidify the cytoplasm and must be extruded to exterior by using energy in the form of ATP, the constant influx of these protons will eventually deplete cellular energy. It must be noted that system or by inhibiting active transport of nutrients into the cell. Sheu et al. (111) found that active transport inhibition influenced energy metabolism only indirectly cells were not necessarily ATP depleted. They suggested that inhibition of active transport was due to destruction of the proton motive force (PMF), which in turn caused active transport to cease. To summarize, the organic acids have significant affect on bacterial cytoplasmic membranes, interfering with metabolite transport and maintainance the membrane potentail. There is also considerable evidence that many organic acids affect activities of microbial enzymes. However, because many of the studies are done with whole cell, it is not clear whether these are direct or indirect effects (110, 113)

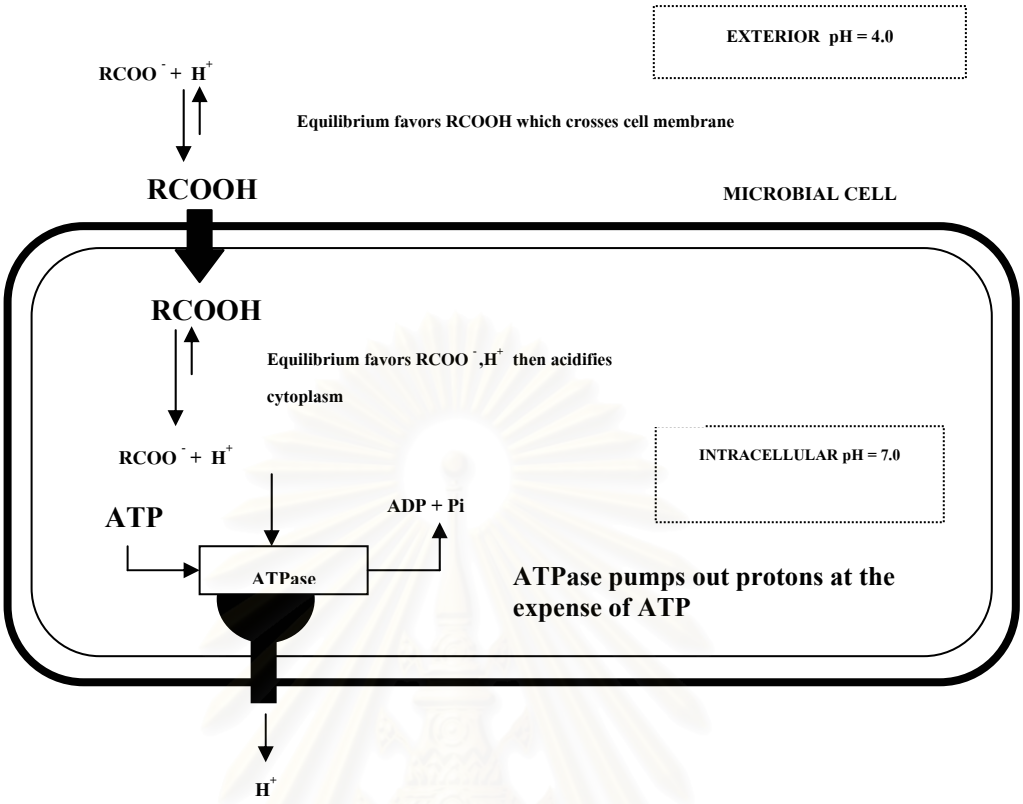

Figure 9 Fate of an organic acid (RCOOH) in a low-pH environment in the presence of a microbial cell**.** (110)

Acetic acid

The chemical compound acetic acid comes from the Latin word "*acetum*" meaning vinegar, systematically called ethanoic acid, it gives vinegar sour taste. It is a carboxylic acid with chemical formula $C_2H_4O_2$, often written as CH_3COOH. Pure acetic acid is a colorless, corrosive, flammable liquid that boils at 118 ^oC and freeze at 16.6 ^oC. It is miscible with water in all proportion. In aqueous solution, acetic acid can lose the proton of its carboxyl group, turning into the acetate ion CH_3Coo^- . The pK_a of acetic acid is about 4.75 at 25 ^oC. (114, 115)

Antimicrobial properties

One of primary functional uses of acetic acid in food has been that of an acidulant. The bacteriocidal activity has been attributed to a lowering of the pH below that needed for optimal growth. Acetic acid has shown variable success as antimicrobials in food application. In 1939 Levine and Fellers demonstrated a broad

range of susceptibilities of microorganisms to acetic acid. *Bacillus,* Salmonella, and Staphylococcus species were inhibited by lower concentrations of acid than *Saccharomyces* and *Aspergillus* (116). *Pseudomonas aeruginosa* was found to be sensitive to 1 % acetic acid (117) . Acetic acid can increase poultry shelf life when added to cut-up chicken parts in cold water at pH 2.5 (118). Addition of acetic acid at 0.1 % to scald tank water used in poultry processing decrease the heat resistance of *Salmonella* Newport, *Salonella* Typhimurium, and *Campylobacter jejuni* (119). In contrast, Lillard et al. (37) found that 0.5 % acetic acid (pH 3.6) in the scald water has no significant effect on *Salmonella spp.*, total aerobic bacteria, or member of the family *Enterobacteriaceae* on unpicked poultry carcasses. At 1 to 3 % as a dip for beef or lamb, acetic acid reduces counts of both pathogenic and spoilage imcroorganisms (120, 121). Acetic acid has shown variable effectiveness as an antimicrobial for use as a spray sanitizer on meat carcasses. Dickens and Whittemore exposed that the broiler to the same 10 min, pre-chill acid treatment, but at two concentrations of acetic acid (0.3 and 0.6 %) with and without the use of air injection to agitate the chill water. Aerobic plate count were unaffected by the treatment, but *Enterobacteriaceae* counts were significantly reduced by 0.86 log MPN/ml for the 0.3 % acid and 2.35 log MPN/ml for the 0.6 % acid solutions. Air injection did not affect reduction of these counts. There was no significant difference in texture or sensory characteristics between the treatments although the skin of the 0.6 % acetic acid treated carcasses was darkened or yellowed (32). Water pockets occurred under the skin of chicken carcasses with air-agitated samples (122). Dickson demonstrated that contaminated lean and beef tissue surface with *Salmonella* Typhimurium followed by treatment with 2 % acetic acid. *Salmonella* Typhimurium was reduced by 0.5 to 0.8 log CFU/cm^2, however this was not significantly different from the controls. It was noted that the use of acetic acid as a rinse for beef tissue did lead to sublethal injury of bacterial cells. An increase in organic material, such as rumen fluid, dirt, or manure, led to less effective reduction of *Salmonella* Typhimurium. (32) In 1994 Kotula and Thelappurate compared the efficacy of acetic acid and lactic acid solution (0.6 or 1.2 %) as a dip for rib-eye steaks. Acids were applied for 20 or 120 s at 1-2 ^{o}C and stored for up to 9 days. Although total plate counts were significantly lower for beef dipped in a 1.2 % acetic acid solution for 120 s. compared with water-dipped control, only a 0.8 log reduction was achieved at day 1. Similar result was seen with *E. coli* counts for the same parameters with a 0.7 log reduction. For lactic acid-treated

samples, total plate counts and *E. coli* counts were significantly reduced, but only by 0.4 log for the same parameters, A residual effect was noted for the lactic acid-treated tissue in the microbial counts were still significantly decreased compared to the control tissue after 9 days storage, but this effect was not seen with acetic acid- treated tissue. Acid-treated samples were lighter in color due to leaching of the pigment during immersion, but the shear values, moisture content, and sensory analysis were not affected by acid treatment. (123)

The general mechanism by which acetic acid inhibits microorganisms is related to that of other organic acids discussed previously. Sheu and Freese (124) and Freese at el. (110, 125) observed that acetic acid inhibits oxygen uptake and resultant ATP production by 76 to 77 % in whole cells of *B. subtilis*. The compound does not, however, inhibit NADH oxidation by isolated membranes. Further, they found that α-glycerol phosphate- or NADH-energized uptake of serine transport in membrane vesicals of *B. subtilis* is inhibited by acetic acid. *E. coli* whole cells and vesicals give similar results. They concluded that acetate inhibits growth by uncoupling substrate transport and oxidative phosphorylation from the electron transport system. This inhibits uptake of metabolites into the cell. Later, Sheu et al. (111) determined that the short-chain fatty acids, such as acetic acid may also act on cellular enzymes by reducing the intracellular pH (126).

Application and regulatory use in foods

Acetic acid is a monocarboxylic acid with a pungent odor and taste which limits its use. It is a principle component of vinegars and such is primarily used for its flavoring abilities. It is highly soluble in water. It is characteristically used in condiments such as mustard, catsup, salad dressings, and mayonnaise, and maybe found in pickled products such as sausages and pig feet. Because of cost and antimicrobial action, it has been added to infant feeding formulas to replace lactic acid (34, 127)

Acetic acid is generally regarded as safe (GRAS) for miscellaneous and general – purpose usage (21 CFR 182.1005) and when used in accordance with a good manufacturing practice (21 CFR 184.1005). It can be use as a curing and pickling agent, a pH control agent, flavor enhancer, flavoring agent and adjuvant, solvent, and vehicle (34, 35). The acceptable daily intake is listed in Table 3.

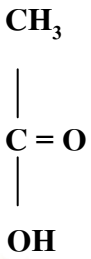

Figure 10 Structure of acetic acid

Citric acid

Citric acid or 2-hydroxy-1, 2, 3 propanetricarboxylic acid, its chemical formula is $C_6H_8O_7$. It is a carboxylic acid containing three carboxylic groups so citric acid has three pK_a values that are pK_{a1} 3.14, pK_{a2} 4.77, pK_{a3} 6.39 at 25^{o}C. At room temperature, citric acid is a white crystalline powder, melts at 153°C, and decomposes at higher temperatures. It is a weak acid as a good natural preservative and also use to add an acidic (sour) taste of foods and soft drinks. (128, 129)

Antimicrobial properties

Citric acid and its salts have been investigated for their effects on inhibition of bacteria, yeasts, and molds. In 1950 Murdock reported that citric acid was particulary inhibitory to flat-sour organism isolated from tomato juice and this inhibition appeared to be related to the inherent pH of the product (130). Citric acid was found to the most inhibitory to salmonellae, followed by lactic and hydrochloric acids (131). As a little as 0.3% citric acid lowered the level of salmonellae on poultry carcasses (33).Citric acid reduced growth and toxin production by *A. parasiticus* and *A. versicolor* but not *penicillium expansum* (132). It is inhibitory to *Salmonella spp.* In media and on poultry carcasses, growth and toxin production by *C. botulinum* in shrimp and tomato products, and *S. aureus* in microbiological medium (133-135). Branen and Keenan (136) were the first to suggest that inhibition by citrate may be due to chelation, in studies with *Lactobacillus casai*. In contrast, Buchanan and Golden (137) found that while undissociated citric acid is inhibitory against *L. monocytogenes*, the dissociated molecule protects the microorganism. They

theorized that this protection is due to chelation by the anion. Citric acid, rather than acetic or lactic acids, was also shown to have an effect on the inhibition of thermophilic bacteria (138), *Salmonella* Typhimurium (133), lactic acid bacteria such as *Streptococcus agalactiae* (139), and *S.* Anatum and *S.* Oranienburg (140) . As little as 0.3 % citric acid has been shown to be particularly effective in decreasing native levels of salmonellae on poultry carcasses (33). Conner (1990) found that *Listeria monocytogenes* was inhibited at pH 5.0 by propionic acid, 4.5 for acetic and lactic acids, and 4.0 for citric and hydrochloric acids when added in trypicase yeast extract soya broth. The effect was temperature dependent in that survival of *L. monocytogenes* decreased to undetectable levels within 1-3 weeks at 30 ^{o}C wherease 10 ^{o}C. *L. monocytogenes* was still surviving after 11-12 weeks in media adjusted with acetic, citric, and propionic acids and for 6 weeks in media containing HCL or lactic acid (141).Temperature dependency also played a role in inhibition of *L. monocytogenes* by citric acid. Minimum pH values for growth of *L. monocytogenes* were 4.66 at 30 ^{o}C, 4.36 at 10 ^{o}C, and 4.19 at 5 ^{o}C (142).

Application and regulatory use in foods

Citric acid is tricarboxylic acid having a pleasant sour taste and is found in a variety of natural foods. It is highly water soluble and enhances the flavor of citrus – based foods. It is approved for use in ice cream, sherbets and ices, beverages, salad dressings, fruit preserves, and jams and jellies, and it is used as an acidulant in canned vegetables and dairy products. It is precursor of diacetyl and therefore indirectly improves the flavor and aroma of a variety of cultured dairy products. It can control the pH, to prevent acidity by chelating metal ions (143). Citric acid, sodium citrate, and isopropyl citrate can be used for a multitude of proposes in meat and poultry products (9 CFR 318.7, 381.147) .

Citric acid is approved as a GRAS with no limitation when used in accordance with good manufacturing practice (21 CFR 184.1033) and used as substance for miscellaneous and general – purpose usage, in the acid form (21 CRF 182.1033) or as the calcium (21 CRF 182.1195). Citric acid also acts synergistically with antioxidants potassium (21 CRF 182.1625), or sodium salt (21 CRF 182.1751) (34, 35). The acceptable daily intake is listed in the Table 3.

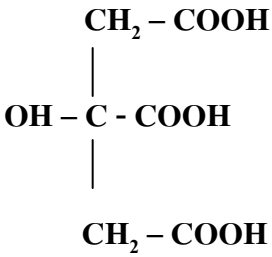

Figure 11 Structure of citric acid

Lactic acid

Lactic acid or 2-hydroxypropanoic acid, is a carboxylic acid and its chemical formula is $C_3H_6O_3$. At room temperature lactic acid is a colorless liquid organic acid. It is miscible with water or ethanol. The pK_a is about 3.08 at 25 ^oC. Lactic acid is a fermentation product of lactose, it present in sour milk, koumiss, and yogurt. Citric acid is produced in the muscle during intense activity. (144)

Antimicrobial properties

The microbial activity of lactic acid depend on the food application and target microorganism. Lactic acid was an excellent inhibitor of spore-forming bacteria at pH 5.0 but was totally ineffective against yeasts on molds (145). In 1954 Rice and Pederson found that lactic acid is more effective than malic, citric, propionic, or acetic acid in inhibiting growth of *Bacillus coagulans* in the tomato juice (146). Smulders et al. (147) and Snijders et al (148) showed that 1 to 2 % lactic acid reduces *Enterobacteriaceae* and aerobic microphillic microorganisms on beef, veal, pork, and poultry and delays growth of spoilage microflora during long-term storage of products. Lactic acid sprays have been effective in the rang of 1-1.25 % for lowering microbial loads on veal carcasses (149), but 2 % concentration of lactic acid led to discoloration (150). Rinse solution combining acetic acid and lactic acid coupled with higher temperature and packaging under vacuum also provided increased destruction of microbial load and extended shelf-life in beef carcasses (151-154).

A 1% concentration of lactic acid, pH 2.8 at 55 ^oC had little effect on the aerobic plate counts taken of the surface of pork carcasses. *Salmonella* spp. and *Listeria* spp. were not recovered, nor were sensory characteristic affected (155). By increasing the

concentration of lactic acid to 2 %, number of *Salmonella* spp. and *Campylobacter* spp. were reduced immediately and remain lower 24 h. after slaughter (156). Poultry carcasses have also been successfully decontaminated with lactic acid using dips or sprays (157, 158). Izat et al demonstrated that 1 % of lactic acid added to both chill water at 0-1.1 ^{o}C and scald water at 54 ^{o}C reduced the bacterial level of broilers artificially contaminated with *Salmonella* Typhimurium to almost non-detectable numbers. Lactic acid added to scald water alone had minimal effect on reducing the numbers of contaminated birds. The number of *Salmonella*-positive birds was also reduced as a function of time of the dip (39). Lactic acid added to broiler chill water resulted in the development of brown coloration most likely due to blood coagulation. In an effort to reduce carcass discoloration, lower levels of lactic acid as 0.25 %, pH 2.88 or 0.5 %, pH 2.62 were combined with 20 % of propylene glycol in chill water result in eliminated salmonellae from broiler carcasses after exposed 1 h, however lactic acid promoted discoloration and propylene glycol contributed an objectionable flavor (159).

Very little research has been done specifically on the mechanism of action o f lactic acid against foodborne microorganisms. Presumably, it functions similarly to other organic acids and has a primary mechanism involving distruption of the cytoplamic membrane PMF (113). Chen and Shelef (160) and Weaver and Shelef (161) measured the water activity of cooked meat model systems and liver sausage, respectively, containing lactate salts up to 4 % and concluded that water activity reduction is not sufficient to inhibit *L. monocytogenes*. It is most likely that at the high concentrations of lactate used, sufficient undissociated lactic acid is present, possibly in combination with a slightly reduced pH and water activity, to inhibit some microorganisms.

Application and regulatory use in foods

Lactic acid is a hygroscopic, syrupy liquid having a moderately strong acid taste. It is one of the most widely distributed acids in nature. Lactic acid is one of the primary acids formed during the natural fermentation process and is found in sauerkraut, pickles, green olives, fermented milk, cheese, certain sausages, and in other fermented foods of plant origin. The inhibitory capacity of this acid lies in its reduction of pH to level below the growth of many bacteria. In fermented foods, the

inhibitory action of lactic acid maybe coupled with other antigrowth factors excreted by lactic acid microorganisms (108) .

Lactic acid is used in the manufacture of jam, jellies, sherbets, confectionary products, and beverages. It is used to adjust acidity in brines for pickles and olives. Calcium lactate can used as a firming agent for apple slices, to prevent discoloration in fruit, and in baking powders (108).

Lactic acid is approved as a GRAS substance for miscellaneous or general – purpose usage (21 CFR 182.1061) and also used in accordance with manufacturing practice. It can be use as antimicrobial and pH control agents, curing and pickling agent, flavor enhancer, flavoring agent and adjust, solvent, and vehicle (21 CFR 184.1061). Lactic acid and its calcium, potassium, and sodium salts can be used for a multitude of purposes in meat and poultry products (21 CFR 318.7, 381.47) (34, 35) . The acceptable daily intake is listed in the Table 3.

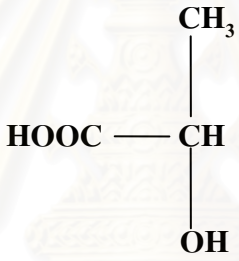

Figure 12 Structure of lactic acid

Table 1 Dissociation constants of organic acids in aqueous solutions (34).

Acids	pKa_1	pKa_2	pKa_3
Acetic acid	4.75		
Dehydroacetic acid	5.27		
Sodium diacetate	4.75		
Adipic acid	4.43	4.41	
Caprylic acid	4.89		
Citric acid	3.14	4.77	6.39
Fumaric acid	3.03	4.44	
Lactic acid	3.08		
Malic acid	3.40	5.11	
Propionic acid	4.87		
Succinic acid	4.16	5.61	
Tartaric acid	2.98	4.34	

Table 2 Proportion of total acid undissociated at different pH values[a] (42).

Organic acids	pH values				
	3	4	5	6	7
Acetic acid	98.5	84.5	34.9	5.1	0.54
Benzoic acid	93.5	59.3	12.8	1.44	0.144
Citric acid	53.0	18.9	0.41	0.006	<0.001
Lactic acid	86.6	39.2	6.05	0.64	0.064
Propionic acid	98.5	87.6	41.7	6.67	0.71
Sorbic acid	97.4	82.0	30.0	4.1	0.48

[a]Values given as percentage.

Table 3 Acceptable Daily Intake for Men (34).

Acids	**Limitations (mg/kg bodyweight)**		**References**
	Unconditional	**Conditional**	
Acetic	Not limited		FAO (1965)
Acetate, Ca^+, K^+, Na^+	Not limited		FAO (1963,1973)
Sodium diacetate	0 – 15		FAO (1973)
Adipic		0 – 5	FAO (1965)
Citric[a]	Not limited		FAO (1966)
Citrate, Ca^+, K^+, Na^+	Not limited		FAO (1963)
Fumaric	0 – 6		FAO (1974)
Lactic	Not limited		FAO (1965)
DL-Lactic		0 – 100[b]	FAO (1965)
Lactate, Ca^+, K^+, NH_4^+, Na^+	Not limited		FAO (1973)
Malic	Not limited		FAO (1965)
DL-Malic		0 – 100[c]	FAO (1966)
Propionic	Not limited		FAO (1965)
Propionates, Ca^+, K^+, Na^+	Not limited		FAO (1973)
Tartalic[a]	0 – 30		FAO (1973)
Tartrate, K^+, Na^+	0 – 30		FAO (1973)

[a] Naturally ocurring substances; the estimated acceptable daily intakes listed here do not include amounts occurring naturally.

[b] Refers to content of D (-) -lactic acid.

[c] Refers to content of D (-) – malic acid; the maleic acid content of malic acid should not exceed 0.05%

Table 4 Antimicrobial spectra of organic acids used in foods[a,b] (42).

Organic acids	Concentrations of undissociated acid required to inhibit growth of most strains in microbiological media				
	Yeast	**Mold**	**Enterobacteriaceae**	**Micrococcaceae**	**Bacillaceae**
Acetic acid	0.5	0.1	0.05	0.05	0.1
Benzoic acid	0.05	0.1	0.01	0.01	0.02
Citric acid	>0.005[d]	>0.005	>0.005	0.001[e]	>0.005
Lactic acid	>0.01	>0.02	>0.01	>0.01	>0.03
Methyl paraben[c]	0.1	0.1	0.2	0.4	0.2
Ethyl paraben[c]	0.1	0.05	0.1	0.1	0.1
Propyl paraben[c]	0.01	0.02	0.1	0.05	0.05
Propionic acid	0.2	0.05	0.05	0.1	0.1
Sorbic acid	0.02	0.04	0.01	0.02	0.02[f]

[a] From Chichester and Tanner, 1972 and unpublished data of S. Warren and B. Freame.

[b] Values given as Percentage in solution.

[C] Paraben = p-hydroxybenzoic acid.

[d] Actual inhibitory concentrations are probably far in excess of values given here.

[e] Staphylococcus aureus; micrococci are much more resistant.

[f] Clostridia are generally more resistant.

Table 5 Types, functions, and limitations of cleaning agents used in the food industries (42) .

Categories of cleaners	**Concentrations for use (%)**	**Examples of chemical used**	**Functions**	**Limitation**
Clean water	100	Usually contains dissolved air and soluble minerals in small amounts	- Solvent and carrier for soils, as well as chemical cleaners	- Residual moisture may allow microbial growth on washed surfaces - Promotes rusting of iron - Hard water leaves deposit on surfaces
Oraganic acids	0.1-2	Acetic, Gluconic, Lactic, Saccharic, Citric, Levulinic, Tartaric	- Remove inorganic precipitates and other acid-soluble substances from surfaces	- Moderately corrosive, but can be inhibited by various organic nitrogen compounds
Chlorinated compounds	1	Dichlorocyanuric acid, Trichlorocyanuric acid, Dichlorohydantoin	- Used with alkaline cleaners to increase peptizing of proteins and minimize milk stone deposits	- Not germicidal because of high pH. - Concentrations very depending on the alkaline cleaner and conditions of use
Strong alkalis	1-5	Sodium hydroxide, Sodium orthosilicate, Sodium sesquisilicate	- Detergents for fat and protein - Precipitate water hardness -Produce alkaline pH	- Highly corrosive - Difficult to remove by rinsing - Irritating to skin and mucous membranes.
Inorganic acids	0.5	Hydrochloric acid, Sulfuric acid, Nitric acid, Phosphoric acid, Sulfamic acid	- Produce pH 2.5 or below - Remove precipitates from surfaces	- Very corrosive to metals, but can be partially inhibited by amines - Irritating to skin and mucous membranes

CHAPTER IV

MATERIALS AND METHODS

Methodology scheme

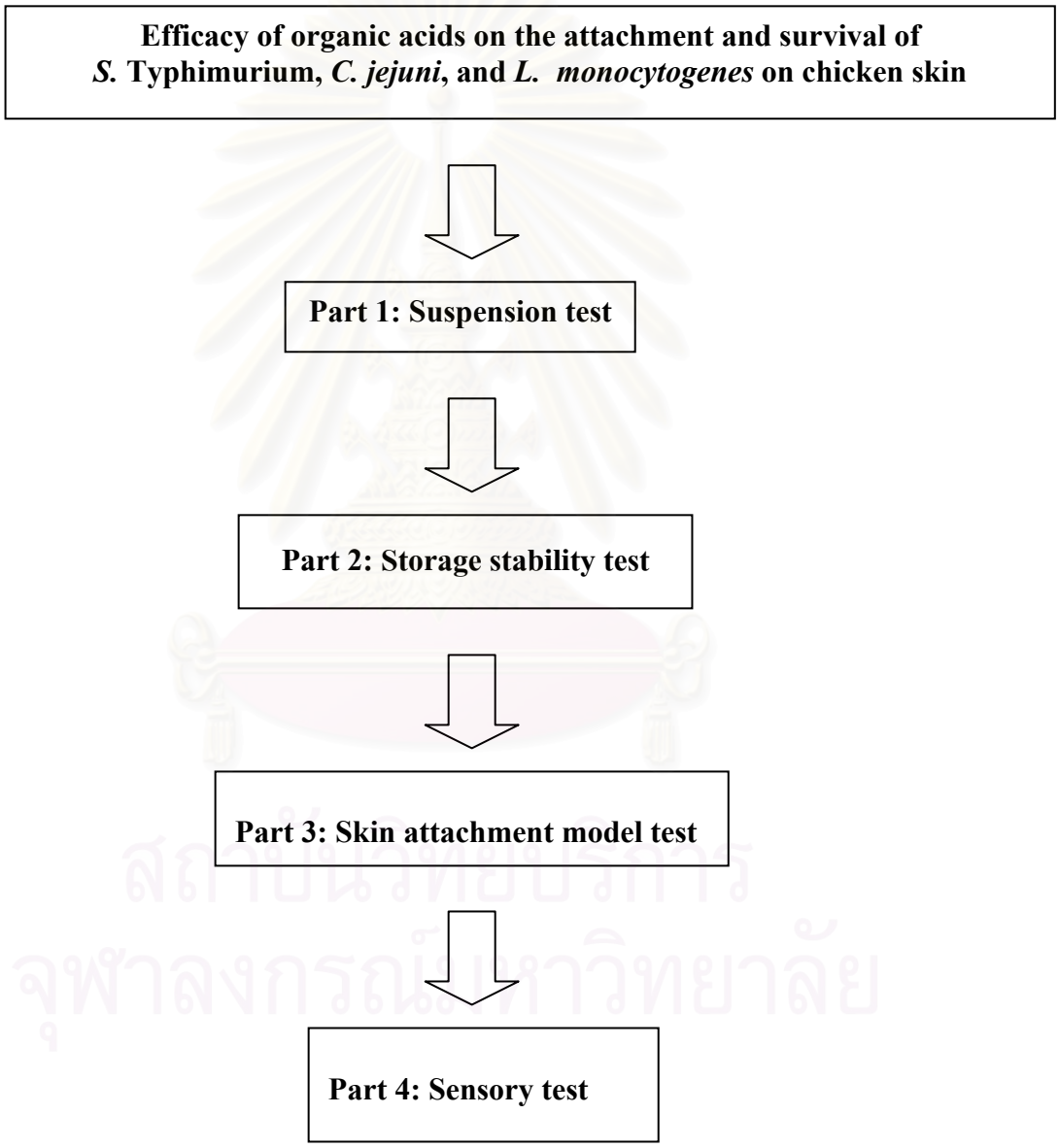

Figure 13 Methodology scheme

Part 1: Efficacies of organic acids to eliminate *Salmonella*Typhimurium, *Campylobacter jejuni*, and *Listeria monocytogenes* in suspension test.

1.1 Bacterial culture

In this experiment used 3 strains of *Salmonella enterica* serovar Typhimurium DT104 that were *S.* Typhimurium DT104 4587and 30820 which obtained from the WHO National *Salmonella* and *Shigella* Center, National Institute of Health, Department of Medical Sciences, Ministry of Public Health and *S.* Typhimurium DT104 9822099 were obtained from Danish Institute for food and Veterinary Research Department for epidermiology and Risk Assesment, the Danish Zoonosis center and Animal Health Section. Three strains of *Campylobacter jejuni* that were *C. jejuni* DVL 20, DVL 21, and ATCC 700819 were obtained from Center for Antimicrobial Resistance Monitoring in Foodborne Pathogens (in cooperation with WHO), Department of Microbiology, Faculty of Veterinary Science, Chulalongkorn University. *Listeria monocytogenes* 3 strains that were *L. monocytogenes* DMST 17303, 8-5, 17-45 were obtained from DMST Culture Collection, Department of Medical Sciences, Ministry of Public Health and Center for Antimicrobial Resistance Monitoring in Foodborne Pathogens (in cooperation with WHO), Department of Microbiology, Faculty of Veterinary Science, Chulalongkorn University, respectively.

For experiments, each bacterial strain was cultured separately in 10 ml of brain heart infusion broth (BHI) (Difco Laboratories, U.S.A.) at 37 ^{o}C overnight without shaking except *C. jejuni* twice day transfer and incubated in microphilic condition. Following incubation, 10 ml of each culture was harvested by centrifugation (7000 x g for 15 min), washed twice with sterile phosphate buffer saline (PBS) and resuspended in 10 ml of sterile PBS. Bacterial suspension were adjusted as a final cell concentration of 10^{8} CFU/ml measuring by spectrophotometer Novaspec II (Phamacia LKB, U.S.A.) at 600 nm (as value 0.5 $\pm$ 0.1). Then pooled 5 ml of 3 strains of each bacterial specie into a common test tube that had 3 tubes individualy and confirmed bacterial numbers by plating 0.1 ml portion of appropriately diluted culture on tryptic soy agar (TSA) (Difco, U.S.A.) plates and inocubating the plates at 37 ^{o}C for 24 h. *C. jejuni* was confirmed using modified charcoal cefoperazone desoxycholate (mCCDA) (Oxoid, U.S.A.) agar incubated at 37 ^{o}C for 48 h in microaerophilic condition.

1.2 Preparation of acidifiers

The experiments were performed with crystalline technical citric acid (molecular weight 210.14 g/mol. Ajex Finechem, Australia); glacial acetic (molecular weight 60.05 g/mol. Merck, Germany); 85 % lactic acid (molecular weight 90.08 g/mol. Ajex Finechem, Australia). Acetic, citric and lactic acids were used in final concentration of 0.25, 0.5, 1, 2 and 4 % in each acid. Preparing of mixed organic acids were divided in 6 formula. The first formular was applied in 0.5 % lactic and 0.5 % citric acids. The second formula was applied in 0.5 % lactic and 1 % citric acids. The third, forth, fifth, and sixth formula were assessed in 1 % lactic: 0.5% citric acids, 1 % lactic: 1 % citric acids, 1 % lactic: 2 % citric acids and 2 % lactic: 1 % citric acids, respectively. The concentration of organic acids in this study were that considered generally recognized as safe for use on food. The pH of the acid treatments ranged from 2.09 to 2.94**,** depending on the concentration and type of acids.

1.3 Determination of pH

A digital pH meter with a glass pH electrode cyberscan 500 (Beckman, U.S.A) was used to measure pH of organic acid solution.

1.4 Testing bactericidal activity of organic acids and mixed organic acids

Place each test tube that contained organic acid and mixed organic acids on the race. Add 0.5 ml of tested culture to each tube acid at 30 s intervals to provide an initial population of 10^7 CFU/ml (4 replications/test culture/acid application). Final concentration of acids after added culture suspension were 0.25, 0.5, 1, 2, and 4 % of acetic, lactic, citric acids, mixture of lactic and citric acids in concentration of 0.5:0.5, 0.5:1, 1:0.5, 1:1, 1:2, and 2:1 %, respectively. After 5, 10, 15 and 20 minutes, 1 loopful (10 μl) of inoculated tubes as above were transferred into BHI incubated at 37 ^{o}C for 48 h except *Campylobacter* used preston broth and incubated in microaerophilic environment.The broth culture tubes were evaluated for growth by tubidity. Comfirmed by restreak subculture that showed positive result on selective agar plate as modified Oxford agar (MOX) (Oxoid, U.S.A.) for *L. monocytogenes*, xylose lysine deoxycholate (XLD) (Oxoid, U.S.A.) agar for *S.* Typhimurium. In case of *Campylobacter* have to restreak all subculture tubes on mCCDA plates because cannot suspect the tubidity of broth.

Part 2: Storage stability of organic acids to eliminate *Salmonella* Typhimurium, *Campylobacter jejuni*, and *Listeria monocytogenes* in suspension test.

2.1 Bacterial culture as above (1.1)

2.2 Preparation of acidifiers

Selected organic acid or mixed organic acids from part 1 that show the most effective to eliminated 3 species of tested culture in suspension test and prepared as described above. Storage these organic acid or mixed organic acids at 25 ^{o}C (room temperature) and 4 ^{o}C for 1, 3, 5, and 7 before used in experiment.

2.3 Testing bactericidal activity of organic acids and mixed organic acids

Place selected organic or mixed organic on the race. Add 0.5 ml test culture to each tube of acid at 30 s intervals to provide an initial population of 10^7 CFU/ml (4 replications/tested culture/acid application). After 5, 10, 15 and 20 min, 1 loopful of inoculated tubes as above were transferred into BHI, incubated at 37 ^{o}C for 48 h except *Campylobacter* used preston broth and incubated in microaerophilic environment.The broth culture tubes are evaluated for growth by tubidity. Comfirmed by restreak subculture that showed positive result on selective agar plate as MOX for *L. monocytogenes*, XLD agar for *S.* Typhimurium.In case of *Campylobacter* have to restreak all subculture tubes on mCCDA plates because cannot suspect the tubidity of broth. (protocol as 1.4).

Part 3: Efficacy of organic acids on the attachment and survival of *Salmonella*Typhimurium, *Campylobacter jejuni*, and *Listeria monocytogenes* on chicken skin.

3.1 Bacterial culture

Preparing as 1.1

3.2 Skin preparation

Chicken breast samples were collected from Big C Department store, aseptically cut into 5x10 cm, using a template. Each sample had a 50 cm^2 exposure area. All samples were individually packaged in plastic bag and gamma irradiated at 10 kGa by cobalt 60 source (at Office of Atomic for Peace, Bankok) to inactivate naturally occurring microflora. Irradiated samples were stored at -20 ^{o}C until used.

Figure 14 Chicken skin sample after gamma irradiation

3.3 Determination of pH

A digital pH meter with a glass pH electrode cyberscan 500 (Beckman, U.S.A), was used to measure pH of chicken skin surface before and after treatment for 0, 1, 24, and 48 h.

3.4 Skin attachment experiments and spraying treatments

In a 1 st trial (Experiment 1) 216 chicken breast skins were used in this trial (72 chicken skins/ test culture). Irradiated chicken breast samples were thawed at refrigeration temperature, 0.2 ml of the standardized bacterial culture were gently spreaded on the skin surface to give approximately $10^5 – 10^6$ CFU/cm^2 and leaving it for 30 min to allow for bacterial cells attachment prior to application of treatment (8). Loosely attached bacterial were washed of by rinsing the skin 3 times with 100 of PBS. The skin was placed on sterile plate for spraying treatment. Eight chicken breast skins (replicates) were each sprayed with 0 ^{o}C of sterile distilled water, 8 with 0 ^{o}C of selected organic acids from part 1, and 8 were not sprayed. Spraying was performed by using low pressure spray gun for 10 s at 40-45 psi at room temperature. During spraying, each skin was hold in a vertical, the distance between the skin and nozzle was about 30 cm, and excess acid or water was drain off. After the organic acid spraying, leaving it in sterile petri dish for 1 h at room temperature and then the skin was rinsed to remove chemical residues by rinsing 100 ml of water before detection of viable tested bacteria. The skin samples were treated as above for 25 and 55 ^{o}C of water or selected organic acids per tested organism.

In a 2 nd trial (Experiment 2) 144 chicken breast skins were used, for each tested organism 48 pieces were inoculated as describe above. Sixteen skins sprayed with water, 16 with selected organic acids in selected temperature of suspension from 1 st trial, with the least 16 not sprayed. To maintain stable conditions and avoid dehydration, chicken skins were store in individually sterile plastic bag at 4 ^{o}C. Enumeration of viable tested organism of 24 skins (8 skins per treatment), was performed 24 h later and the other after 48 h.

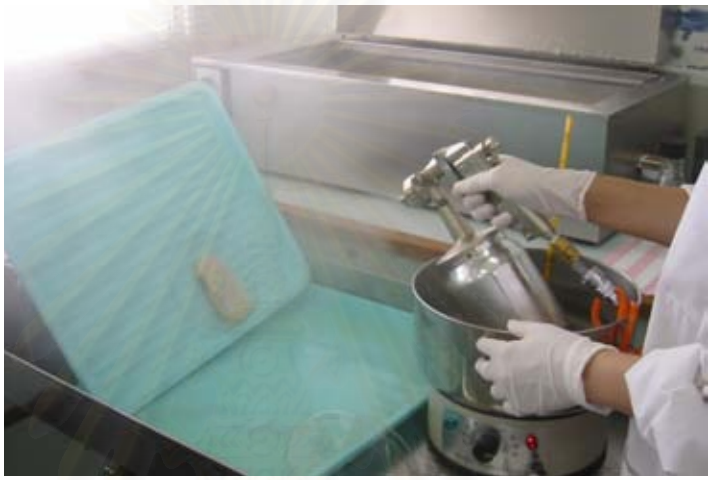

Figure 15 Spraying treatment

3.5 Microbial enumeration

Skin samples were transferred by sterile forcep and scissor to 50 ml of fresh PBS in sterile plastic bag and blended with a automatically masticator (IUL instrument, Thailand) for 2 min. Samples were obtained following blending to enumerate population of target bacteria, which were defined as firmly attached cells. One milliliter of the blending solution in each sample was serially diluted (1:10) in 9 ml of PBS and the population of *Salmonella* Typhimurium or *Campylobacter jejuni* or *Listeria monocytogenes* were determined by plating 0.1 ml each dilution in triplicate on selective medium of each culture and incubated in aerobic condition at 37 ^{o}C except *C. jejuni* incubated in microaerophilic environment at 37 ^{o}C for 48 h. Plates were colonies counted and confirmation tests run.

S. Typhimurium was determined using XLD agar and represented dark colonies that were further screened using gram strain, TSI (triple sugar agar), LIM (lysine indole motile agar) slants and serological typing by slide agglutination test.

Confirmation of *C. jejuni* was on the basis of microscopic examination (motile Gram negative non-sporing curved S-shaped rods), and the isolates being positive to catalase and oxidase tests. *C. jejuni* was also determined using mCCDA agar and represented small gray droplike or gray slimey colonies, were further screened using hippurate hydrolysis test for identify specie.

L. monocytogenes was determined using MOX agar and representative colonies were 2-3 mm in diameter with dark brown or black haloes, the colonies have sunken centers, further screened using motility testing which *Listeria* shows tumbling motility, β-hemolysis, CAMP test, and fermentation of manital, rhamnose and xylose.

3.6 Expression of antimicrobial activity

Efficacies of the treatments were assessed by determining the reduction in viable population of attached *S.* Typhimurium or *C. jejuni* or *L. monocytogenes*. Reductions ($\log_{10}$ CFU/cm^2) were obtained by subtracting the number of cells recovered from the treated skin from the number of cells recovered from the inoculated skin (non-sprayed skin, control). The difference was the reduction due to treatment. Percentage of bacterial reduction was performed by this formular

$$\% \text{ Reduction} = \frac{\begin{array}{c}\text{(Average of bacterial number of control group -} \\ \text{Average of bacterial number of treated group)}\end{array}}{\text{Average of bacterial number of control group}} \text{ x } 100$$

3.7 Data analysis

Microbiological count data was transformed into logarithm before obtaining mean and performing statistical. Cell populations on chicken skins not treated and treated with water and mixed organic acids solution were compared for significant difference ($P<0.05$) using Analysis of Varience (one-way ANOVA) and any statistical differences among different treatment were determined by Duncan's multiple range test. The SPSS program was used to carry out the computation.

Part 4: Effect of organic acids on physical properties and sensory quality of chicken

4.1 Difference of chicken skin color between non-sprayed and mixed acids-sprayed chicken skin in various temperatures of solution.

4.1.1 Skin sample preparation

Chicken thigh samples used in this study were obtained from a commercial broiler processing plant. All chicken thigh samples were collected before exposing into chiller tank (chlorinated water). Skin was removed from broiler carcass and cut into 5x5 cm, using a template. All samples were individually packed in sterile plastic bags, maintained at -20 ^{o}C, and thawed at 4 ^{o}C prior to the application of treatments.

All thigh skins were separated into 4 treatments. Treatment 1 to 3 sprayed with mixed organic acids that contained 2 % lactic acid and 1 % citric acid for 10 s, temperatures of solution were 0, 25 and 55 ^{o}C. Treatment 4 was control group, non-mixed organic acids sprayed. Skin samples were individually packed in sterile plastic bags. Color of skin samples were measured color by using Spectro-sensor II (Applied Color System, Inc., Princeton, New Jersey) before spraying (day 0) and after treatment 1 to 3.

4.1.2 Color Measurement

CIE color values were measured on the surface of sample using a Spectro-sensor II (Applied Color System, Inc., Princeton, New Jersey) that had been calibrated against a black and a white reflectance tile. The CIE L* value represented lightness (0 = dark to 100 = light), a* and b* values measured chromaticity coordinates, where positive a* value indicates redness and positive b* value indicates yellowness. Color values were recorded by placing the hand-held colorimeter directly in contact with the skin. Color was measured at three locations of skin surface. Triplicate measurements were taken from each skin sample and averaged for analysis.

Figure 16 A Spectro-sensor II colorimeter using for mearsure chicken skin

4.1.3 Determination of pH

The pH of skin chicken samples were measured by direct placing of the electrode (Beckman, U.S.A.) into the homogenized skins, the mean value was used as the final pH value.

4.1.4 Statistical analysis

Use One-way ANOVA to analyze whether significance exists for a difference among the sample. If the *F*-statistic is significant, apply LSD. Color differentials (Δ) were calculated by subtracting pretreatment color values from post-treatment values.

4.2: Evaluation of the differences of color and odor of 0, 25, and 55^{o}C of mixed organic acids treated compared to non-sprayed raw chicken samples before cooking.

4.2.1 Panelist

Sensory multiple comparison tests were conducted by 17 trained panelists. Panelists were recruited from the staffs of the Institute of Food Research and Product Development, Bangkok. Criteria for recruitment were that the individual was: (1) not allergic to any food; (2) a consumer of chicken; (3) available and willing to participate during testing dates. The panelists were trained to use the spectrum intensity scoring and to calibrate scores of selected aroma in standard chicken

samples. This test was performed in partition boots inside controlled environment of sensory laboratory.

Figure 17 and 18 Sensory laboratory and partition booths.

4.2.2 Sample preparation and sensory evaluation

Chicken samples used in this study collected from the Big C department store, Ratjadumri. Samples were cut into 5x5 cm, using a template. All samples were individually packed in sterile plastic bags, maintained at -20 ^{o}C, and thawed at 4 ^{o}C prior to the application of treatments. All skin samples were separated into 4 treatments.

Treatment 1 to 3 were sprayed mixed organic acids that contained 2 % lactic acid and 1 % citric acid on chicken skin for 10 s, temperature of suspension were 0, 25 and 55 ^{o}C. Treatment 4, as a control group, non-mixed organic acids sprayed chicken. All samples were individually packed in sterile plastic bags. The samples were kept at 4 ^{o}C for a day.

Each sample cup was labeled by a random three digit code number, contained a piece of raw chicken from each treatment and covered a cup by a lid before serving. Panelists were asked to evaluate each group of samples in randomized order. For each group of four pieces, one piece as a control, panelists were asked to assign scores for sour odor of mixed organic acids on skin and external skin color compared to the control chicken before cooking using a nine-point scale. For a scoring test using a nine-point scale: 1 means extremely lighter/mild, 2 means much lighter/mild, 3 means lighter/mild, 4 mean slightly lighter/mild, 5 means no

difference, 6 means slightly darker/stronger, 7 means darker/stronger, 8 means much darker/stronger, and 9 means extremely darker/stronger.

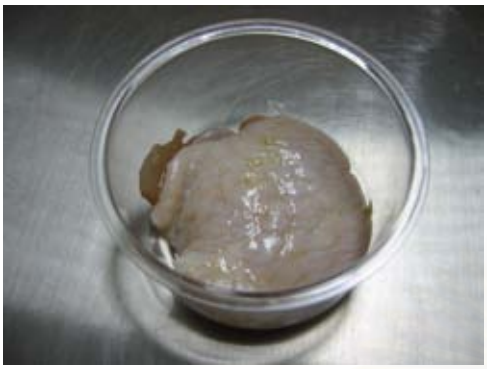

Figure 19 and 20 Serving size of raw chicken sample to panelist.

4.2.3 Statistical analysis

One-way ANOVA was used for analyzing whether significance exists for a difference among the sample. If the *F*-statistic is significant, apply LSD test will be applied.

4.3 Evaluation of the differences of flavor and taste of 0, 25, and 55^{o}C of mixed organic acids treated compared to non-sprayed cooked chicken samples.

4.3.1 Panelist

Used panelists as describe in section 4.2.1.

4.3.2 Cooking method and sensory evaluation

After evaluating color and sour odor of raw chicken of all four groups of treatments in part 2, samples were cooked. The chicken samples, covered with the skin, were cooked in microwave (Hitachi, Japan) at the highest electric power as 220 Volt for 3 min. The chicken samples were cooked in an area separated from the testing area to avoid cooking odors influencing the panelist and kept warm as about 50-55 ^{o}C until they were served to panelists. The holding period did not exceed 1 h after cooking. Each sample cup was labeled by a random three digit code number. Panelists were served with four cups of each treatment group, contained about 15 g of cooked chicken sample for each group. Panelists were asked to evaluate sour flavor and taste of the control sample compared to treated samples after cooking using a nine-point scale. Panelists were provided with water, an expectoration cup, unsalted

crackers to clean the palate between samples. A scoring test using a nine-point scale: 1 means extremely mild, 2 means much mild, 3 means mild, 4 means slightly mild, 5 means no difference, 6 means slightly stronger, 7 means stronger, 8 means much stronger, and 9 means extremely stronger.

Figure 21 Presentation of chicken sample

Figure 22 Serving size of chicken sample after cooking**.**

4.3.3 Statistical analysis

One-way ANOVA was used for analyzing whether significance exists for a difference among the sample. If the *F*-statistic is significant, LSD test will be applied.

4.4 Acceptance and Preference of customer to non-sprayed and mixed acids-sprayed chicken skin

4.4.1 Panelist

For acceptance and preference test, panelists were selected from volunteers at a test site (Daokanong and Bangprakaew markets) as 125 persons who were willing to participate.

4.4.2 Sample preparation

Breast chicken samples used in this study collected from Big C department store, Ratjadumri. Samples were cut into 10x5 cm, using a template. Samples were divided into two groups. First group sprayed mixed organic acids that contain 2 % lactic acid and 1 % citric acid, temperature of suspension was 55 ^{o}C on chicken skin for 10 s. Another group, as a control group, non-mixed organic acids

sprayed chicken. Three pieces of chicken samples of each group were packed in sterile plastic bags, maintained at 4 ^{o}C for a day. This test was performed in Dawkanong and Bangprakaew markets.

Panelists were asked about the acceptance, preference and attitudes to mixed acids sprayed chicken samples. This experiment separated into two parts; part 1 showed the acceptance of consumers to mixed acids sprayed chicken samples. Only mixed acids sprayed chicken samples were presented to panelists and then panelists were asked a question. The question was “Would you like to buy these chickens, why not?” There were two choices that were (1) would buy or (2) would not buy. Panelists selected the choice and provided the reason why they selected that choice. Part 2 showed the preference of consumers to mixed acids sprayed and non-mixed acids sprayed chicken samples. Both mixed acids sprayed and non- mixed acids sprayed chicken samples were presented to panelists and then panelists were asked to select one of chicken sample that they prefered more than another one and provided the reason why they prefered. All answers were presented in percentage of consumers who picked mixed acids sprayed or non- mixed acids sprayed chicken samples and percentage of consumers who prefered mixed acids sprayed or non-mixed acids sprayed chicken samples.

CHAPTER IV

RESULTS

Part 1: Efficacies of organic acids to eliminate *Salmonella* Typhimurium, *Campylobacter jejuni*, and *Listeria monocytogenes* in suspension test.

Bactericidal efficacies of acetic, citric, lactic acid and mixed acids to eliminate *Salmonella* Typhimurium, *Campylobacter jejuni*, and *Listeria monocytogenes* were investigated by varying the concentration that were 0.25, 0.5, 1, 2, and 4 % of each acid and mixed organic acids that consist of lactic and citric acids; 0.5:0.5, 0.5:1, 1:0.5, 1:1, 1:2, and 2:1 %. For comparison, the activity of each organic treatment was determined against freely suspended *Salmonella* Typhimurium, *Campylobacter jejuni*, and *Listeria monocytogenes* were presented in Table 6. The following ranges of activity (time to kill bacteria in suspension) for all organic acids treatments at the following concentrations were observed. All concentration of acetic acid that are 0.25, 0.5, 1, 2, and 4 % of acid could not eliminate suspended *L. monocytogenes* in concentration 10^7 CFU/ml within 20 min but 2 and 4 % of acid could destroy *S.* Typhimurium within 10 and 5 min, respectively. Acetic acid in concentration 0.25 % could not eliminate *C. jejuni* within 20 min but 0.5% of acetic acid could kill this organism within 10 min, whereas 1, 2, and 4 % of acid could eliminate *C. jejuni* within 5 min.

As citric acid, 0.25 to 4 % of acid could not eliminat suspended *L. monocytogenes* within 20 min. Citric acid in concentration 0.25 to 1 % could not kill *S.* Typhimurium within 20 min but in higher concentration 2 and 4 % could destroy within 15 and 10 min, respectively. Only 0.25 % of citric acid could not kill *C. jejuni* within 20 min, wherease *C. jejuni* was killed within 10 min by 0.5 % of citric. In higher concentration of citric acid, 1, 2, and 4 % could eliminate *C. jejuni* within 5 min.

In case of lactic acid, 0.25, 0.5, and 1 % of acid could not destroy against freely suspended *L. monocytogenes* within 20 min but both 2 and 4 % of its could eliminate *Listeria* within 15 and 5 min, respectively. Low concentration of lactic acid solution (0.25 %) could not eliminate *S.* Typhimurium within 20 min wherease 0.5 %

of lactic acid could eliminate *S.* Typhimurium within 15 min. Since 1 to 4 % of lactic acid solution could destroy *S.* Typhimurium within 5 min. Lactic acid showed high activity to eliminated *C. jejuni*, 0.25 % of lactic acid could kill this organism within 15 min and 0.5, 1, 2 and 4 % of its could destroy *C. jejuni* within 5 min.

Combination of 0.5 to 2 % concentration of lactic and citric acids (0.5:0.5, 0.5:1, 1:0.5, 1:1, 1:2, and 2:1) as mixed organic acids were observed the effectiveness in each formula compared with acetic, citric and lactic acid. All mixed acids treatments could kill *C. jejuni* within 5 min. In contrast, 0.5:0.5, 0.5:1, 1:0.5, and 1:1 % of lactic and citric acids could not kill *L. monocytogenes* within 20 min, wherease 1:2 and 2:1 % of lactic and citric acids could destroy within 15 and 5 min, respectively. Mixed acids in concentration 0.5:0.5 and 0.5:1 % of lactic and citric acids could kill *S.* Typhimurium within 15 min and *S.* Typhimurium was killed within 15 by 1:0.5, 1:1, 1:2, and 2:1 % of lactic and citric acids.

Table 6 Efficacies of acetic, lactic, citric, and mixed organic acids in bacterial elimination at 5, 10, 15 and 20 min.

Organic acid / Time (min)	pH	*L. monocytogenes* (10^7 CFU/cm^2)				*S.* Typhimurium (10^7 CFU/cm^2)				C. jejuni (10^7 CFU/cm^2)			
		5	10	15	20	5	10	15	20	5	10	15	20
Acetic acid (%)													
0.25	2.94	+	+	+	+	+	+	+	+	+	+	+	+
0.5	2.88	+	+	+	+	+	+	+	+	+	-	-	-
1.0	2.82	+	+	+	+	+	+	+	+	-	-	-	-
2.0	2.67	+	+	+	+	+	-	-	-	-	-	-	-
4.0	2.55	+	+	+	+	-	-	-	-	-	-	-	-
Citric acid (%)													
0.25	2.68	+	+	+	+	+	+	+	+	+	+	+	+
0.5	2.52	+	+	+	+	+	+	+	+	+	-	-	-
1.0	2.37	+	+	+	+	+	+	+	+	-	-	-	-
2.0	2.22	+	+	+	+	+	+	-	-	-	-	-	-
4.0	2.09	+	+	+	+	+	-	-	-	-	-	-	-
Lactic acid (%)													
0.25	2.68	+	+	+	+	+	+	+	+	+	+	-	-
0.5	2.52	+	+	+	+	+	+	+	-	-	-	-	-
1.0	2.38	+	+	+	+	-	-	-	-	-	-	-	-
2.0	2.26	+	+	-	-	-	-	-	-	-	-	-	-
4.0	2.15	-	-	-	-	-	-	-	-	-	-	-	-
Mixed acids (%) (Lactic : Citric)													
0.5 : 0.5	2.39	+	+	+	+	+	+	-	-	-	-	-	-
0.5 : 1.0	2.32	+	+	+	+	+	+	-	-	-	-	-	-
1.0 : 0.5	2.29	+	+	+	+	-	-	-	-	-	-	-	-
1.0 : 1.0	2.25	+	+	-	-	-	-	-	-	-	-	-	-
1.0 : 2.0	2.18	+	+	-	-	-	-	-	-	-	-	-	-
2.0 : 1.0	2.15	-	-	-	-	-	-	-	-	-	-	-	-

+ : growth

- : no growth

Number of replications = 4

Part 2: Storage stability of organic acids to eliminate *S.* Typhimurium, *C. jejuni*, and *L. monocytogenes* in suspension test.

Objective of this part to study storage stability of selected organic acids that showed high effectiveness to eliminate suspended *S.* Typhimurium, *C. jejuni*, and *L. monocytogenes* in concentration 10^7 CFU/ml in part 1. Mixed organic acid which combination of 2 % lactic and 1 % citric acids was selected because its showed the most effective in bacterial elimination with in 5 min. After prepared acids, divided into 2 groups. Each group of mixed organic acids suspension was kept since 1 to 7 days at 4 and 25 ^{o}C, respectively. The efficacy of mixed acids was observed at day 1, 3, 5, and 7. The results showed (Table 7) combination of 2 % lactic and 1 % citric acids after storage for 7 days at 4 and 25^{o}C could killed freely suspended *S.* Typhimurium, *C. jejuni*, and *L. monocytogenes* within 5 min as results show in part 1. So the results indicate that no different in storage time (1 to 7 days) and storage temperature between 4 and 25 ^{o}C in efficacy to eliminate three pathogenic bacteria.

Table 7 Efficacy of mixed acids in bacterial elimination at 5, 10, 15 and 20 min after storage at 4 ^{o}C and 25 ^{o}C for 1, 3, 5, and 7 days.

Mixed acids (%) (Lactic : Citric) 2:1	*L. monocytogenes* (10^7 CFU/cm^2)				*S.* Typhimurium (10^7 CFU/cm^2)				*C. jejuni* (10^7 CFU/cm^2)			
	5	10	15	20	5	10	15	20	5	10	15	20
Storage time at 4^{o}C												
1 day	-	-	-	-	-	-	-	-	-	-	-	-
3 days	-	-	-	-	-	-	-	-	-	-	-	-
5 days	-	-	-	-	-	-	-	-	-	-	-	-
7 days	-	-	-	-	-	-	-	-	-	-	-	-
Storage time at 25^{o}C												
2 day	-	-	-	-	-	-	-	-	-	-	-	-
4 days	-	-	-	-	-	-	-	-	-	-	-	-
6 days	-	-	-	-	-	-	-	-	-	-	-	-
7 days	-	-	-	-	-	-	-	-	-	-	-	-

+ : growth

- : no growth

Number of replications = 4

Part 3: Efficacies of organic acids on the attachment and survival of *S.* Typhimurium, *C. jejuni*, and *L. monocytogenes* on chicken skin.

The effectiveness of the selected mixed organic acids in combination of 2 % lactic and 1 % citric acids and water spray treatments in reducing bacterial population on the chicken skins were determined by evaluating the difference in number of *S.* Typhimurium, *C. jejuni*, and *L. monocytogenes* between the treatment and the no-spray control sample (Table 9 to 14). All temperature of suspension of mixed organic acids found to be effective against microorganisms when sprayed on chicken skins.

As showed in Table 9, when skin samples were sprayed with distilled water at 0, 25, and 55^{o}C, the treatments reduced *S.* Typhimurium by 0.69 (80.4 %), 0.67 (78.7 %), and 0.70 (80.6 %) log CFU/cm^2, respectively. There were no differences ($P<0.05$) among 0, 25, and 55^{o}C of water treatment. The number of *S.* Typhimurium in mixed acids spray treatment with different temperatures were reduce by 1.34 (95.6 %) to 1.55 (97.3 %) log reduction compared to the control. There were no significantly different between 0 and 25 ^{o}C of mixed acid treatment but 55 ^{o}C of acids showed significant differences from other temperature. The most effective spray temperature for mixed organic acids spray was 55 ^{o}C.

A similar trend was observed in the ability of water and mixed acids spray in varies temperature of suspension to detach *L.monocytogenes* to chicken skins, the results were presented in Table 10. Firmly attached *L.monocytogenes* cell count was significantly reduced from 5.44 for the control to 5.07, 5.06, and 5.03 $\log_{10}$ CFU/cm^2 (0.37, 0.38, and 0.41 $\log_{10}$ reduction) for 0, 25, and 55^{o}C of water spray treatment, respectively. In water spraying, there were no statistically significant differences in *Listeria* reduction among the different temperature of suspension. After application of 0, 25, and 55^{o}C of mixed acids spray reduced *Listeria* contamination by 0.62 (76.0 %), 0.67 (78.8 %), and 0.82 (83.9 %), respectively. As *Salmonella* reduction, there were no significantly different between 0 and 25 ^{o}C of mixed acid treatment but 55^{o}C of acids showed significant differences from other temperature. Maximum reduction of *L. monocytogenes* obtained in 55 ^{o}C of mixed acids spray.

The efficacy of mixed organic acids and water spray treatments in reducing *C. jejuni* were presented in Table 11. Treatment with 0, 25, and 55 ^{o}C of water spray resulted in significantly lower average bacterial count on chickens skins as compared to the control ($P<0.05$), reduced by 0.67 (79.1 %), 0.76 (83 %), and 0.79 (83.9 %) log

reduction. There were no differences ($P<0.05$) among 0, 25, and 55 ^{o}C. A large reduction in the bacterial count was observed in mixed acids treatment. The 0, 25, and 55^{o}C of mixed acids solution reduced *Campylobacter* by 1.59 (97.5 %), 1.61 (97.6 %), and 1.71 (98.1 %) $\log_{10}$ CFU/cm^2. There were no significant differences in *Campylobacter* reduction between 0 and 25 ^{o}C of suspension but 55 ^{o}C of mixed acids suspension gave a significantly greatest bacterial reduction value and was significantly different from other mixed acids spray groups. The reduction of all tested bacteria on the chicken skins, however, was significantly different among the different treatments and maximum reduction of all tested bacteria obtained in 55 ^{o}C of mixed organic acids treatment.

During storage at refrigeration temperature 4 ^{o}C for time periods of various duration. In the present study, the ability of water and mixed acids spraying to reudce *S.* Typhimurium, *L. monocytogenes*, and *C. jejuni* contamination on chicken skins at refrigerated temperature (4^{o}C) after spray treatment for 24 and 48 h as showed in Table 12 to 14. During storage, water treatment resulted in a reduction of *S.* Typhimurium declines by less than a log cycle that 0.75 (82.4 %) and 0.72 (80 %) log reduction compared to the control. Mixed organic acids treatment with 55^{o}C reduced firmly attached *S.* Typhimurium by 1.62 (97.6 %) to 1.84 (98.5 %) log reduction for 24 and 48 h, respectively. Due to reduction of *L. monocytogenes* on the chickens showed in Table 13. When the skins were sprayed with 55 ^{o}C of distilled water and stored at 4 ^{o}C for 24 and 48 h resulted in small decrease in viable counts to 0.41 (61.5 %) and 0.38 (60.4 %) log reduction. Under these conditions, the number of bacterial recovered after mixed organic acids treatment reduced to 1.01 (90.1 %) and 1.18 (92.9 %) $\log_{10}$ CFU/cm^2. Spraying 55^{o}C of mixed organic acids 10 s after inoculation with *C.jejuni*, resulted in 1.75 (98.6 %) and 1.99 (99 %) $\log_{10}$ reduction in numbers during storage at 4 ^{o}C for 24 and 48 h. *C.jejuni* contamination reduced to 0.89 (91.1 %) and 1.14 (93 %) log reduction by water spray trreatment.

These results indicated that when the temperature of the mixed organic acids solution was raised from 25 to 55 ^{o}C and the storage time at the refrigerated temperature increase from 1 to 48 h, there was a general trend towards a decrease in the number of *Salmonella* Typhimurium, *Campylobacter jejuni*, and *Listeria monocytogenes* recovered from acid treated chicken skins. Comparing the reduction in the number of tested organisms by mixed acid spray were *C. jejuni* > *S.* Typhimurium > *L. monocytogenes.*

Spray treatment with mixed acids caused changes in pH on the chicken skin surface with time of storage after treatment was shown Table 8. Although the pH dropped to 4.15 immediately post-treatment with mixed acids, it rose to about 5.36, 5.50, and 5.75 within 1, 24, and 48 h, respectively. In comparision to water spray did not resulted in changes of pH on chicken skin surface as a control group.

Table 8 Effect of mixed acids spray treatment on the pH of chicken skin after treatment for 0, 1, 24, 48 h.

Treatment	pH values			
	0 h	1 h	24 h	48 h
Control	6.01	6.09	6.28	6.33
Water spray	6.05	6.09	6.23	6.26
Mixed acids spray	4.15	5.36	5.50	5.75

n = 8/treatment

Table 9 Reduction of numbers of *Salmonella* Typhimurium on chicken skins sprayed with mixed organic acids and water at different temperature of suspension (0, 25, and 55°C) for 10 sec, at room temperature and 40-45 psi spray pressure after sprayed 1 h.

Treatment	Temperature of suspension (°C)	Bacterial number (Log CFU/cm^2)	Log reduction	Bacterial reduction (%)
Control (no-spray)	-	5.27 ± 0.14^{A}	-	-
Water spray	0	4.58 ± 0.08^{B}	0.69	80.4
	25	4.60 ± 0.12^{B}	0.67	78.7
	55	$4.57 + 0.09^{B}$	0.70	80.6
Mixed acids spray	0	3.93 ± 0.05^{C}	1.34	95.6
	25	3.85 ± 0.08^{C}	1.42	96.3
	55	$3.72 + 0.06^{D}$	1.55	97.3

n = 8/treatment, except control group n = 24

[a]Mean of tested groups. Same superscripts are non significant diference, different superscripts are significantly different ($P<0.05$)

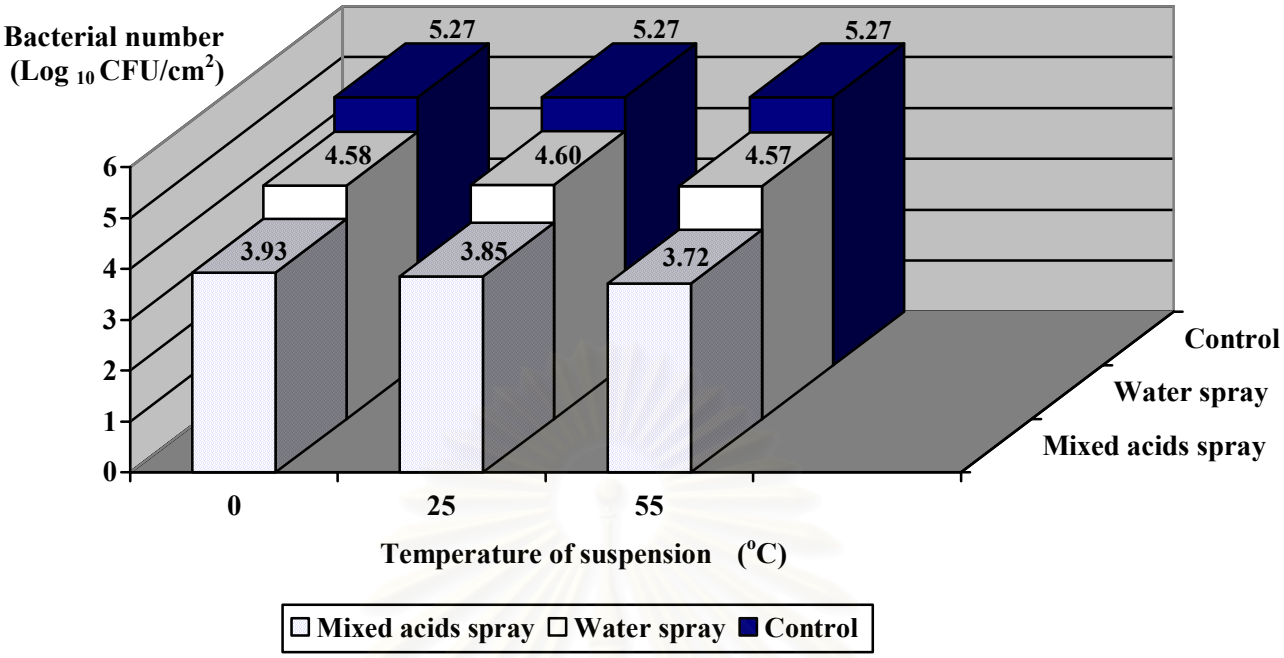

Figure 23 Summarizes the number of *S.* Typhimurium found on the chicken skins treated three ways: not sprayed, water sprayed and mixed organic acids sprayed after sprayed 1 h.

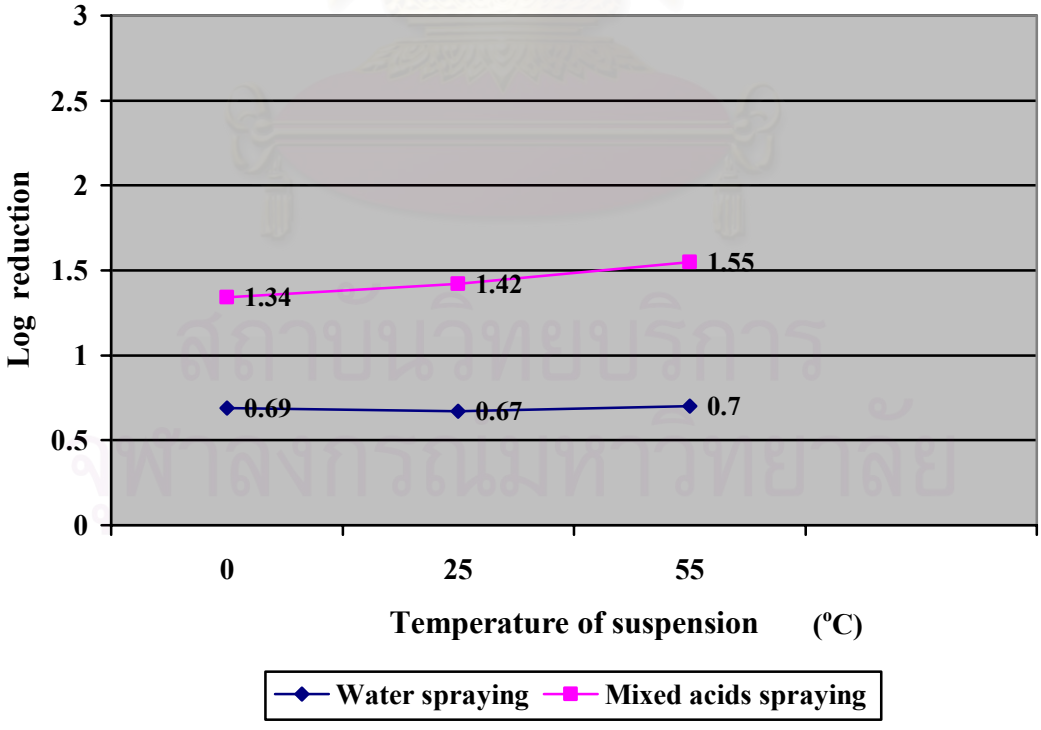

Figure 24 Reduction of *S.* Typhimurium on chicken skins after treated with water spray and mixed organic spray after treated 1 h.

Table 10 Reduction of number of *Listeria monocytogenes* on chicken skins sprayed with mixed organic acids and water at different temperature of suspension (0, 25, and 55 ^{o}C) for 10 sec, at room temperature and 40-45 psi spray pressure after sprayed 1 h.

Treatment	Temperature of suspension (^{o}C)	Bacterial number (Log CFU/cm^2)	Log reduction	Bacterial reduction (%)
Control (no-spray)	-	5.44 $\pm$ 0.10 ^{A}	-	-
Water spray	0	5.07 $\pm$ 0.06 ^{B}	0.37	57.6
	25	5.06 $\pm$ 0.14 ^{B}	0.38	58.6
	55	5.03 + 0.14 ^{B}	0.41	60
Mixed acids spray	0	4.82 $\pm$ 0.12 ^{C}	0.62	76
	25	4.77 $\pm$ 0.09 ^{C}	0.67	78.9
	55	4.62 + 0.19 ^{D}	0.82	83.9

n = 8/treatment, except control group n = 24

[a]Mean of tested groups. Same superscripts are non significant diference, different superscripts are significantly different (P<0.05).

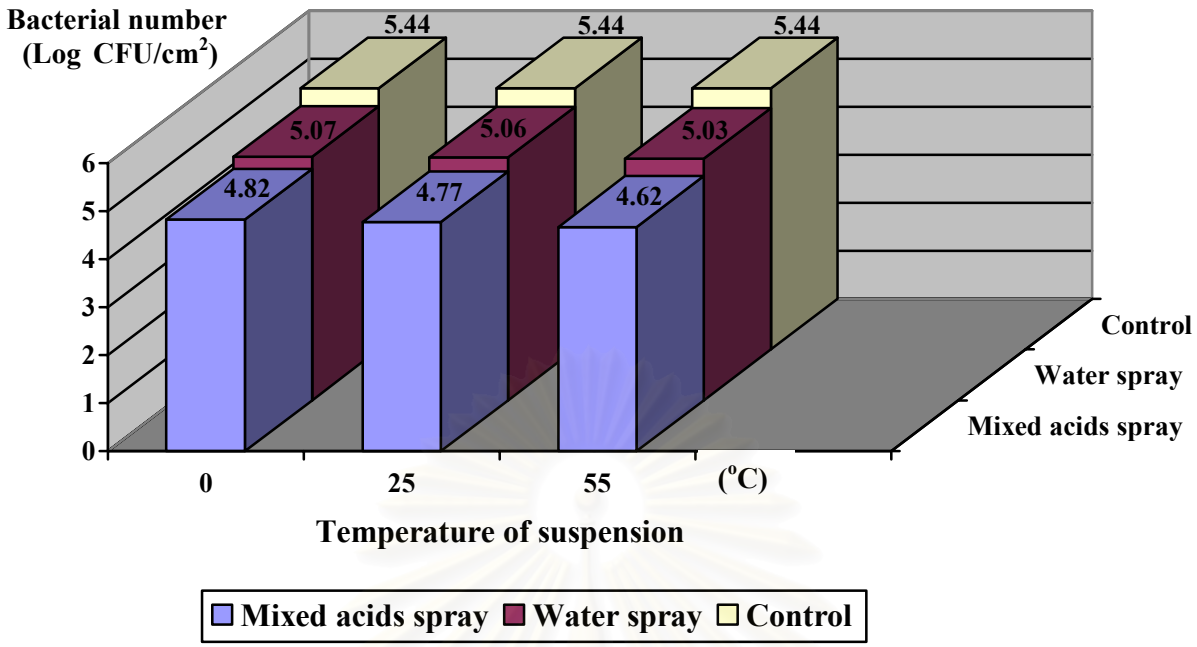

Figure 25 Summarizes the number of *L. monocytogenes* found on the chicken skins treated three ways: not sprayed, water sprayed and mixed organic acids sprayed after sprayed 1 h.

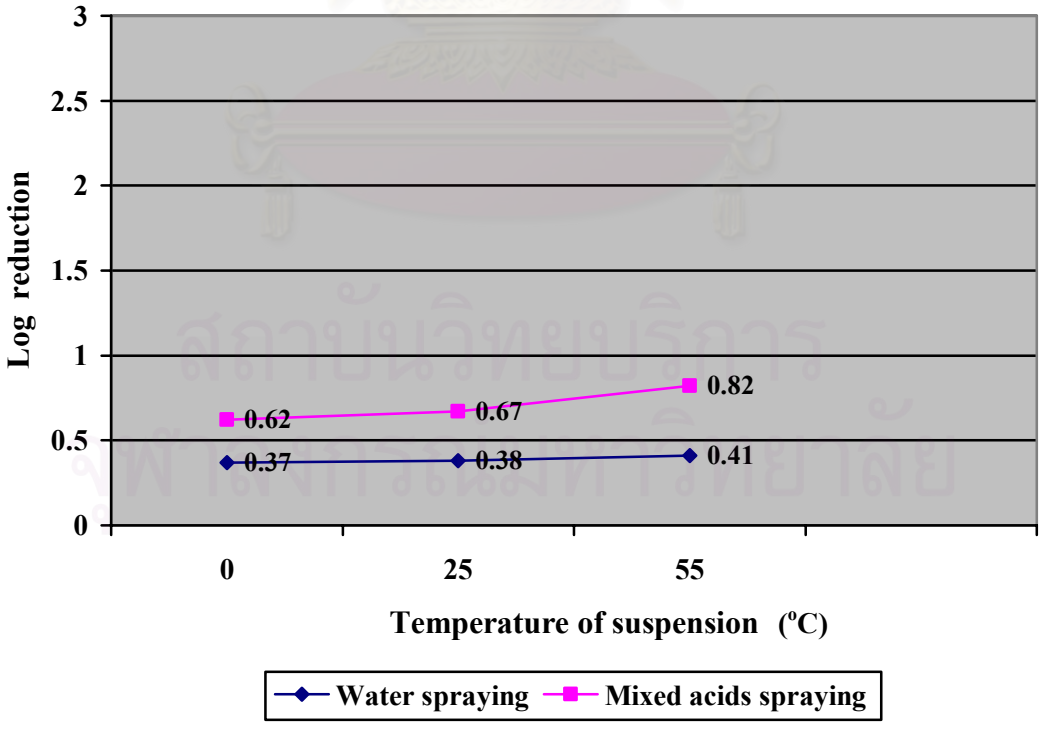

Figure 26 Reduction of *L. monocytogenes* on chicken skins after treated with water spray and mixed organic spray after treated 1 h

Table 11 Reduction of number of *Campylobacter jejuni* on chicken skins sprayed with mixed organic acids and water at different temperature of suspension (0, 25, and 55 ^{o}C) for 10 sec, at room temperature and 40-45 psi spray pressure after sprayed 1 h.

Treatment	Temperature of suspension (^{o}C)	Bacterial number (Log CFU/cm^2)	Log reduction	Bacterial reduction (%)
Control (no-spray)	-	5.27 $\pm$ 0.12 ^{A}	-	-
Water spray	**0**	4.60 $\pm$ 0.05 ^{B}	0.67	79.1
	25	4.51 $\pm$ 0.08 ^{BC}	0.76	83
	55	4.48 + 0.10 ^{C}	0.79	83.9
Mixed acids spray	**0**	3.68 $\pm$ 0.10 ^{D}	1.59	97.5
	25	3.66 $\pm$ 0.10 ^{D}	1.61	97.6
	55	3.56 + 0.10 ^{E}	1.71	98.1

n = 8/treatment, except control group n = 24

[a]Mean of tested groups. Same superscripts are non significant diference, different superscripts are significantly different (P<0.05).

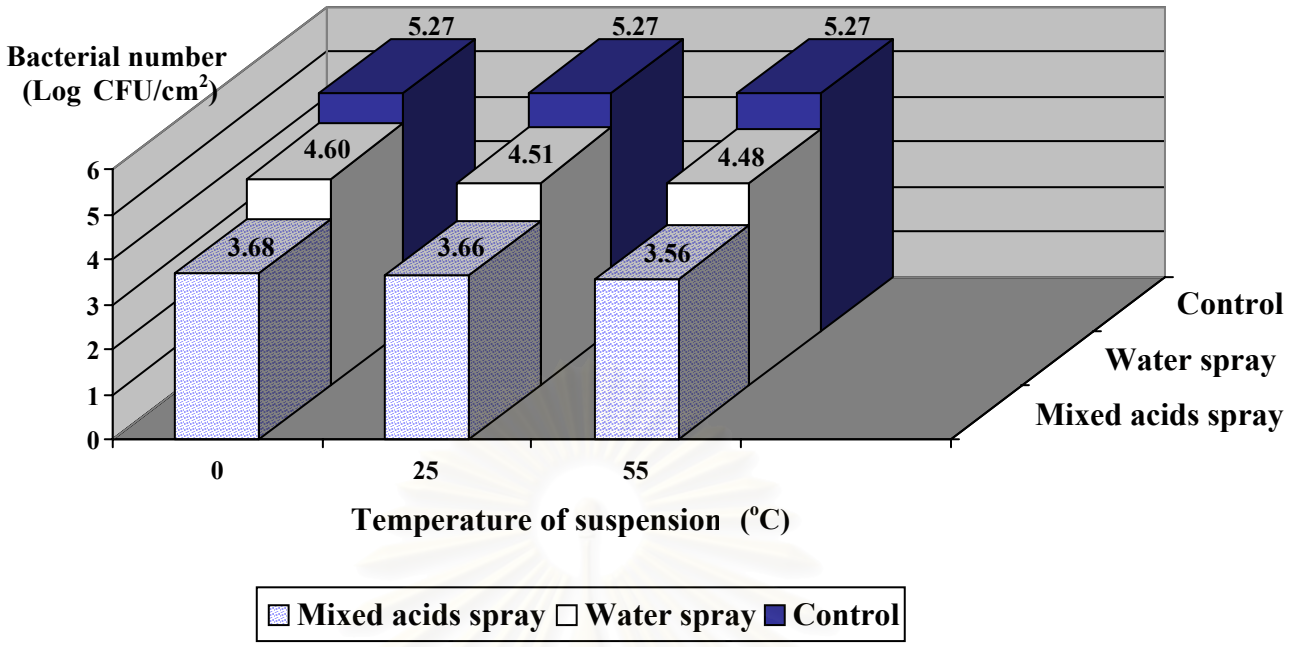

Figure 27 Summarizes the number of *C. jejuni* found on the chicken skins treated three ways: not sprayed, water sprayed and mixed organic acids sprayed after sprayed 1 h.

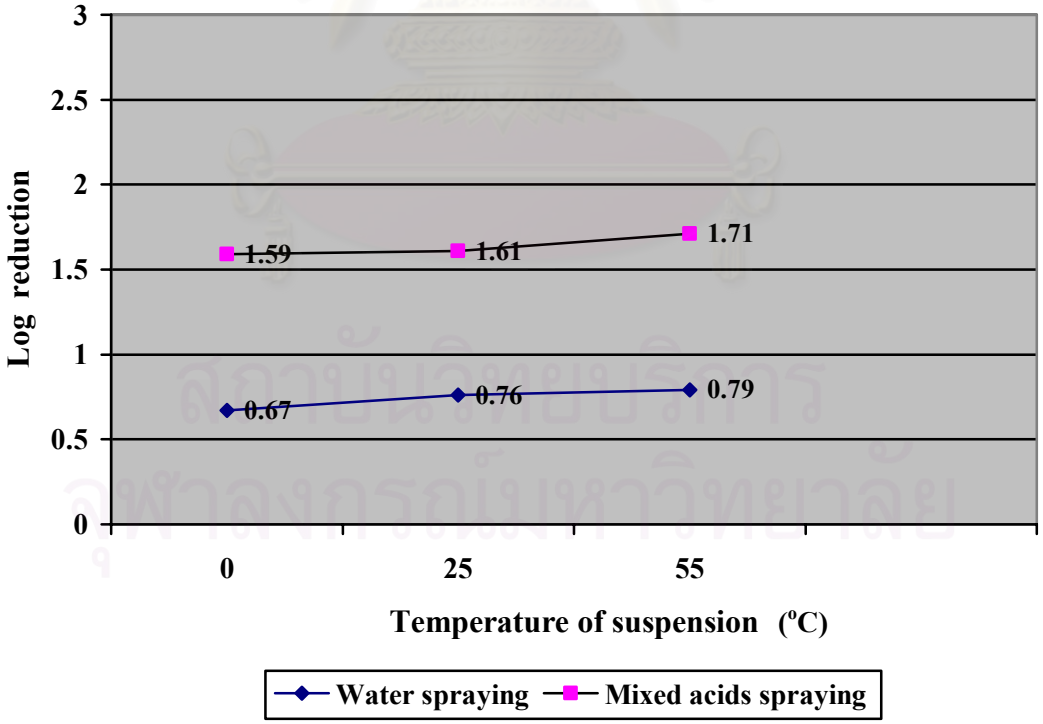

Figure 28 Reduction of *C. jejuni* on chicken skins after treated with water spray and mixed organic spray after treated 1 h.

Table 12 Reduction of number of *S.* Typhimurium on chicken skins sprayed with 55 ^{o}C of mixed organic acids and water for 10 sec, at room temperature and 40-45 psi spray pressure after sprayed 1 h and storage at 4 ^{o}C for 24 and 48 h.

Storage time (h)	Treatment	Bacterial number (Log CFU/cm^2)	Log reduction	Bacterial reduction (%)
1	**Control**	5.27 $\pm$ 0.13	-	-
	Water spray	4.57 $\pm$ 0.07	0.70	80.6
	Mixed acids spray	3.72 $\pm$ 0.06	1.55	97.3
24	**Control**	5.32 $\pm$ 0.05	-	-
	Water spray	4.57 $\pm$ 0.03	0.75	82.4
	Mixed acids spray	3.70 $\pm$ 0.05	1.62	97.6
48	**Control**	5.38 $\pm$ 0.05	-	-
	Water spray	4.66 $\pm$ 0.15	0.72	80
	Mixed acids spray	3.54 $\pm$ 0.15	1.84	98.5

n = 8/treatment, except control group (1 h) n = 24

[a] Time in hours after spraying when samples were taken and examined after stored at 4^{o}C, except at time 1 h, stored at room temperature.

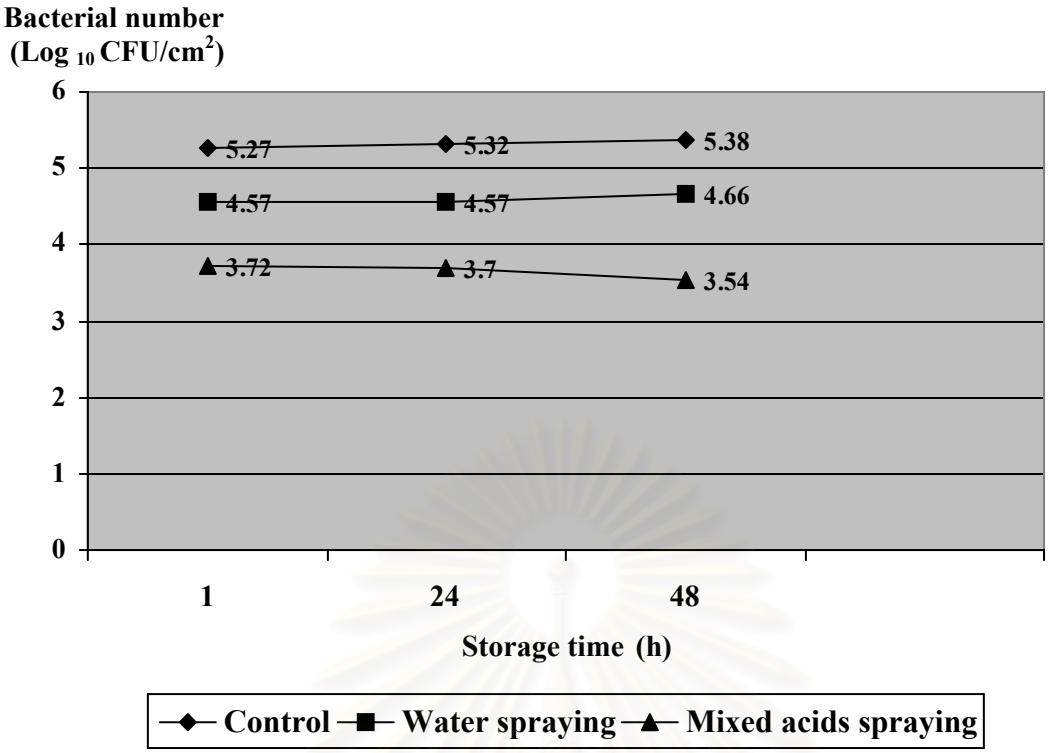

Figure 29 Summarize the number of *S.* Typhimurium found on the chicken skins treated three ways: not sprayed, water sprayed and mixed organic acids sprayed after sprayed 1 h and storage at 4 °C for 24 and 48 h.

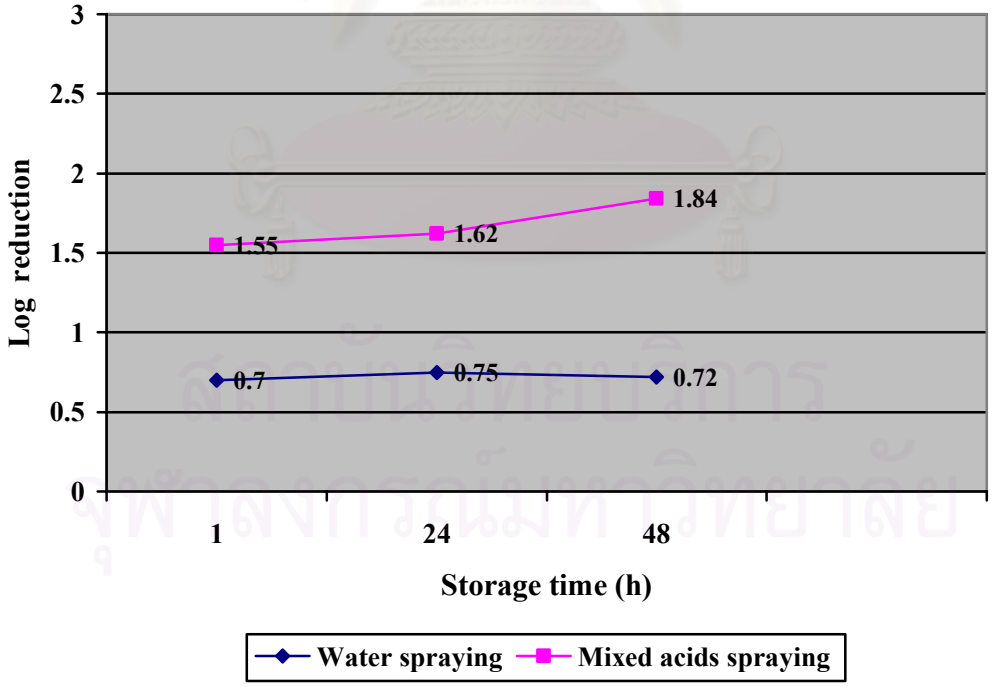

Figure 30 Reduction of *S.* Typhimurium found on the chicken skins treated with water sprayed and mixed organic acids sprayed after sprayed 1 h and storage at 4 °C for 24 and 48 h.

Table 13 Reduction of number of *L. monocytogenes* on chicken skins sprayed with 55 ^{o}C of mixed organic acids and water for 10 sec, at room temperature and 40-45 psi spray pressure after sprayed 1 h and storage at 4 ^{o}C for 24 and 48 h.

Storage time (h)	Treatment	Bacterial number ($Log_{10}CFU/cm^2$)	Log reduction	Bacterial reduction (%)
1	**Control**	5.44 ± 0.10	-	-
	Water spray	5.03 ± 0.14	0.41	60.1
	Mixed acids spray	4.62 ± 0.19	0.82	83.9
24	**Control**	6.48 ± 0.10	-	-
	Water spray	6.07 ± 0.13	0.41	60.7
	Mixed acids spray	5.47 ± 0.13	1.01	90.1
48	**Control**	6.73 ± 0.14	-	-
	Water spray	6.35 ± 0.18	0.38	60.4
	Mixed acids spray	5.55 ± 0.25	1.18	92.9

n = 8/treatment, except control group (1 h) n = 24

[a] Time in hours after spraying when samples were taken and examined after stored at 4 ^{o}C, except at time 1 h, stored at room temperature.

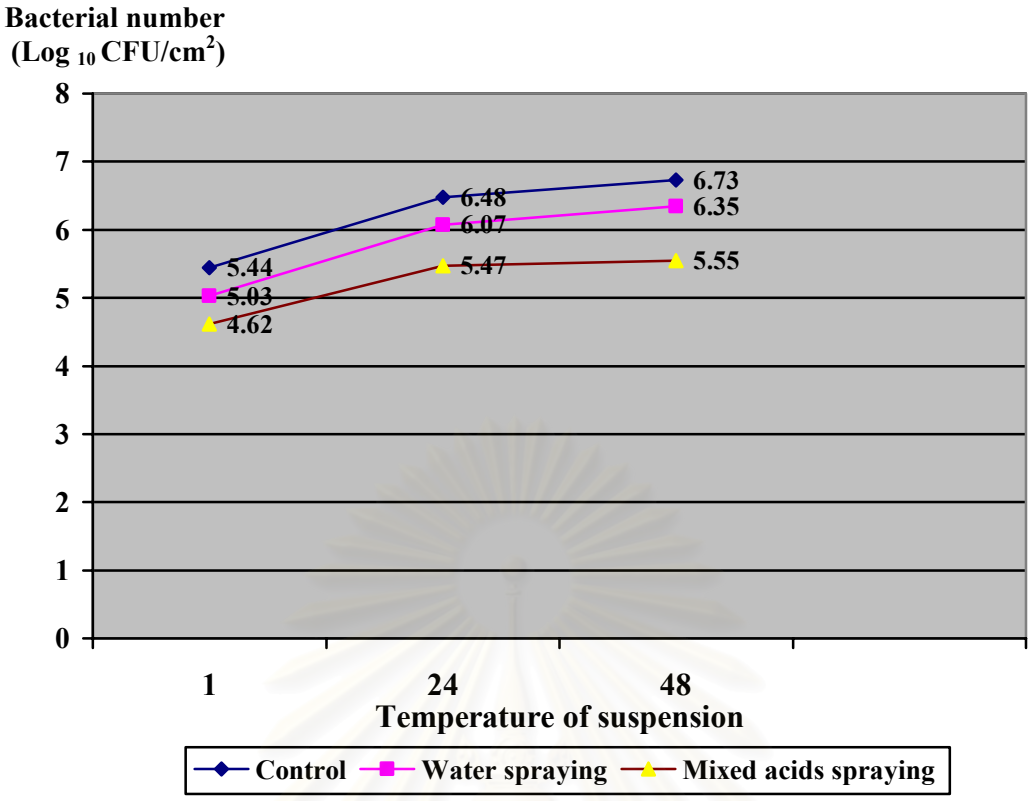

Figure 31 Summarize the number of *L. monocytogenes* found on the chicken skins treated three ways: not sprayed, water sprayed and mixed organic acids sprayed after sprayed 1 h and storage at 4 °C for 24 and 48 h.

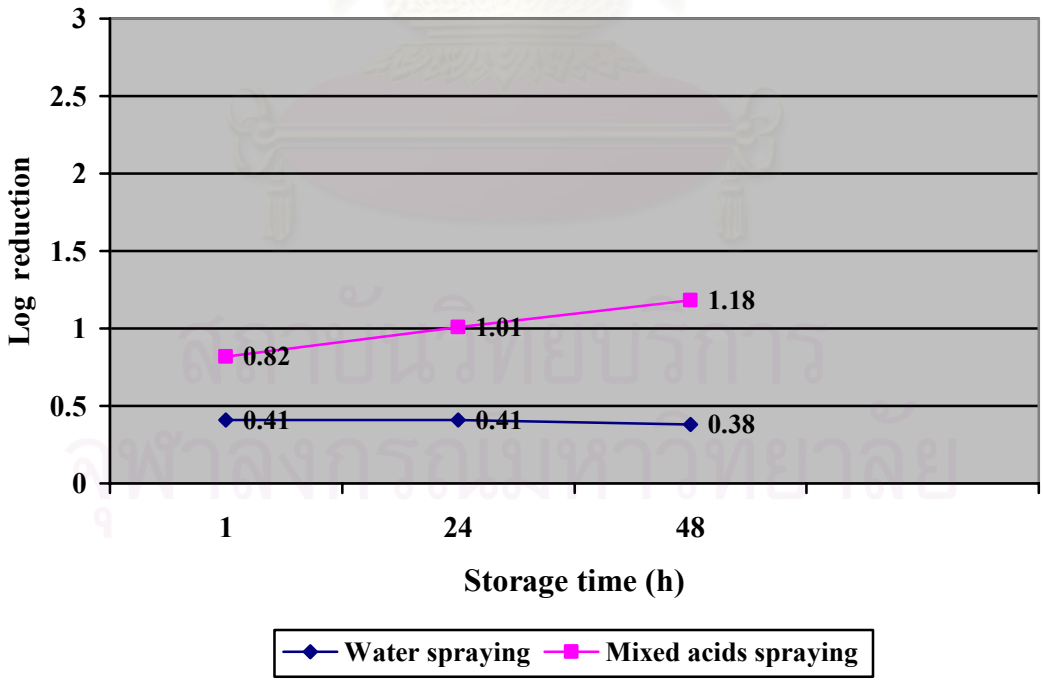

Figure 32 Reduction of *L. monocytogenes* found on the chicken skins treated with water sprayed and mixed organic acids sprayed after sprayed 1 h and storage at 4 °C for 24 and 48 h.

Table 14 Reduction of number of *Campylobacter jejuni* on chicken skins sprayed with 55 ^{o}C of mixed organic acids and water for 10 sec, at room temperature and 40-45 psi spray pressure after sprayed 1 h and storage at 4 ^{o}C for 24 and 48 h.

Storage time (h)	Treatment	Bacterial number (Log CFU/cm^2)	Log reduction	Bacterial reduction (%)
1	**Control**	5.27 $\pm$ 0.12	-	-
	Water spray	4.48 $\pm$ 0.10	0.79	83.9
	Mixed acids spray	3.56 $\pm$ 0.08	1.71	98.1
24	**Control**	5.19 $\pm$ 0.06	-	-
	Water spray	4.30 $\pm$ 0.18	0.89	91.1
	Mixed acids spray	3.44 $\pm$ 0.07	1.75	98.6
48	**Control**	4.81 $\pm$ 0.07	-	-
	Water spray	3.67 $\pm$ 0.04	1.14	93
	Mixed acids spray	2.82 $\pm$ 0.05	1.99	99

n = 8/treatment, except control group (1 h) n = 24

[a] Time in hours after spraying when samples were taken and examined after stored at 4 ^{o}C, except at time 1 h, stored at room temperature.

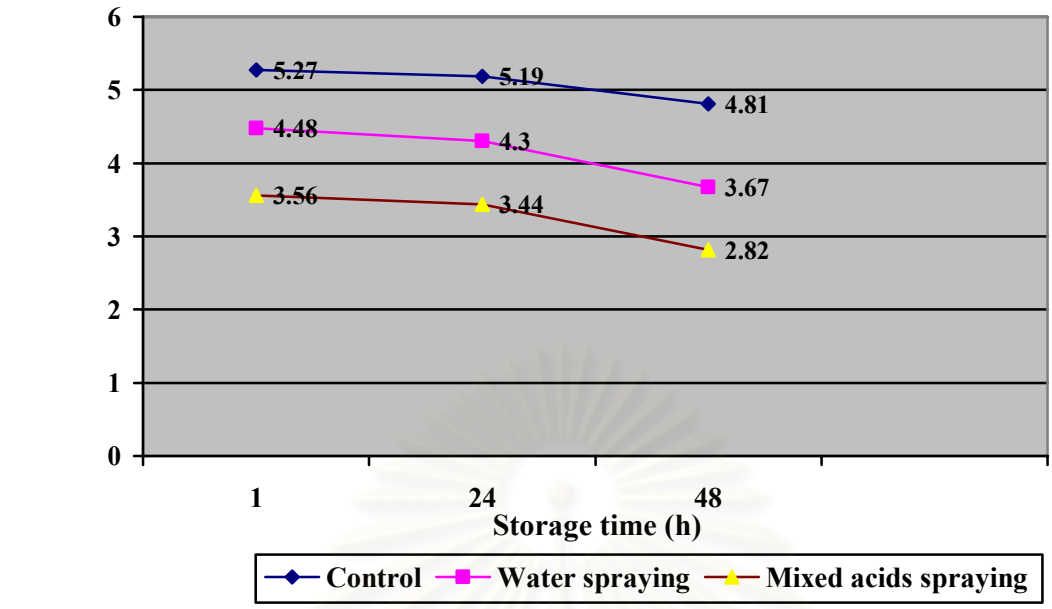

Figure 33 Summarize the number of *C. jejuni* found on the chicken skins treated three ways: not sprayed, water sprayed and mixed organic acids sprayed after sprayed 1 h and storage at 4 ^{o}C for 24 and 48 h.

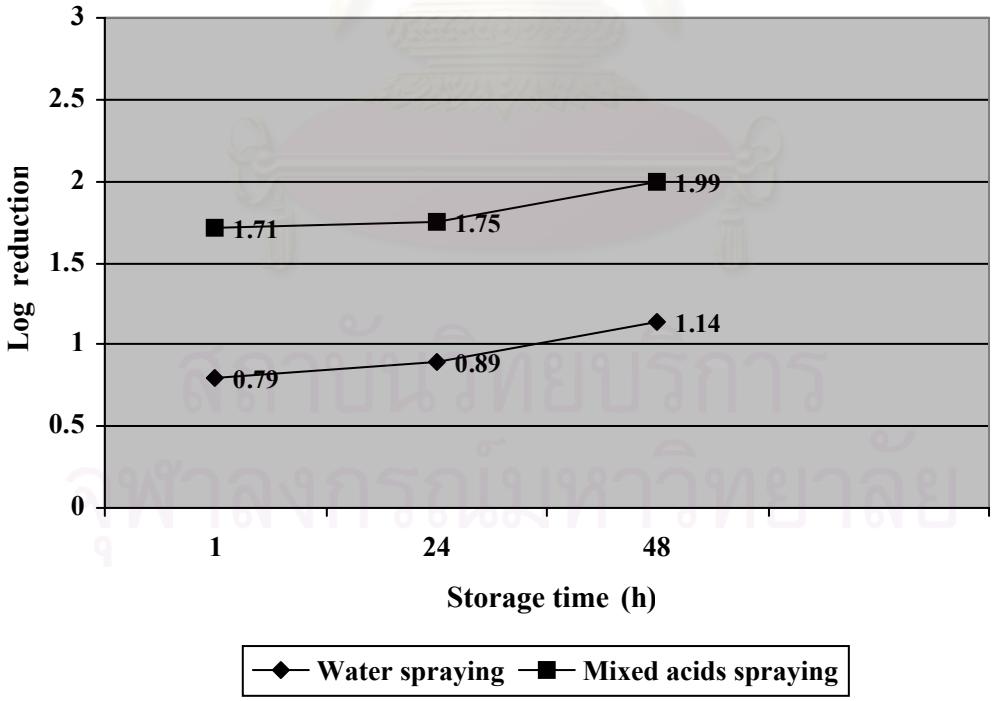

Figure 34 Reduction of *C. jejuni* found on the chicken skins treated with water sprayed and mixed organic acids sprayed after sprayed 1 h and storage at 4 ^{o}C for 24 and 48 h.

Part 4 Effect of mixed organic acids on physical properties and sensory quality of chicken

4.1 Difference of chicken skin color between non-sprayed and mixed acids-sprayed chicken skin in various temperatures of solution.

Forty chicken skin samples were used in this experiment and divided into 4 treatments. Each treatment used 10 chicken skin samples. Treatment 1 to 3 sprayed mixed organic acids that contain 2 % lactic acid and 1 % citric acid on chicken skin for 10 s, temperatures of solution were 0, 25 and 55 ^{o}C, respectively. Treatment 4, as a control group, non-mixed organic acids sprayed chicken. All samples were measured at day 0 before spraying then treatment 1 to 3 were sprayed with mixed organic acids as condition as describe above. All samples were kept at 4 ^{o}C for 2 days and measured color values after storage at day 1 and 2 by a Spectro-sensor II (Applied Color System, Inc., Princeton, New Jersey). Color values (L*, a*, b*) of chicken skin treated with mixed organic acids sprayed and control were presented in table 15.

The L* values (lightness) of control samples within group both before storage at day 0 and after stored at 4 ^{o}C at day 1 and 2 were not significantly different. The L* values of control samples were 96.75 $\pm$ 2.20, 96.54 $\pm$ 2.10, and 97.44 $\pm$ 2.02 respectively. As treatments 1 to 3 which sprayed with mixed organic acids and temperature of acids were 0, 25 and 55 ^{o}C, respectively. There were also no significant differences within group both before storage at day 0 and after stored at 4 ^{o}C at day 1 and 2. The L* values of treated samples with 0 ^{o}C of mixed organic acids before spraying at day 0, after spraying and stored at 4 ^{o}C at day 1 and 2 were 97.61 $\pm$ 2.29, 97.46 $\pm$ 2.19, and 98.34 $\pm$ 2.13 respectively. The L* values of treated samples with 25^{o}C of mixed organic acids before spraying at day 0, after spraying and stored at 4^{o}C at day 1 and 2 were 98.12 $\pm$ 2.29, 97.50 $\pm$ 2.19, and 98.20 $\pm$ 2.13 respectively. The L* values of treated samples with 55 ^{o}C of mixed organic acids before spraying at day 0, after spraying and stored at 4^{o}C at day 1 and 2 were 97.98 $\pm$ 1.38, 98.59 $\pm$ 1.80, and 99.60 $\pm$ 1.71 respectively. Comparison between the temperatures of mixed organic acids (0, 25, and 55^{o}C) within each treatment that were not significantly different.

The a* values (redness) of control samples within group both before storage at day 0 and after stored at 4^{o}C at day 1 were not significantly different but

were significantly different after stored at 4 ^{o}C for 2 days . Before storage a* values of control group was 19.99 $\pm$ 1.46 and after storage at day 1 and 2 were 19.60 $\pm$ 1.56, and 17.84 $\pm$ 1.60 respectively. The a* values (redness) of each mixed organic acids treated group after sprayed were significantly lower than before spraying. Differences in redness of treated samples with 0 ^{o}C of mixed organic acids ranged from 19.70 $\pm$ 1.50 at day 0 to 14.52 $\pm$ 0.50 at day 2 , treated samples with 25 ^{o}C of mixed organic acids ranged from 19.50 $\pm$ 1.63 to 14.38 $\pm$ 0.58, and treated samples with 55^{o}C of mixed organic acids ranged from 19.58 $\pm$ 1.11 to 13.96 $\pm$ 0.45. The difference of a* value was still significantly different between control and three mixed organic acids sprayed groups.

The b* values (yellowness) for control samples before storage at day 0 and after stored at 4^{o}C at day 1 and 2 were 20.39 $\pm$ 1.24 , 20.25 $\pm$ 1.04, and 20.08 $\pm$ 1.20, respectively. The b* values of treated samples with 0 ^{o}C of mixed organic acids were 20.01 $\pm$ 0.55, 20.72 $\pm$ 0.67, and 20.81 $\pm$ 0.60, respectively. The b* values of treated samples with 25^{o}C of mixed organic acids were 20.14 $\pm$ 0.51, 22.73 $\pm$ 0.94, and 22.33 $\pm$ 1.30, respectively. The b* values of treated samples with 55 ^{o}C of mixed organic acids were 20.52 $\pm$ 0.71, 22.66 $\pm$ 1.01, and 22.56 $\pm$ 0.91, respectively. There were no significant differences among the 25 and 55 ^{o}C of mixed acids treated samples. However, both samples treated with mixed acids at 25 ^{o}C and 55 ^{o}C were significantly different from control group for the b* values. The b* values of 0 ^{o}C of mixed acids treated and control groups were no significant differences. For control group, there was no significant differences within group both before storage at day 0 and after stored at 4 ^{o}C at day 1 and 2. In case, all mixed treated groups were significantly different within group between before spraying and after spraying and stored at 4 ^{o}C at day 1 and 2. Color values (L*, a*, b*) and color differentials were presented in Table 15.

Table 15 Color values (L*, a*, b*) and color differentials of non-sprayed and mixed acids sprayed skin chicken in various temperatures of solution for 10s before and after spraying.

Treatment	L* (Lightness)		a* (Redness)		b* (Yellowness)	
	Value[a] ± SD	Δ[b]	Value[a] ± SD	Δ[b]	Value[a] ± SD	Δ[b]
Control						
Before storage (day 0)	96.75 ± 2.20 [A]		19.99 ± 1.46[A]		20.39 ± 1.24[A]	
After storage						
day 1	96.54 ± 2.10 [A]	0.21	19.60 ± 1.56[A]	0.39	20.25 ± 1.04 [AC]	0.14
day 2	97.44 ± 2.02 [A]	-0.69	17.84 ± 1.60[D]	2.15	20.08 ± 1.20 [AC]	0.31
Mixed acids sprayed, 0 °C						
Before spraying (day 0)	97.61 ± 2.29[A]		19.70 ± 1.50[A]		20.01 ± 0.55[A]	
After storage						
day 1	97.46 ± 2.19 [A]	0.15	15.44 ± 0.40[B]	4.26	20.72 ± 0.67[C]	-0.6
day 2	98.34 ± 2.13 [A]	-0.73	14.52 ± 0.50[C]	5.18	20.81 ± 0.60[C]	-0.8
Mixed acids sprayed, 25 °C						
Before spraying (day 0)	98.12 ± 2.29 [A]		19.50 ± 1.63[A]		20.14 ± 0.52[A]	
After storage						
day 1	97.50 ± 2.19 [A]	0.62	15.19 ± 0.82[B]	4.31	22.73 ± 0.97[B]	-2.59
day 2	98.20 ± 2.13 [A]	-0.08	14.38 ± 0.58[C]	5.12	22.33 ± 1.30[B]	-2.19
Mixed acids sprayed, 55 °C						
Before spraying (day 0)	97.98 ± 1.38 [A]		19.58 ± 1.11[A]		20.52 ± 0.71[A]	
After storage						
day 1	98.59 ± 1.80 [A]	-0.61	14.68 ± 0.58[B]	4.90	22.66 ± 1.00[B]	-2.14
day 2	99.60 ± 1.71 [A]	-1.62	13.96 ± 0.45[C]	5.62	22.56 ± 0.91[B]	-2.04

n = 10/treatment

[a] Pretreatment color value.

[b] Color differential (pretreatment – post treatment)

[c] Mean within column and samples with differing superscripts are significantly different.($P<0.05$)

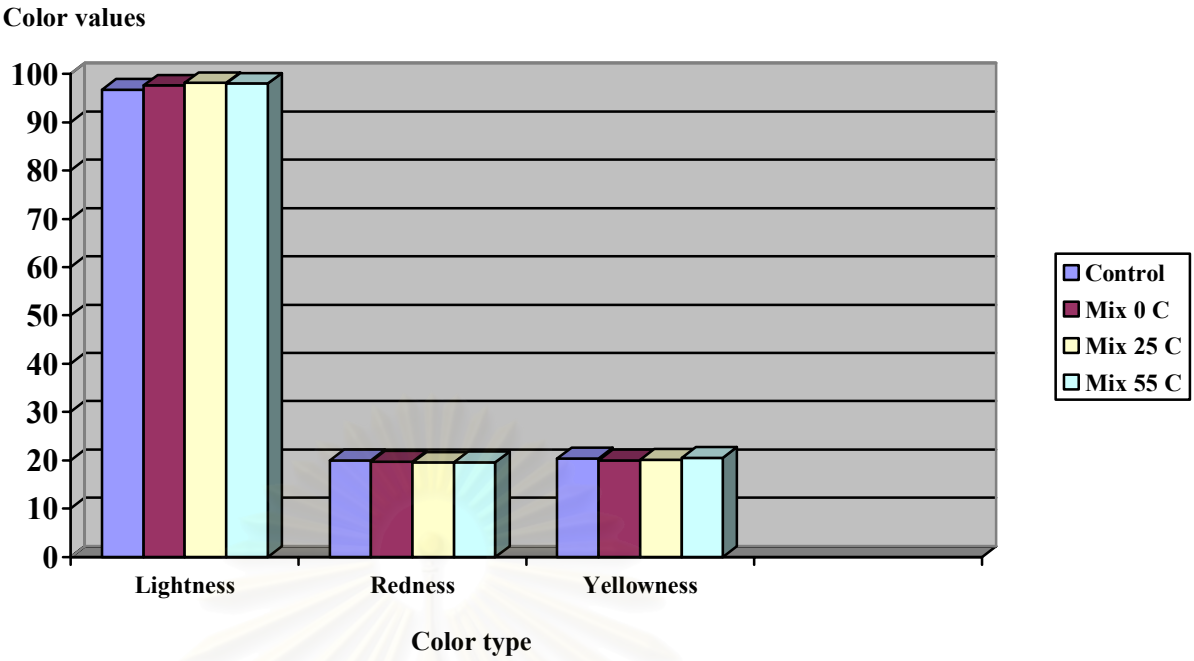

Figure 35 Color values of chicken skin samples before mixed organic acids spraying at day 0.

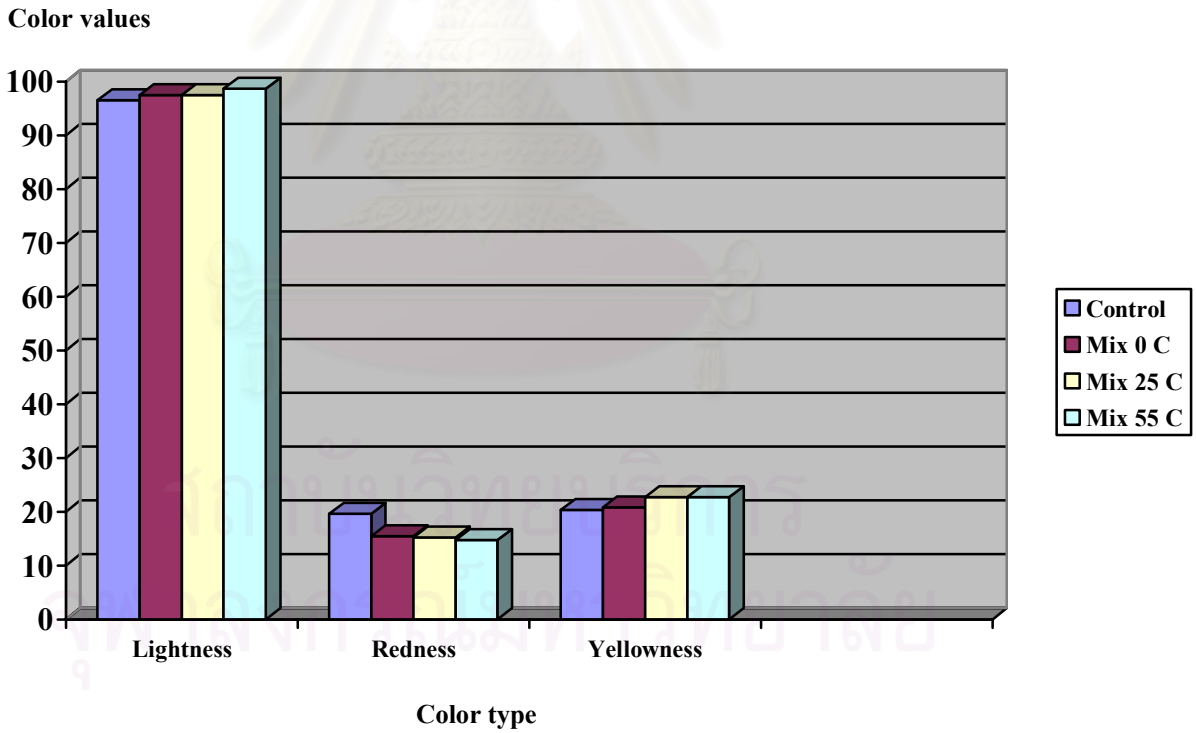

Figure 36 Color values of chicken skin samples after mixed organic acids spraying for 10 s, temperatures of solution were 0, 25, and 55 ^{o}C and stored at 4 ^{o}C for a day.

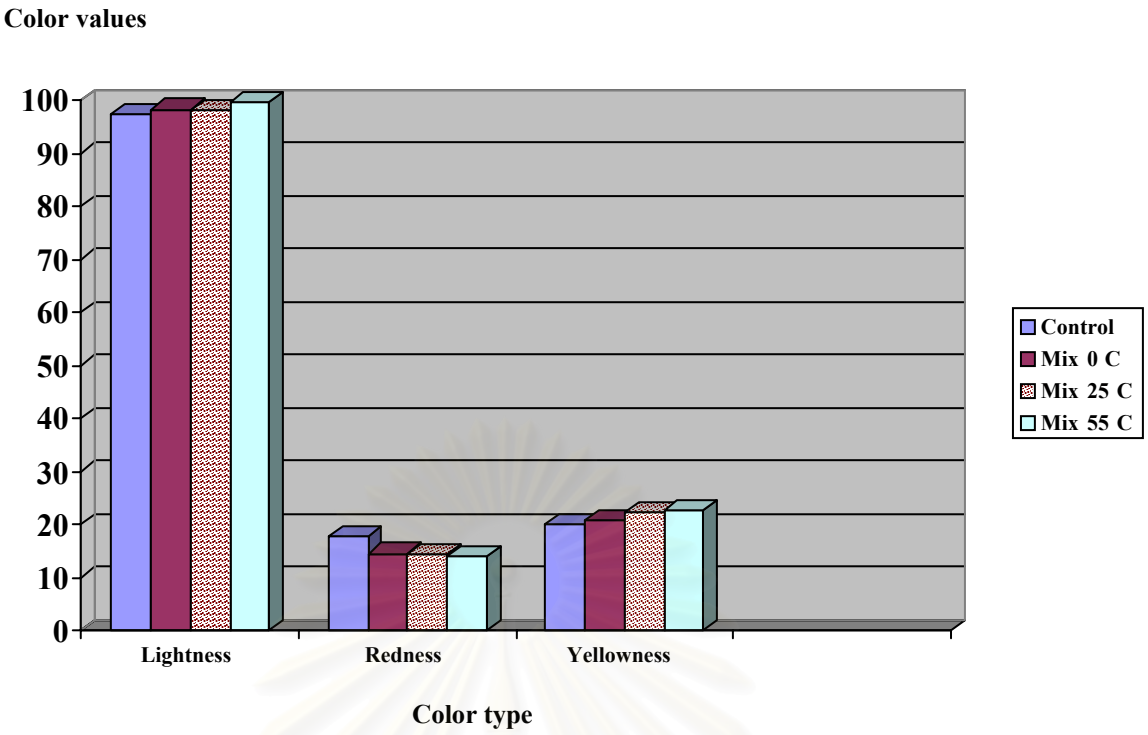

Figure 37 Color values of chicken skin samples after mixed organic acids spraying for10 s, temperatures of solution were 0, 25, and 55 ^{o}C and stored at 4 ^{o}C for 2 days.

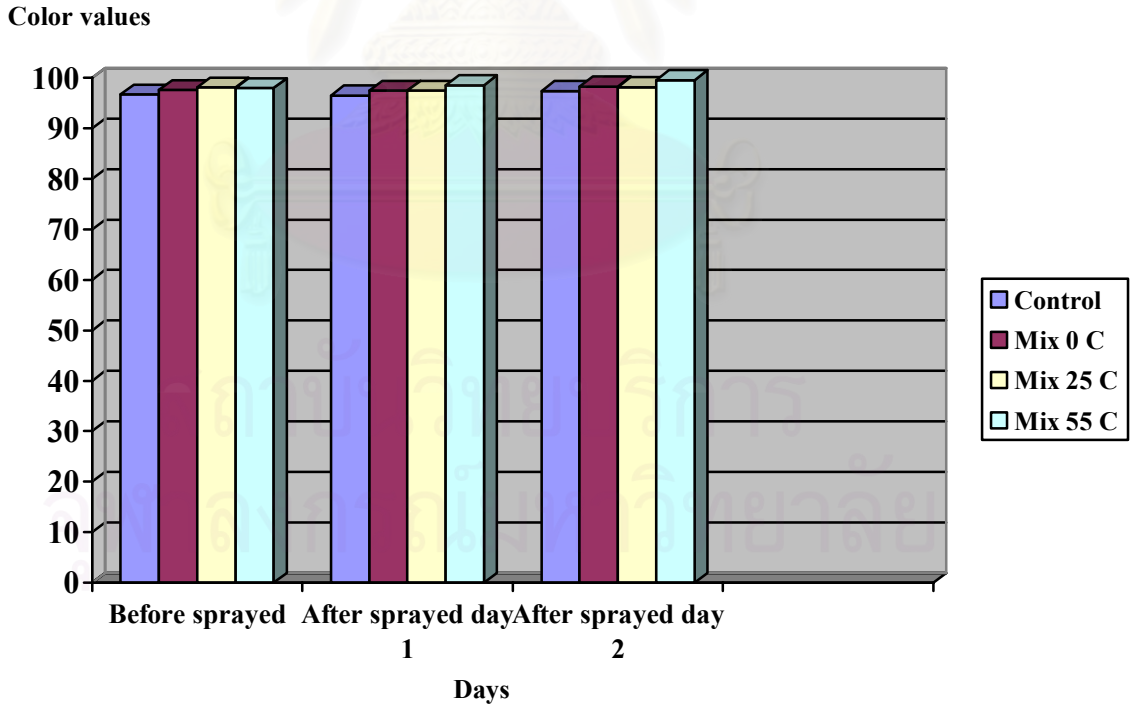

Figure 38 Lightness (L*) values of chicken skin samples before and after mixed organic acids spraying for 10 s, temperatures of solution were 0, 25, and 55 ^{o}C and stored at 4 ^{o}C at day 1 and 2.

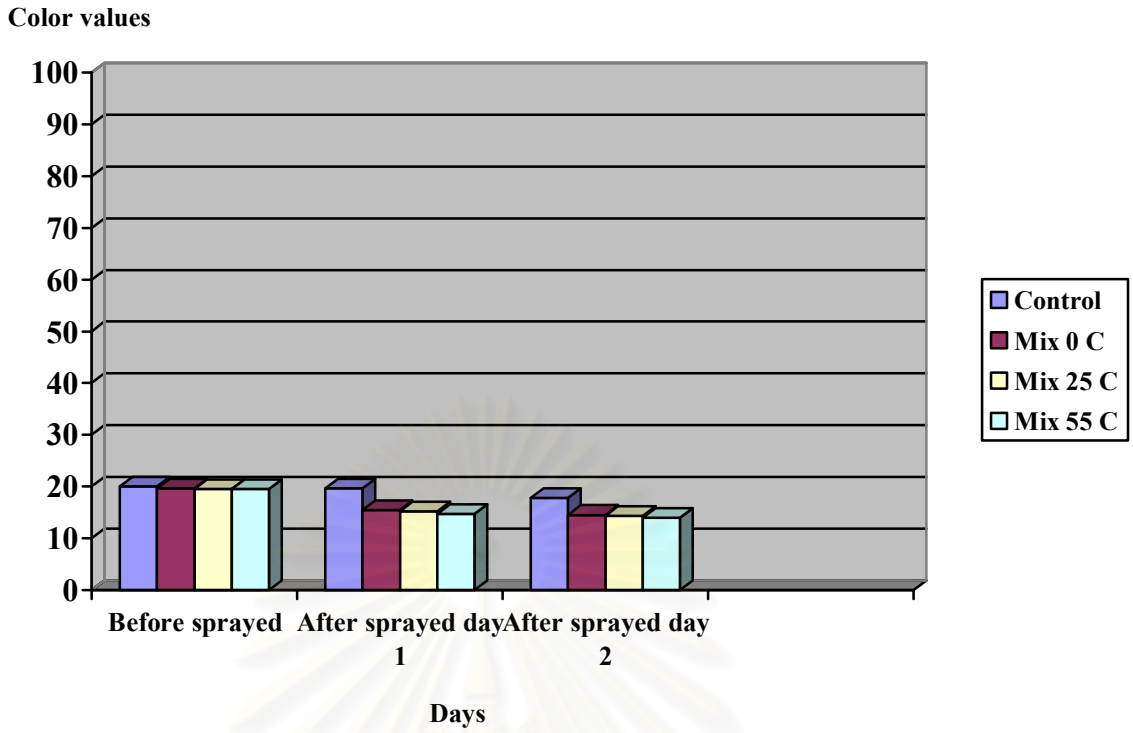

Figure 39 Redness (a*) values of chicken skin samples before and after mixed organic acids spraying for 10 s, temperatures of solution were 0, 25, and 55 ^{o}C and stored at 4 ^{o}C at day 1 and 2.

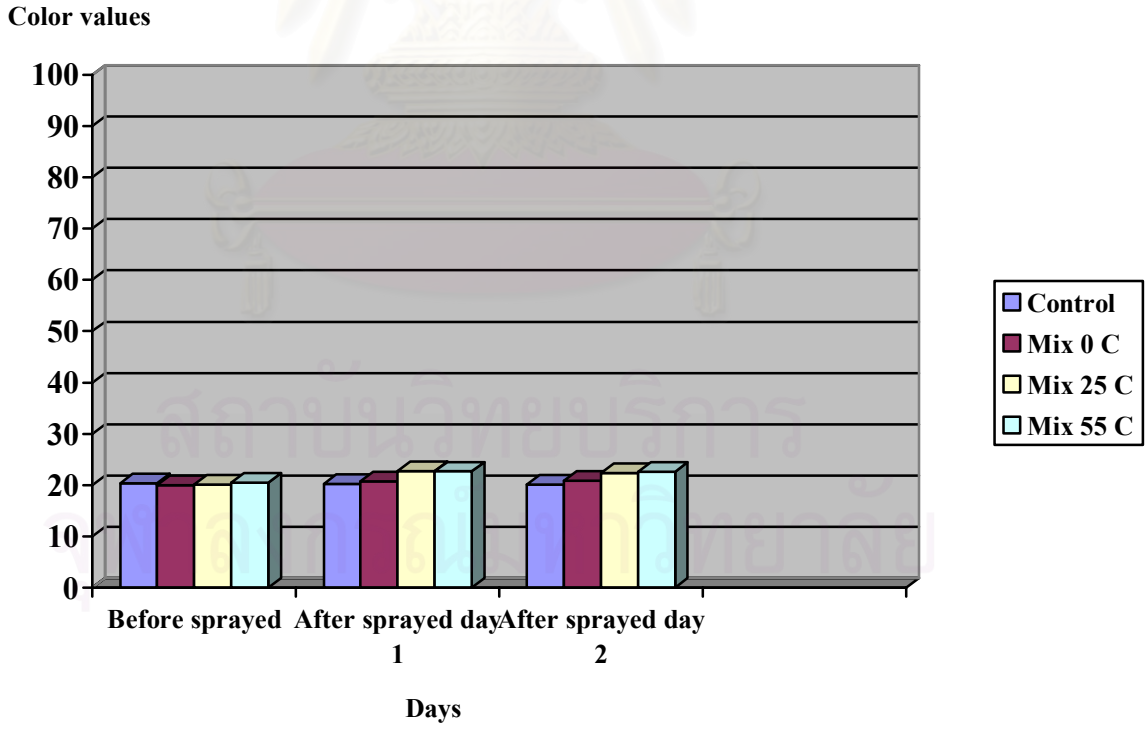

Figure 40 Yellowness (b*) values of chicken skin samples before and after mixed organic acids spraying for 10 s, temperatures of solution were 0, 25, and 55 ^{o}C and stored at 4 ^{o}C at day 1 and 2.

4.2 Evaluation of the differences of color and odor of 0, 25, and 55^{o}C of mixed organic acids treated compare to non-sprayed raw chicken samples before cooking.

All chicken skin samples were separated into 4 treatments. Treatment 1 to 3 were sprayed with mixed organic acids (2 % lactic acid and 1 % citric acid) for 10 s, the temperature of mixed organic acids were 0, 25 and 55 ^{o}C. Treatment 4 was control group, chicken skin samples were sprayed with non-mixed organic acids. All samples were individually packed in sterile plastic bags. The samples were kept at 4 ^{o}C for a day. Panelists were asked to evaluate each group of samples in randomized order. For each group of four pieces, one piece as a control, panelists were asked to assign scores sour odor of mixed organic acids on skin and external skin color compared to control chicken before cooking on a nine-point scale. The color and odor scores were shown in Table 2 and 5, respectively.

The average color scores of each 0, 25, and 55 ^{o}C of mixed organic acids treated samples compared to control group were 6.00 $\pm$ 1.00, 6.12 $\pm$ 0.70, and 5.71 $\pm$ 1.16, respectively. There were not significantly different among treated groups in various temperature of acid treatment, the values and statistic test were presented in Table 16, 17 and 18. Similarly, the odor scores of each 0, 25, and 55 ^{o}C of mixed organic acids treated samples compared to control group were 6.71 $\pm$ 1.11, 7.00 $\pm$ 1.06, and 7.18 $\pm$ 1.19, respectively. There were not significantly different among treated groups in various temperature of acid treatments, the values and statistic test were presented in Table 19, 20 and 21.

Table 16 Color differential score of 0, 25, and 55 ^{o}C of mixed organic acids sprayed compared to non-sprayed raw chicken samples before cooking by 17 panelists.

Judge number	Sample scores		
	A 55 ^{o} C	B 25 ^{o} C	C 0 ^{o} C
1	7	6	6
2	6	7	6
3	6	7	4
4	7	6	5
5	6	6	5
6	7	5	3
7	5	6	3
8	7	6	6
9	6	7	6
10	7	6	6
11	4	7	4
12	6	5	7
13	7	6	6
14	4	6	7
15	5	6	6
16	6	5	6
17	6	7	4
Total	102	104	90

Nine-point scale:
1 = extremely lighter
2 = much lighter
3 = lighter
4 = slightly lighter
5 = no difference
6 = slightly darker
7 = darker
8 = much darker
9 = extremely darker

Table 17 Descriptive color differential score of 0, 25, and 55 ^{o}C of mixed organic acids sprayed compare to non-sprayed raw chicken samples before cooking.

Temperature of suspension	N	Mean	Std. Deviation	Std. Error	95 % Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
55 ^{o} C	17	6.00[a]	1.000	0.243	5.49	6.51	4	7
25 ^{o} C	17	6.12[a]	0.697	0.169	5.76	6.48	5	7
0 ^{o} C	17	5.71[a]	1.160	0.281	5.11	6.30	3	7
Total	51	5.94[a]	0.968	0.136	5.67	6.21	3	7

[a]Mean within column and samples with differing superscripts are significantly different (P<0.05)**.**

Table 18 The statistic test for a color difference among the 0, 25, and 55^{o}C of mixed organic acids sprayed groups of raw chicken samples by One – way Anova.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.529	2	0.765	0.810	**0.451**
Within Groups	45.294	48	0.944		
Total	46.824	50			

Table 19 Sour odor differential score of 0, 25, and 55 °C of mixed organic acids sprayed compared to non-sprayed raw chicken samples before cooking from 17 panelists.

Judges	Sample scores		
	A 55° C	B 25 ° C	C 0 ° C
1	7	8	6
2	6	7	8
3	8	8	7
4	5	7	8
5	8	6	6
6	8	7	6
7	7	5	7
8	8	7	6
9	7	9	9
10	8	7	9
11	5	5	5
12	7	7	6
13	5	6	8
14	6	8	8
15	6	8	7
16	7	7	8
17	6	7	8
Total	106	119	122

Nine-point scale:
1 = extremely mild
2 = much mild
3 = mild
4 = slightly mild
5 = no difference
6 = slightly stronger
7 = stronger
8 = much stronger
9 = extremely stronger

Table 20 Descriptive sour odor differential score of 0, 25, and 55 ^{o}C of mixed organic acids sprayed compare to non-sprayed raw chicken samples before cooking.

Temperature of suspension	N	Mean	Std. Deviation	Std. Error	95 % Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
55 ^{o} C	17	6.71[a]	1.105	0.268	6.14	7.27	5	8
25 ^{o} C	17	7.00[a]	1.061	0.257	6.45	7.55	5	9
0 ^{o} C	17	7.18[a]	1.185	0.287	6.57	7.79	5	9
Total	51	6.96[a]	1.113	0.156	6.65	7.27	5	9

[a]Mean within column and samples with differing superscripts are significantly different (P<0.05).

Table 21 The statistic test for a sour odor difference among the 0, 25, and 55 ^{o}C of mixed organic acids sprayed groups of raw chicken samples by One – way Anova.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.922	2	0.961	0.769	**0.469**
Within Groups	60.000	48	1.250		
Total	61.922	50			

4.3 Evaluation of the differences of flavor and taste of 0, 25, and 55 ^{o}C of mixed organic acids treated compare to non-sprayed cooked chicken samples.

All treated and control chicken meat samples, covered with the skin, were cooked by Microwave at the highest electric power for 3 min. Panelists were served with four cups of each treatment groups, contained as about 15 g of cooked chicken sample for each group. Panelists were asked to evaluate flavor and taste of control samples compared to treated samples after cooking using a nine-point scale. The flavor and taste scores were shown in Table 8 and 11, respectively.

The average flavor scores of each 0, 25, and 55 ^{o}C of mixed organic acids treated compared to group were 5.13 $\pm$ 0.89, 5.44 $\pm$ 0.89, and 5.31 $\pm$ 0.79, respectively. There were not significantly different among treated groups in various temperature of acid treatments, the values and statistic test were presented in Table 22, 23, and 24. Similarly, the taste scores of each 0, 25, and 55 ^{o}C of mixed organic acids treated compared to group were 5.25 $\pm$ 0.93, 5.19 $\pm$ 0.91, and 5.31 $\pm$ 1.08, respectively. There were not significantly different among treated groups in various temperatures of acid treatment. The values and statistic test were presented in Table 25, 26 and 27.

Table 22 Sour flavor differential score of 0, 25, and 55 ^{o}C of mixed organic acids sprayed compared to non-sprayed cooked chicken samples from 16 panelists.

Judge number	Sample scores		
	A 55^{o} C	B 25 ^{o} C	C 0 ^{o} C
1	6	6	6
2	5	5	5
3	4	4	5
4	5	6	6
5	5	6	5
6	4	6	6
7	6	4	5
8	5	4	5
9	4	7	5
10	4	6	5
11	5	6	5
12	5	6	6
13	6	5	7
14	7	5	4
15	6	5	4
16	5	6	6
Total	82	87	85

Nine-point scale:
1 = extremely mild
2 = much mild
3 = mild
4 = slightly mild
5 = no difference
6 = slightly stronger
7 = stronger
8 = much stronger
9 = extremely stronger

Table 23 Descriptive sour flavor differential score of 0, 25, and 55 ^{o}C of mixed organic acids sprayed compare to non-sprayed cooked chicken samples.

Temperature of suspension	N	Mean	Std. Deviation	Std. Error	95 % Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
55^{o} C	16	5.13[a]	0.885	0.221	4.65	5.60	4	7
25^{o} C	16	5.44[a]	0.892	0.223	4.96	5.91	4	7
0^{o} C	16	5.31[a]	0.793	0.198	4.89	5.74	4	7
Total	48	5.29[a]	0.849	0.123	5.05	5.54	4	7

[a]Mean within column and samples with differing superscripts are significantly different.($P<0.05$)

Table 24 The statistic test for sour flavor difference among the 0, 25, and 55 ^{o}C of mixed organic acids sprayed groups of cooked chicken samples by One – way Anova.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.792	2	0.396	0.538	**0.588**
Within Groups	33.125	45	0.736		
Total	33.917	47			

Table 25 Sour taste differential score of 0, 25, and 55 °C of mixed organic acids sprayed compared to non-sprayed cooked chicken samples from 16 panelists.

Judge number	Sample scores		
	A 55° C	B 25 ° C	C 0 ° C
1	5	5	5
2	6	4	6
3	5	4	4
4	5	6	5
5	4	6	4
6	4	4	6
7	6	7	5
8	6	6	3
9	7	5	7
10	6	5	6
11	5	5	5
12	5	6	7
13	4	4	6
14	6	6	5
15	6	5	5
16	4	5	6
Total	84	83	85

Nine-point scale:
1 = extremely mild
2 = much milder
3 = mild
4 = slightly milder
5 = no difference
6 = slightly stronger
7 = stronger
8 = much stronger
9 = extremely stronger

Table 26 Descriptive sour taste differential score of 0, 25, and 55 °C of mixed organic acids sprayed compare to non-sprayed cooked chicken samples.

Temperature of suspension	N	Mean	Std. Deviation	Std. Error	95 % Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
55° C	16	5.25[a]	0.931	0.233	4.75	5.75	4	7
25° C	16	5.19[a]	0.911	0.228	4.70	5.67	4	7
0° C	16	5.31[a]	1.078	0.270	4.74	5.89	3	7
Total	48	5.25[a]	0.957	0.138	4.97	5.53	3	7

[a]Mean within column and samples with differing superscripts are significantly different.($P<0.05$)

Table 27 The statistic test for sour taste difference among the 0, 25, and 55 °C of mixed organic acids sprayed groups of cooked chicken samples by One – way Anova.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.125	2	0.063	0.066	**0.937**
Within Groups	42.875	45	0.953		
Total	43.000	47			

4.4 Acceptance and Preference of customers to non-sprayed and mixed acids-sprayed chicken skin samples.

Six chicken samples were used in this experiment and divided into two groups. First group sprayed with mixed organic acids that contained 2 % lactic acid and 1 % citric acid, temperature of solution was 55 ^{o}C and duration of spray was 10 s. Another group, as a control group, non-mixed organic acids sprayed chicken. Three pieces of chicken samples of each group were packed in sterile plastic bags, maintained at 4 ^{o}C for a day. This test was performed in Daokanong and Bangprakaew. Sensory panelist selected from people who were a consumer of chicken and willing to participate Daokanong and Bangprakaew markets, were 125 persons divided into male 17 and female 108 persons. Panelists were asked about the acceptance, preference and attitudes to mixed acids sprayed chicken samples. In part of acceptance test, panelist answered the question "Would you like to buy these chickens, why not?" There were two choices that were (1) would buy or (2) would not buy. Panelists who answered "would buy" were amounts 118 persons (male 17 and female 101 persons). Panelists who answered "would not buy" were amounts 7 persons (male 0 and female 7 persons). The Percentage of panelists who would buy and would not buy were 94.4 and 5.6, respectively. Data was presented in Table 28.

Part 2 showed the preference of consumers on mixed organic acids sprayed and non- mixed acids sprayed chicken samples. Both mixed acids sprayed and non- mixed acids sprayed chicken samples were presented to panelists at the same time and panelists were ask to select one of chicken sample that they prefered more than another one and provided the reason why they prefer it. The answers were presented in percentage of consumers who prefered the group of mixed acids sprayed or non- mixed acids sprayed. Panelists who prefered mixed organic acids sprayed samples were amounts 52 persons divided into male 9 and female 43 persons. Panelists who preferred non-sprayed samples were amounts 73 persons, male 8 and female 65 persons. The Percentage of panelists who prefered mixed organic acids sprayed and non-sprayed samples were 41.6 and 58.4 %, respectively. Data was classified by occupation of consumers as presented in Table 29.

Table 28 Acceptance of consumers to mixed organic acid sprayed chicken after stored at 4 ^{o}C for a day considered by external appearance of chicken skin.

Occupation	Amount	Mixed acids sprayed chicken	
		Accept	Reject
Housewife	15	13 (87 %)	2 (13 %)
Government official	9	7 (78 %)	2 (22 %)
female	8	6	2
male	1	1	0
Private official	36	35 (97 %)	1 (3 %)
female	27	26	1
male	9	9	0
Merchant	51	50 (98 %)	1 (2 %)
female	45	44	1
male	6	6	0
Others	14	13 (93 %)	1 (7 %)
female	13	12	1
male	1	1	0
Total	125	118/125 (94 %)	7/125 (6 %)

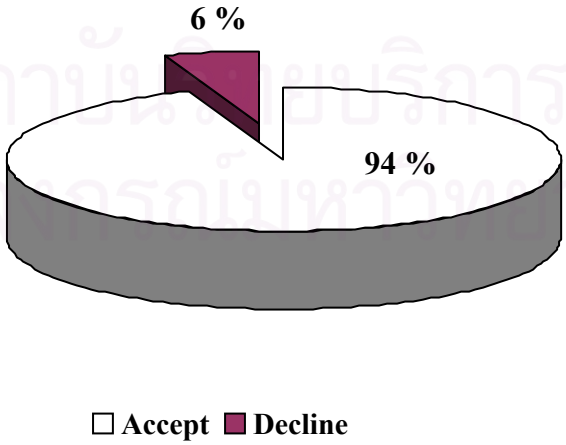

Figure 41 Percentage of consumers acceptance to mixed organic acid sprayed chicken.

Table 29 Preference of consumers to non-sprayed and mixed organic acid sprayed chicken after stored at 4 ^{o}C for a day considered by external appearance of chicken skin.

Occupation	Amount	Score	
		Non – sprayed chicken	Mixed acids sprayed chicken
Housewife	15	10 (67 %)	5 (33 %)
Government official	9	5 (56 %)	4 (44 %)
female	8	4	4
male	1	1	0
Private official	36	25 (69 %)	11 (31 %)
female	27	20	7
male	9	5	4
Merchant	51	25 (49 %)	26 (51 %)
female	45	23	22
male	6	2	4
Others	14	8 (57 %)	6 (43 %)
female	13	8	5
male	1	0	1
Total	125	73/125 (58 %)	52/125 (42 %)

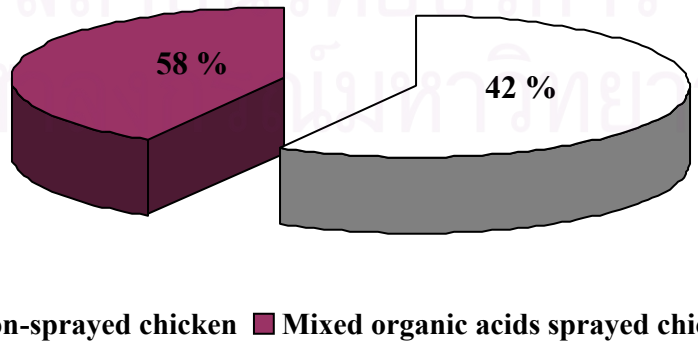

Figure 42 Percentage of consumers preference to mixed organic acid sprayed chicken compare with non sprayed chicken.

CHAPTER VI

DISCUSSION

This study had evaluated the bacterial reducing efficacies of acetic acid, citric acid, lactic acid at the concentrations of 0.25 to 4 %. Also evaluation bacterial reducing efficacies of mixed organic acids which consisted of lactic and citric acids, concentrations of 0.5 to 2 %. The target pathogenic bacteria for eliminating by organic acids in this study were *Salmonella* Typhimurium, *Campylobacter jejuni*, and *Listeria monocytogenes*. For comparison, the method for testing modified from phenol co-efficiency test that can show bactericidal activity of each acid with directly contact to tested bacterium for 5, 10, 15 and 20 min. Under condition as describe above, mixed acids (2 % lactic acid and 1 % citric acid) and 4 % lactic acid were the most effectiveness which eliminated 3 species of tested bacteria within 5 min. Furthermore, the nature of these interactions was not the same for each bacterium tested. These results indicate that bacterial eliminations were greatest with higher concentration of acids. Antimicrobial effects of organic acid vary depending on the acids, microbial species. The results showed *L. monocytogenes* was the most resistant to acids follow by *S.* Typhimurium, while *C. jejuni* was the most acid-sensitive microorganism which was similar to report by Van Netten *et al*., 1994 (162). In comparison with other microorganism, *C. jejuni* is considered fragile and sensitive to many environmental parameters, including oxygen, low pH. In contrast to *L. monocytogenes*, the cell is relatively resistant to harsh environment.

In addition, stability of mixed acids was studied by storage at 4 and 25 ^{o}C for 1, 3, 5 and 7 days. The stored mixed organic acids were able to kill all tested cultures within 5 min as well. These results indicated that mixed organic acids are stable at room temperature in close container under normal storage for several days after preparing that suitable for application in commercial processing.

Section of evaluation the effectiveness of bacterial reducing of mixed organic acids on chicken skin using a skin attachment model (8) had been changed on the part of inoculation technique by using directly spreading of tested culture onto the skin instead of using a coarse aerosol spraying which maight not be appropriate. Since, it could cause a wide spread of pathogenic bacteria in laboratory environment. The trial

on the skin attachment model in this study had utilized irradiated chicken skin as a carrier for the tested bacteria. Irradiating of all samples prior using can eliminate all background microflora and assure the validity of our study. Conner and Bilgili, 1994, indicated that the skin model techniques make it easier to evaluate the efficacy of a potential carcass disinfectant (8). The numbers of Bacterial contamination used in this experiment would be considered a very high-level of contamination which is quiet far from the natural contamination in the poultry slaughtering line that were essential in determining the effectiveness of carcass interventions.

Previous research studies of using organic acids to reduce or eliminate the numbers of bacterial contamination by artificially or naturally inoculated chicken skin had shown the various degrees of effectiveness (9, 19, 22, 32, 37, 38, 41, 107, 118, 121, 141, 143, 151, 153, 158, 163-175). In our study, the skin samples were sprayed with 0, 25, and 55^{o}C of mixed organic acids, significant reduction were achieved 1 h after spraying. The results complement previous researchers by demonstrating that mixed organic acids (mixture of 2 % lactic acid and 1 % citric acids) produced reduction in the population of meatborne pathogens at artificially inoculated chicken skin surfaces. Of mesophilic pathogens as *S.* Typhimurium reduce by 1.34 to 1.55 log, psychotrophic pathogen as *L. monocytogenes* reduce by 0.62 to 0.82, and *C. jejuni* reduce by 1.59 to 1.71 log CFU/cm^2.

As expected, Anderson et al., 1990 reported that acids were most effective bactericides at a higher temperature (55^{o}C) (152, 174). There are situations where acid decontamination of chicken can be enhanced. That are, mixed acids have been found to be more effective in the destruction of *Salmonella*, *Listeria monocytogenes*, *Campylobacter jejuni*. In different studies, the temperature of the acid solution has been found to have a profound effect on the magnitude of reductions in bacterial counts on carcass surfaces (151, 176, 177). Therefore, the effectiveness of these interventions may vary, depending on whether they are applied onto hot or cold carcass surfaces.

Other investigators have observed similar bactericidal efficacies (0.5-1.5 log cycle reduction) with *Salmonella*, *L. monocytogenes* and *C. jejuni* as tested microorganisms (152, 178). In accordance with the view of Dickson, 1992 and Dickson and Anderson, 1992, the limited magnitude of these reductions would not

likely improve the safety of raw meat under commercial processing conditions (32, 179).

Spray washing the carcasses with water is suggested to be the effective method for reducing the microbial load on the carcass surfaces (19). In the current study, it was also demonstrated that spray treatment with water resulted in the reductions, ranging from 0.37 to 0.79 log depend on types of tested bacteria. Other researchers have reported that water reduced bacterial populations on meat (176, 177). Spray treatment with water may effectively reduce bacterial population by physically removing cells from the chicken skin surface. Carcass washing reduces the population of bacteria on carcasses by removal of liquid film containing microorganism before they bacame more closely attached with the skin surface (180). Spray washing replaces the contaminated water film on carcasses with clean film of water (181).

The current study evidence that bacterial reduction steadily increased with time following refrigerating of chicken samples. The reduction of *Salmonella* recovered from skins steadily increase from 1.55 log immediately after spraying 1 h to 1.84 log after 48 h. Due to reduction of *Listeria* increase from 0.82 to 1.18, *Campylobacter* reduction increase from 1.71 to 1.99 log, respectively. These results indicated that mixed acids may exhibit an extended antibacterial effect during storage, adding continued protection against the survival of pathogenic bacteria such as *Salmonella*, *Campylobacter* and *Listeria*. This effect, however, become manifest only several hours after the organic acid treatment. This observation was different from Stern et al, 1985, which their study found that organic acids had been effective only a period of few seconds after exposing to chicken meat samples. But our research and many previous studies were well agreed that organic acids still contained bactericidal properties for a long period of time. Besides their effectiveness was shown to better as longer exposing time (171, 172). Cudjoe et al, 1991, showed that lethal effect of lactic acid diminished probably due to diffusion of the acid into the carcasses after 48 h. A continued antimicrobial effect also been observed by other during storage of meat after spraying with lactic acid solution (173, 177) . Also, the effects of mixed organic acids in bacterial reducing followed on consistent trend and varied with the nature of the test bacterium.

In this study, the application of mixed acids spray reduced the surface pH of skin immediately after treatment, thereby creating an unfavorable environment for

bacterial growth. However, the surface pH of the treated skin was less than the control after 24 and 48 h incubation at 4^{o}C. The proportion of undissociated lactic acid and citric acid are greater at lower pH values. The pH differences observed between the two days may be explained by a buffering mechanism exerted by the tissue. It has been reported that the pH of food play a critical role in the injury and survival of bacteria following refrigerated and frozen storage (182).

The antimicrobial effect of organic acids in inhibiting microbial growth or killing cells due to that interfere with cellular metabolism or a decrease in biological activity as a result of pH changes of the cell's environment (35). In case of interfering cellular metabolism has been attributed to undissociated acid molecules of lipophilic organic acids that are responsible for antimicrobial activity. Since they are readily soluble into cell membranes, undissociated molecules interfering with the permeability of the microbial cell membrane, causing uncoupling of both substrate transport and oxidative phosphorylation from the electron transport system (108). The inhibitory effect of the acid observed in this study was likely due to the synergistic effects of low pH resulted in a high concentration of the undissociated molecule, temperature of application , microbial species and reaction time caused high endogenous levels of organic acid within the muscle.

The bactericidal activity of mixed acid when compared to freely suspended cells, mixed organic acids rapidly killed more than a million free-floating bacteria. It is clear that test bacterium firmly attached to chicken skin have increase resistance to or are protected from organic acids. Protective chemical components, such as proteins, fatty acids, and oils that contained within the surface structures of chicken skin (including follicles) may enhance the survival of contaminating bacteria by interact with a mixed organic acids, so the acid be less available to interact with bacterial cells. Attachment in a crevice, fold, or follicle provide bacteria additional protection from surface sanitizer.

This study confirm previous reports that, while spray treatment with organic acids reduce population of *Salmonella* Typhimurium, *Listeria monocytogenes*, *Campylobacter jejuni* on chicken skin, depending upon the strain of pathogen, temperature of solution and reaction time, reduction ranging from 1 to 2 log CFU/cm^2 may not be sufficient as the only mean to improve the overall microbiological safety of chicken carcasses. However, organic acid spray treatment may be beneficial as part

of an overall hazard analysis critical control point (HACCP) approach that can be implemented in order to enhance the microbiological safety and extend the shelf life of post rigor chicken. The practical use of mixed acids solution to kill or control the growth of pathogenic and spoilage microorganisms on chicken carcasses need to be further studied in terms of effectiveness over a wide range of packaging condition and storage temperature.

Sensory evaluation was used to evaluate the effect of mixed organic acid to physical and sensory properties of chicken. From the observation of the changes of color skin using colorimeter, there were no significant differences among control and all mixed organic acids sprayed groups for the L* (lightness). The L* values of control and mixed acids treated samples within group both before and after spraying and stored for 2 days, were not significantly different. These results showed that mixed organic acids spraying with 0, 25, and 55^{o}C of acids did not affect to lightness value of chicken skin. In contrast, a* and b* values, the differences of a* and b* values were still significantly different across control to mixed acids treated groups. The a* values of all mixed organic acids treated groups were significant differences within both before and after spraying samples resulted in the redness values of chicken skins decreased, the range of redness differential from 4.26 to 5.62. There were no significant differences among the 25 and 55^{o}C of mixed acids treated but that were significantly increased to compared with control group for the b* values. The b* values of 0 ^{o}C of mixed acids treated and control groups were no significant differences. Control group was no significant differences within group both before storage at day 0 and after stored at 4^{o}C at day 1 and 2. In case, all mixed treated groups were significantly increased of b* values within group between before spraying and after spraying and stored at 4^{o}C at day 1 and 2, the difference values were small and range from 2.04 to 2.59. These results support to previous reported by Bilgili *et al*, 1998 showed that broiler breast chicken treated with lactic and citric acids in chilling (1^{o}C for 60 min) and scalding (50^{o}C for 2 min) conditions increased yellowness values but different from these data in lightness and redness values (183). Because of the differences in application and skin samples, Bilgili's samples were collected after chilling process as chlorinated samples and used breast skin. Processing condition, such as scalding temperature and duration (184, 185) and immersion chilling (186), have been showed to affect broiler skin color. Results of this study indicate that spraying 0, 25, and 55^{o}C of mixed organic acids,at spray

pressure 40-45 psi for 10 s resulted in significantly decrease a* (redness) but increase b* (yellowness), except 0 ^{o}C of acids and no affect to L* (lightness) values by comparing the same chicken skin before and after spraying. So, chicken skin can be further altered by use of mixed organic acids as carcass disinfectants. The published literature also demonstrates some disparity in the reported the effecet of organic acids to color changes of treated skin. Izat *et al*, 1989, found that treatmentof carcasses with 1 % of lactic solution for 1 h resulted in bleaching and mild discoloration. Spraying with 2 % of lactic solution at 70 ^{o}C caused swelling of tissue and irreversible discoloration were reported by Xiong et al, 1998 (9). The study of Yang et al, 1998, supported that 2 % of lactic acid caused slight discoloration in the part of the chicken skin. Contrary to those reported, Cudjoe and Kapperud, 1991, observed no color change in 2 % of lactic acid sprayed fresh broiler chickens but they were noteworthy that frozen and throwed chicken appeared to show a graying of the skins immediately after spraying with lactic acid, slightly stronger with 2 % lactic acid, but the color reverted to normal after 24 h. However, the degree of subcutaneous fat cover including, the mode of applying the acid onto the carcasses, the time elapsed after spraying, and the subjective evaluation of color development could account for the differences in results. Other visual defects frequently attributed to organic acids, such as hardening, bloating, or puckering of the skin were not evaluated in this study.

As effects on color (L*, a*, b*) attributes were measured, objective color value could not be correlated to consumers acceptability. In raw chicken samples, no significant differences ($P>0.05$) of color and odor among groups were observed by 17 sensory trained panels in response to 0, 25, and 55 ^{o}C of mixed acids spraying. Color evaluation scores of 0, 25, and 55 ^{o}C of mixed acids sprayed samples were 6.00 ± 1.00, 6.12 ± 0.70, and 5.71 ± 1.16 that mean chicken skin color of each group slightly darker than control (non-sprayed sample) as detected by reflectance colorimeter, as describe above. As in case of odor score were scored 6.71 ± 1.11, 7.00 ± 1.06, and 7.18 ± 1.19, were not significantly different in all mixed organic treated group that mean chicken samples had sour odor of mixed acids combined with meathy odor of chicken result in stronger odor than control. Flavor and taste differences detected in cooked chicken treated with 0, 25, and 55 ^{o}C of mixed acids spraying and untreated could not be distinguished by trained panels.

The sensorial data indicated that 0, 25, and 55 ^{o}C of mixed acid spraying treatment slightly affect to color and odor of chicken skin but not affect to flavor and taste of chicken after cooking like previous reported by Dickens et al. (41)

Changing of poultry meat color and uniformity of poultry skin had influenced on both consumers' decision on purchasing of fresh meat at the retail-market level and their final evaluation and acceptance of a meat product at time of consumption. Considerable attention has been focus on the acceptance of consumers to mixed acid sprayed chicken. Sensory evaluation by consumer panel is the best method of measuring consumers response to chicken product color. Research on ground meat from the other species indicated that color is perhaps the most important influence in consumer decision-making regarding acceptability of fresh meat products for consumtion (187). Lynch *et al* reported that 74 % of consumers indicated that color was important in ground meat purchase decisions (188).

As effects on color (L*, a*, b*) attributes were measured, objective color values could not be correlated to consumers acceptability. In this study show the most of consumers (94.4 %) accepted to mixed organic acids sprayed chicken because of they like color of chicken skin that seemed fresh chicken and the color was not different from chicken meat which sold in the market. In contrast, a few consumers (5.6 %) did not accepted to mixed organic acids sprayed chicken because of they mention that chicken skins were darker and more yellow than typical chicken. Consequently, these results indicate that the most of consumers did not have negative attitude to mixed organic acids sprayed chicken. The preference of consumers to non-sprayed and mixed organic acids sprayed chicken showed 58.4 % of consumer who prefered non-sprayed more than mixed organic acids sprayed chicken. Comparison to Percentage of consumers who prefered mixed organic acids sprayed more than non-sprayed chicken showed 41.6 %. Usually, the most of chickens which sell in the market obtain from commercial processing plant, processed chickens have light skins (cause by chlorinated water in chilling process) and it became a trend for buying chicken meat that is the reason why most consumers prefer non-sprayed chicken more than another one.

Therefore, alteration in sensory properties (color, odor, and taste), acceptability of consumers, in addition efficacy against surface microorganism, should be taken into account in selecting and applying organic acids as disinfectants for chicken carcasses. Furthermore, the cost of mixed organic acids were calculated

in this study. The cost of mixed organic acids are approximately 3.23 bath/L which equivalent to 0.64 bath/ chicken carcass. This expense might be not well acceptable for the chicken meat processing plants. However, it is the best alternative carcass disinfectant for substitute the use of chlorine which is not allowed to be use in food.

CHAPTER VII

CONCLUSION

1. In suspension test, mixed acids (2 % lactic and 1 % citric acids) and 4 % lactic acid were the most effectiveness which eliminated *S.* Typhimurium, *C. jejuni*, and *L. monocytogenes* of tested bacteria within 5 min.
2. Mixed organic acids were stable at 4 and 25 ^{o}C in close container for 7 days.
3. Antimicrobial effects of organic acid vary depending on the acids, microbial species and the temperature of application. Spraying mixed organic acids with combination of 2 % lactic and 1 % citric acid at 55 ^{o}C for 10 seconds were maximally reduced *S.* Typhimurium, *C. jejuni*, and *L. monocytogenes* by 1.55, 1.71, and 0.82 log CFU/cm^2, respevtively.
4. Antibacterial effects of mixed organic acids on bacterial reduction tend to be increased with time.
5. Mixed organic acids caused slight color-change on chicken skin to be yellow and left slight sour odor on the skin. However, there were not be able to notice after cooking.
6. Purchasing decision of raw chicken meat, the consumer survey found that 94.4 % accepted the mixed acid-treated chicken when they did not have non-acid treated chicken comparison. However, when displaying both acid-treated and non-acid treated chicken meat without revealving the information of treatment, 58.4 % of consumers prefer to buy non-acid treated chicken meat.

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APPENDICES

APPENDIX I

Medium, Chemical agents, Materials, Instruments and Confermation procedures.

A. Medium **g/L**

A1. Brain heart infusion (Oxoid, U.S.A.)

Calf brain infusion solids	12.5
Beef heart infusion solids	5.0
Proteose peptone	10.0
Glucose	2.0
Sodium chloride	5.0
Disodium phosphate	2.5

pH 7.4 $\pm$ 0.2

Dissolve 39 g in 1 liter of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121^{o}C for 15 minutes.

A2. 7 % Blood Agar

Blood Agar Base (Mast Diagnostics, U.K.)	**g/L**
Peptone mixture	16.0
Yeast extract	2.0
D-Glucose	0.5
Sodium chloride	7.0
Agar	12.0

pH 7.3

Dissolve 37.5 g in 1 liter of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121^{o}C for 15 minutes. Add 7% sheep blood at 50^{o}C. Mix well before pouring.

A3. MIL Medium (Difco, U.S.A.) **g/L**

Peptone	10.0
Pancreatic digest of casein	10.0
Yeast extract	3.0
L-Lysine HCL	10.0
Dextrose	1.0
Ferric ammonium citrate	0.5

	g/L
Bromcresol purple	0.02
Agar	2.0

Suspend 36.5 g of medium in 1 liter of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121°C for 15 minutes.

A4. Modified Charcoal Cefoperazone Desoxycholate Agar (Oxoid, U.S.A.)

Campylobacter blood free selective medium	**g/L**
Nutrient Broth No.2	25.0
Bacteriological charcoal	4.0
Casein hydrolysate	3.0
Sodium desoxycholate	1.0
Ferrous sulphate	0.25
Sodium pyruvate	0.25
Agar	12.0
pH 7.4 ± 0.2	
Cefoperazone selective supplement	
Cefoperazone	16 mg

Suspend 22.75 g of Campylobacter blood free selective agar base in 500 ml of distilled water and bring to boil to dissolve. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add 1 vial of cefoperazone selective supplement reconstituted with 2 ml of sterile distilled water. Mix well and pour into sterile Petri dishes.

A5. Modified Oxford Agar (Oxoid, U.S.A.)	**g/L**
Listeria selective agar base	
Columbia blood agar base	39.0
Aesulin	1.0
Ferric ammonium citrate	0.5
Lithium chloride	15.0
pH 7.4 ± 0.2	
Listeria selective supplement	
Colistin	5.0 mg
Moxalactam	7.5 mg

Aseptically add 2 ml of sterile distilled water to a vial and mix well to dissolve. Add the contents to 500 ml of sterile Listeria selective agar base at 50°C. Mix well and pour into sterile Petri dishes.

A5. Preston broth (Oxoid, U.S.A.)

Nutrient broth No.2	**g/L**
'Lab-Lemco' powder	10.0
Peptone	10.0
Sodium chloride	5.0

Campylobacter growth supplement (SR084)

Vial contents (each vial is sufficient for 500 ml of medium)

Sodium pyruvate	0.125 g
Sodium metabisulphite	0.125 g
Ferroous sulphate (hydrated salt)	0.125 g

Campylobacter selective supplement (preston, SR117)

Vial contents (each vial is sufficient for 500 ml of medium)

Polymyxin B	2,500 IU
Rifampicin	5 mg
Trimethoprim Lactate	5 mg
Cycloheximide	50 mg

Dissolve 12.5 g of Nutrient broth No.2 in 475 ml of distilled water and sterilize by autoclaving at 121°C for 15 min. Cool to 50°C or below. Aseptically add 25 ml of lysed horse blood, 1 vial of Preston Campylobacter Selective Supplement SR117 and and 1 vial of Campylobacter Growth Supplement SR084 both reconstituted as directed. Aseptically dispense 10 ml volumes in sterile screw-capped test tubes. The Selective Enrichment Broth may be stored for up to 7 days at 4°C

A6. Three Sugar Iron Agar (Scharlau Microbiology, U.N.) g/L

Casein peptone	15.0
Meat extract	3.0
Yeast extract	3.0
Sodium chloride	5.0
Lactose	10.0
Sacarose	10.0
Dextrose	1.0

	g/L
Iron ammonium sulphate	0.3
Phenol red	0.025
Agar	12.0

pH 7.4 $\pm$ 0.2

Suspend 59.7 g in 1 liter of distilled water. Mix well and bring to boil to dissolve completely. Distribute into tubes. Sterilize by autoclaving at 121^{o}C for 15 minutes.

A7. Tryptic Soy Agar (Difco, U.S.A.)	**g/L**
Pancreatic digest of casein	15.0
Enzymatic digest of soybean meal	5.0
Sodium chloride	5.0
Agar	15.0

pH 7.3 $\pm$ 0.2

Suspend 40.0 g of medium in 1 liter of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121^{o}C for 15 minutes.

A8. Xylose Lysine Desoxycholate	**g/L**
Yeast extract	3.0
L-Lysine HCI	5.0
Xylose	3.75
Lactose	7.5
Sucrose	7.5
Sodium desoxycholate	1.0
Sodium chloride	5.0
Sodium thiosulphate	6.8
Ferric ammonium citrate	0.8
Phenol red	0.08
Agar	12.5

pH 7.4 $\pm$ 0.2

Suspend 53 g in 1 liter of distilled water. Heat with frequent agitation until the medium boils. Do not overheat. Transfer immediately to a water bath at 50^{o}C. Pour into plates as soon as the medium has cooled.

B. Chemical agents

Sodium chloride (Merch, USA)

Anti-serum for *Salmonella* species (SAP, Thailand)

Ninhydrin reagent

1 % hippurate solution

1 % Tetramethyl-p-phenylenediamine

3 % H_2O_2

Glacial acetic acid (Merck, Germany)

Citric acid (Ajex finechem, Australia)

Lactic acid (Ajex finechem, Australia)

C. Reagents

Phosphate buffer saline	**g/L**
Sodium chloride	8.0
Potassium chloride	0.2
Disodiumhydrogenphosphate	1.15
Potassiumdihydrogenphosphate	0.2

pH 7.4 $\pm$ 1.0

D. Materials

Acissors

Beakers

Bunsen burner

Centrifuge tube

Disposable loops (1 µl and 10 µl)

Eppendorf tube

Flasks

Forceps

Glass bottle (Duran, Germany)

Loops

McFarland standard 0.5

Petri disk

D. Materials (con.)

Ruler

Spreader

Screw-capped test tubes (Pyrex, USA)

Test tubes (Pyrex, USA)

Tips

Volumetric cylinders size 500, 250, 100, 50, 25 and 10 ml

E. Instruments

Automatic pipette, p200/p1000/p5000 (Gilson Medical Electronic, France)

Freezer (-20°C) (Sanyo, Japan)

Homoginizer PT 3100 Polytron (Kinematica, Japan)

IEC Clinical Centrifuge (International equipment company, USA)

Incubator 37 and 42°C (Termarks, Bergen-Norway)

Masticator (IUL instrument, Thailand)

Plastic sealer (Santo, Thailand)

pH meter cyberscan 500 (Beckman, U.S.A.)

Refrigerator (Sanyo, Japan)

A Spectro-sensor II (Applied Color System, Inc., Princeton, New Jersey)

Vortex mixer (Scientific, U.S.A.)

Water bath (Tokyo rikakikkai, Japan)

APPENDIX II

Identification procedures

A. Gram straining procedure

Gram crystal violet solution

Gram iodine solution

95% ethanol

Gram safranin solution

Staining procedure: Smear the organism on a clean slide and allow to dry. Heat a slide with a flam to fix the smear. Drop gram crystal violet solution on the smear. After minute, wash a slide with water and drain. Next, drop gram iodine solution on the smear, and wash with water after a minute. Decolorize a smeared slide with 95% ethanol and then wash with water. Drop Gram safranin solution on the smear in order to use as counterstain for 30 s. Allow a smeared slide to dry and then examine by microscopy under 100X objective lens over the entire smear.

B. Catalase test

Principle: To detect the presence of the enzyme catalase. Catalase enzyme is found in most bacteria. It catalyses the breakdown of hydrogen peroxide (H_2O_2) with the release of free Oxygen

Procedure: Smear the observed organism on a clean slide. Drop 3% H_2O_2 and mix well with the organism on a slide.

Result: The positive result show as bubbles formation.

The negative result show as no bubbles.

C. CAMP test

The Christie-Atkins-Munch-Peterson (CAMP) test is useful in confirming species particularly when blood agar stab test results are equivocal.

Procedure: To perform the test, streak a beta-hemolytic *S. aureus* and a *Rhodococcus equi* culture in parallel and diametrically opposite each other on a sheep blood agar plate (Sheep blood agar plates should be as fresh as possible). Streak

several test cultures parallel to one another, but at right angles to and between the *S. aureus* and *R. equi* streaks. Incubation at 35° C for 24-48 h.

Result: examine the plates for hemolysis. *L. monocytogenes* hemolytic reactions are enhanced in the zone influenced by the *S. aureus* streak. The other species remain non-hemolytic. The *L. monocytogenes* reaction is often optimal at 24 h rather than 48 h. To obtain enough *R. equi* to provide a good streak of growth, incubate the slant culture 48 h rather than 24 h. Use of known control isolates of *Listeria* spp. on a separate sheep blood agar plate is recommended.

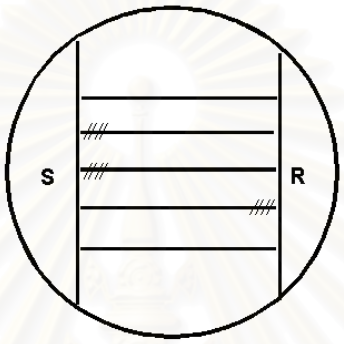

Figure 43 CAMP test for *Listeria monocytogenes*: Inoculation pattern of the sheep blood agar plate. Horizontal lines represent streak inoculations of 5 test strains. Vertical lines represent streak inoculations of *Staphylococcus aureus* (S) and *Rhodococcus equi* (R). Hatched lines indicate (diagrammatically only) the locations of hemolysis enhancement regions. Hemolysis of *L. monocytogenes* and *L. seeligeri* is enhanced near the *S. aureus* streak; *L. ivanovii* hemolysis is enhanced near the *R. equi* streak. Other species are non-hemolytic and do not react in this test.

D. Hippulate hydrolysis

Procedure: Emulsify generous 2 mm loopful of growth from the restreaked pick on the non selective or antibiotic plate to 0.4 ml 1 % hippulate solution in test tube. Incubate 2 h at 37^{o}C water bath. Add 0.2 ninhydrin reagent, agitate, and reincubate 10 min.

Result: Violet (not medium or pale purple) color is positive reaction only *C. jejuni* is hippurate-positive.

E. Motility test

Procedure: Remove organism from agar slant or non selective agar plate with straight inoculating needle and stab tube of EM motility agar Incubate 37^{o}C for 24 to 48 h.

Result: Typical umbrella-like growth of *L. monocytogenes* on motility agar

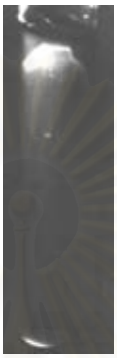

Figure 44 *L. monocytogenes* on motility agar

F. Oxidase test

Principle: Cytochrome oxidase is an enzyme found in some bacteria that transfers electrons to oxygen, the final electron acceptor in some electron transport chains. Thus, the enzyme oxidizes reduced cytochrome c to make this transfer of energy.

Procedure: Pick a good-sized amount of inoculum from a plate culture or slant culture and place it on a piece of filter paper. Add one drop of the reagent as 1% Tetramethyl-p-phenylenediamine (if it is dark blue, it is old and should not be used)

Result: A positive reaction will usually occur within 10-15 seconds, and will be a bluish-purple color that progressively becomes more purple. A positive reaction will occur within 30 seconds. DO NOT READ the reaction after 30 seconds.

G. Triple sugar iron agar test

Purpose: Triple sugar iron (TSI) agar is a screening medium used to identify gram- negative bacilli based on ability to ferment the carbohydrates glucose, sucrose, and lactose to produce H_2S gas.

Principle and interpretation: TSI agar contain protein, NaCl, Lactose, sucrose, dextrose, a sulfur source, an H_2S indicator, a pH indicator, and agar. The medium includes ten times as much lactose and sucrose as glucose. Bacteria that ferment glucose produce a variety of acids, turning the color of the medium from red to yellow. Larger amounts of acid are produced in the butt of the tube (fermentation) than in the slant of the tube (respiration). Organisms growing on TSI also from alkaline products from the oxidative decarboxylation of peptone. These alkaline products neutralize the small amounts of acids present in the slant but are unable to neutralize the large amounts of acid present in the butt. Thus, the appearance of an alkaline (red) slant and an acid (yellow) butt after 24 hours incubation indicates that the organisms is a glucose fermenter but is unable to ferment lactose and sucrose.

Bacteria that ferment lactose or sucrose (or broth), in addition to glucose, reduce such large amounts of acid that the oxidative deamination of protein that may occur in the slant does not yield enough alkaline products to cause a reversion of pH in that region. Thus, these bacteria produce an acid slant and acid butt. It is impossible to determine from the TSI reaction whether both lactose and sucrose are being fermented or only one of these carbohydrates is being fermented; individual carbohydrate fermentation tests are required to make this assessment.

Gas production (CO_2 and hydrogen) is detected by the presence of cracks or bubbles in the medium. These are formed when the accumulated gas escapes.

H_2S gas is produced as results of the reduction of thiosulfate. H_2S is a colorless gas and can be detected only in the presence of an indicator, in this case ferric ammonium sulfate. H_2S combines with the ferric ions of ferric ammonium sulfate to produce the insoluble black precipitate ferrous sulfide. Reduction of thiosulfate proceeds only in an acid environment, and blackening usually occurs in the butt of the tube. Although the black precipitate may frequently obscure the color of the butt, it can be assumed that the organism is a glucose fermenter because of the requirement for an acid environment. The reactions can be summarized as follow:

Alkaline slant/acid butt: glucose only fermented

Acid slant/acid butt: glucose and sucrose fermented or glucose and lactose fermented or glucose, lactose, and sucrose fermented

Bubbles or cracks present: gas produced

Black precipitate present: H_2S gas produced

Procedure: Inoculate test cultures to TSI agar by first touching a sterile bacteriologic needle to a colony and then stabbing the needle into the deep agar region of the medium. Hence withdrawing the needle, move it from side to side over the surface of the medium. Incubate cultures at 37°C for 18 to 24 hours. Examine cultures for color of the slant, butt, gas cracks, and blackening caused by H_2S.

H. Serological tests for *Salmonella*.

Salmonella polyvalent A – 67 antiserum (composed of group A, group B, group C, group D and every group to group 67 antisera)

Salmonella polyvalent A –I antiserum (composed of group A, group B, group C, group D group E, group F, group G, group H, group I antiserum)

Salmonella polyvalent O : 17 – O : 67 antiserum (composed of group J (O :17) , group K (O : 18) to group O : 67)

Procedure: sectioned slides may be used. Emulsify 3 mm loopful of culture from 24-48 h TSI slant or, preferably, TSA with 2 ml 0.85% saline. Add 1 drop of culture suspension to upper portion of each rectangular crayon-marked section. Add 1 drop of saline solution to lower part of one section only. Add 1 drop of *Salmonella* polyvalent somatic (O) to other section only. With clean sterile transfer loop or needle, mix culture suspension with saline solution for one section and repeat for other section containing antiserum. Tilt mixtures in back-and-forth motion for 1 min and observe against dark background in good illumination. Consider any degree of agglutination a positive reaction. Classify polyvalent somatic (O) and flagella (H) test results as follows:

Result: The positive show as agglutination in test mixture; no agglutination in saline control. The negative result show as no agglutination in test mixture; no agglutination in saline control.

APPENDIX III

Scoresheets for sensory testing

A. Scoresheet for Multiple Comparison Test

Code of test…….. **Date**……..**time**……..

Product……. **Name**…….

Characteristic……

Instruction: You are receiving samples of chicken to compare for color and odor difference. You have been given a reference sample, mark R with you are to compare each sample. Test each sample and score degree of difference of each sample to reference in the table.

Characteristic	Sample (code)		
	517	**727**	**464**
Color of skin	……..	……..	……..
Odor	……..	……..	……..

Nine-point scale: 1 = extremely lighter/mild
2 = much lighter/mild
3 = lighter/mild
4 = slightly lighter/mild
5 = no difference
6 = slightly darker/stronger
7 = darker/stronger
8 = much darker/stronger
9 = extremely darker/stronger

Comments:……
……
……

Thank you

Figure 45 Sample of Scoresheet for Multiple Comparison Tests**.**

B. Scoresheet for Acceptance and Preference Test

Code of test…….. **Date**……..**time**……..

Product……. **Name**…….

Occupation……..

Question:

1. Would you like to buy these chickens, why not?

A. Would buy B. Would not buy

Comments:……

……

……

2. Which one of these chickens do you prefer, why not?

A. 517[a] B. 216[a]

Comments:……

……

……

Thank you

Figure 46 Sample of Scoresheet for Acceptance and Preference Test

[a] mean code of mixed or non-mixed acids sprayed chicken skin sample

BIOGRAPHY

NAME:	Miss Jitchawalee Suwannangkoon
DATE OF BIRTH:	5 November 1980
PLACE OF BIRTH:	In Ratchaburi, Thailand.
INSTITUTION ATTENDED:	I graduated with the Bachelor degree of Science in Biology from the Faculty of Science, Silapakorn University in 2001.